VIROLOGY RESEARCH UNIT
Annual Report 1995

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EXECUTIVE SUMMARY 1995
VIROLOGY RESEARCH UNIT

The Virology Research Unit (VRU) pursues two main research objectives: 1) the characterization of plant viruses of economic or quarantine significance affecting CIAT-mandated commodities, and 2) their control.

In 1995, the VRU continued the characterization of selected cucumoviruses collected in representative bean-producing areas of the world. This subproject is financed by the Italian Government and is conducted in collaboration with the Institute of Applied Plant Virology of Turin, Italy. An innovative aspect of this project, is the use of the Polymerase Chain Reaction (PCR) technique for the characterization of the two cucumoviruses known to infect common beans, cucumber mosaic virus (CMV) and peanut stunt virus (PSV). The identification of these viruses is critical to implement effective control measures.

The characterization of cassava vein mosaic virus (CVMV), one of the most pathogenic viruses of cassava in northeastern Brazil, is also conducted as a part of the UNDP Profisma project. Non-infectious clones of the virus are used for the molecular characterization of CVMV, since the virus does not occur in Colombia. Results obtained to date, show that CVMV is a unique pararetrovirus. PCR analyses of CVMV suggest that the pathogenic variability of this pathogen is limited in Northeast Brazil.

Critical epidemiological parameters are being investigated in relation to the variable incidence of rice hoja blanca virus (RHBV) in different rice cultivars and rice production regions. Glasshouse and field surveys conducted in cooperation with FEDEARROZ and CORPOICA have demonstrated that plant age at the time of infection, is a determining factor in the incidence of RHBV. However, some genotypes, such as Colombia 1, become less susceptible as the plant grows past the critical 10 day post-planting period. regarding the planthopper vector of RHBV, it was observed that field-collected vectors are more aggressive and transmit the field isolates of RHBV to a higher percentage of test plants. This finding has important implication for the management of planthopper colonies under artificial conditions for germplasm screening purposes.

The characterization of two viruses affecting tropical forage species was completed in 1995. A potyvirus that infects *Brachiaria* spp. was characterized as a strain of johnsongrass mosaic virus (JGMV). However, this virus exhibits important differences in its pathogenic behaviour with respect to the spectrum of pathogenicity of the JGMV isolates present in Australia and the United States. We are currently investigating the effect of JGMV on seed production by virus-infected *Brachiaria* species.

A second virus characterized in 1995, is a sobemovirus that causes a severe golden mosaic of the forage legume *Calopogonium mucunoides*. This virus is transmitted by beetles and is highly contagious. However, the virus can be indirectly controlled by lowering the populations of its insect vector through integrated control practices. The

Calopogonium sobemovirus is related to but different from southern bean mosaic virus. Highly sensitive and reliable virus detection techniques have already been developed for the above-mentioned tropical forage viruses.

In the area of control of plant viruses affecting CIAT-mandated commodities, the VRU coninues to support the crop improvement activities of all CIAT Commodity Programs. The two main germplasm evaluation activities in the Bean Program are the screening for bean common and bean golden mosaic viruses. The screening is conducted under controlled conditions at CIAT-Palmira to maximize the inoculation efficiency. This service is also provided to NARIs worldwide.

The creation of a virus-free cassava core collection has been a major thrust of the cassava virology subproject. The number of accessions certified as virus-free is currently 437 out of the total core collection of 630 accessions.

The diversification of the genetic basis of rice hoja blanca resistance included the screening of 3,467 rice lines for their reaction to RHBV. Approximately 41 % of the lines were classified as resistant or moderately resistant to RHBV. A total of 1,433 *Brachiaria* spp. plants obtained from seed collected from JGMV-infected plants, was assayed for the presence of seed-borne virus. None of the test plants proved to be infected in this manner. The mechanical inoculation technique is being currently used to evaluate this forage grass for its reaction to JGMV with over 80% inoculation efficiency. Other tropical forage species being evaluated for their resistance to plant viruses is *Arachis pintoi*.

In 1995, the VRU took over the responsibility of indexing all the imported tropical forage species for the presence of viruses, the main concern of the Colombian quarantine authorities. This decision should greatly facilitate the international exchange of tropical forage germplasm.

The VRU is one of the most advanced plant virology laboratories in Latin America, offering support not only to the commodity programs of CIAT and collaborating NARIs, but also to other agricultural research institutions in the host country and many other countries in Asia, Africa, Eastern Europe, and the Americas.

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PROJECT AREA No. 1: Identification and Characterization of Plant Viruses of Economic or Quarantine Significance.

Beans

Subproject 1. Characterization of the main cucumoviruses that infect *Phaseolus vulgaris* worldwide.

Cucumoviruses are becoming widespread in several crops due to their seed transmissibility. Once these viruses are introduced in an agricultural area, they are transmitted efficiently by several aphid species to a very large number of cultivated and wild hosts. The presence of cucumoviruses has been often reported in bean-growing areas around the world. In the past, the general belief was that cucumber mosaic virus (CMV) was the causal agent of the severe distortion and mosaic symptoms observed in beans. However, recent work conducted in collaboration with the Institute of Applied Plant Virology, Turin, Italy, and the Samuel Roberts Noble Foundation, Ardmore, Oklahoma, has shown that two distinct cucumoviruses can be found attacking beans: CMV and peanut stunt virus (PSV). Moreover, cucumovirus isolates collected by CIAT in Chile have been shown to be natural hybrids of CMV and PSV. This unusual hybrid exhibits extreme pathogenicity in *P. vulgaris*.

The following table summarizes the characterization work that has been conducted on selected cucumovirus isolates, using the Polymerase Chain Reaction (PCR).

Table. Molecular characterization of selected cucumovirus isolates from different bean-growing regions of the world.

Cucumovirus Isolate	Origin	RNA 1	RNA 2	RNA 3
CMV-Pinto 114	Chile	PSV	PSV	CMV
CMV-Parral SR	Chile	—	—	CMV
CMV-La Platina	Chile	PSV	—	—
CMV-56	Turkey	—	PSV	PSV
CMV-114	Turkey	PSV	PSV	PSV
CMV-Nahavand	Iran	CMV	—	CMV
CMV-39	China	PSV	PSV	CMV/PSV

Subproject 2. Genetic interaction between bean severe mosaic virus and *Phaseolus vulgaris*

The inheritance of the systemic necrosis, mosaic and apical necrosis reactions induced by bean severe mosaic comoviruses in common bean (*Phaseolus vulgaris* L.), was studied in crosses of Great Northern 123 X Pitouco, Great Northern 123 X Iguaçu and Pitouco X Iguaçu. Cultivar Great Northern 123 reacts with mild mosaic, Iguaçu with systemic necrosis, and Pitouco with apical necrosis to bean severe mosaic comoviruses. The analysis of the F_1 and F_2 generations indicated that systemic necrosis was dominant over mosaic, and apical necrosis was dominant over both the systemic necrosis and mosaic. An independently inherited single dominant gene controlled the expression of systemic and apical necrosis in cultivars Iguaçu and Pitouco, respectively. The dominant gene for apical necrosis (*Anv*) found in Pitouco, was epistatic over the dominant gene (*Snv*) conditioning systemic necrosis in Iguaçu, as suggested by the F_2 of Pitouco X Iguaçu, which yielded a ratio of 12 apical necrosis:3 systemic necrosis:1 mosaic. The genotypes of Pitouco, Iguaçu and GN 123 are: *Anv Anv Snv Snv, anv anv Snv Snv,* and *anv anv snv snv*.

Cassava

Subproject 1. Characterization of cassava vein mosaic virus.

The research on cassava vein mosaic virus (CVMV) is conducted as a part of the UNDP Profisma project. Since CVMV is not present in Colombia, the work at the VRU has been done using non-infectious cDNA clones of the virus. The primary molecular characterization CVMV was completed and the article describing the virus was published during 1995. CVMV was tentatively classified as a caulimovirus, but these studies have determined that CVMV is a unique plant pararetrovirus that is distinct from the caulimoviruses.

Analysis of variation of cassava vein mosaic virus. Five sets of oligonucleotides were developed for the detection and amplification of approximately half the genome using the polymerase chain reaction (PCR). Investigations using PCR were continued to determine the degree of variation within CVMV isolates in the Northeast of Brazil.

Parts of four isolates of CVMV were amplified (using PCR), cloned and sequenced. The isolates were from Fortaleza Ceará, Araripina, and Petrolina, Pernambuco, and Piretiba, Bahia. Including the original cDNA clone of CVMV, five isolates of CVMV were compared. This analysis of variation is continuing and should be finished during 1996.

Approximatly 1200 nucleotides from the Petrolina isolate of CVMV were sequenced, and these were compared with the type isolate. The homology at both the nucleic and amino acid level showed over 95% similarity. Approximately 500 nucleotides of the Araripina

CVMV isolate was compared to the type isolate and the Petrolina isolate. Each of the isolates were approximate by 95% identical.

These results indicate that within the state of Pernambuco, the isolates are closely related. The PCR primers have amplified every isolate of CVMV that has been collected. These two measures of isolate homology make it probable that although the virus is spread over a wide geographical area, there is little pathogen diversity. It also demonstrates the effectiveness of the PCR assay as a method of detection of CVMV.

Subproject 2. Characterization of a defective cassava common mosaic virus RNA species. The presence of a defective cassava common mosaic virus (CsCMV) RNA species has been detected in total RNA extractions and purified preparations of the Brazilian and Paraguay virus isolates. There was a possibility that some of the RNA detected was a subgenomic species of RNA that encodes for the coat protein. The subgenomic coat protein species reported for other potexviruses are about 800-900 nucleotides in lengths. The defective RNA species is just over 100 nucleotides. Experiments were made that clearly demonstrated that the subgenomic RNA was present in all three isolates. The defective RNA did not appear to be present in the Colombian isolate. The defective RNA species was in high concentrations in both the Paraguayan and Brazilian isolates. In the Brazilian isolate, the genomic CsCMV-RNA was in a low concentration. The presence of the defective RNA species did not appear to affect the concentration of the Paraguayan isolate. In both isolates, the concentration of the defective RNA species was much higher than the genomic CsCMV RNA.

There is some evidence that the defective RNA species is interfering with the synthesis of the genomic CCMV.RNA. It is possible that this defective RNA species could be a natural control agent to mitigate the effects of the virus.

Rice

Subproject 1. Characterization of Isolates of *T. orizicolus* and RHBV, and Effect of Plant Age on susceptibility to RHBV.

Effect of plant age on susceptibility to RHBV: One of the factors that influences the degree of damage by a virus is plant age. Plants infected early in the growing season are generaly more severely affected than those that are infected later. In the case of RHBV, there was previous data indicating that younger plants may be more susceptible to virus infection. These studies were carried out to confirm the effect of plant age on the susceptibility to RHBV. Sets of replicated trials were conducted in the greenhouse and a trial was done to confirm the greenhouse results.

Eight varieties of rice were tested for their susceptibility to RHBV at five, ten, fifteen and twenty days post planting. The susceptible control was Bluebonnet-50 and the resistant

control was Colombia 1. The 4 replications of 6 plants were inoculated at each date. Each plant was inoculated in controlled conditions using two adult planthoppers that were proven to be viruliferous. The planthoppers were eliminated after a five day inoculation period and the plants were observed at 2-3 day intervals for the presence of RHBV symptoms.

The data with the percentage of plants infected at the four different plant ages are shown in Table 3. Bluebonnet 50 and Balilla are both highly susceptible and a high percentage of plants became infected with RHBV at all four plant ages. Fanny and IRAT 124 showed an intermediate reaction and over 20% of the plants became infected with RHBV at 10 and 15 days. The only date that Colusa had a moderate level of infection was at 5 days but 4 to 8% of the plants continued to become infected even at 25 days. Colombia 1, Llanos 5 and Blue Rose showed the highest level of resistance with few plants becoming infected with RHBV after the plants were 15 days old. All varieties were partially susceptible at 10 days after planting.

To coincide with the CIAT, INGER-LAC, FLAR Rice Breeders Workshop, July 31 - Aug. 3, 1995, a rice plot was planted to confirm the effect of plant age on the expression of RHBV in the field. In general, the results of the field evaluations reflected what was observed in the greenhouse studies earlier. Resistance to RHBV and % RHBV incidence varied with plant age. Cultivars that were considered to be resistant or moderately resistant (e.g. O. Llanos 5, O. Llanos 4 and Oryzica 1) suffer increased incidence of RHBV when inoculated with RHBV when they were younger (10-15 days post planting).

The susceptibility of the most resistant varieties has important epidemiological implications. If resistant varieties are planted next to established rice with high levels of *T. orizicolus* and RHBV, it is possible that a moderate (10-30) percentage of these plants can become infected with RHBV. Further evaluation is needed to test if crop management strategies can be used to minimize early infections in resistant varieties. Since the "resistant varieties" become less susceptible to the virus with age, the majority of the infections occur when the plants are young. Therefore, the use of chemicals to control the vector probably is not effective more than 15-20 days after planting. Many fields of the resistant variety Llanos 5 have had infection rates that are alarming to the farmers. Most of these fields have less than 10% of the plants affected by RHBV and the early susceptibility of this variety may be an explain these levels of infection.

Greenhouse Experiments for colony and virus variability: In 1994 the program began studies focused on characterizing the variability of *T. orizicolus* populations and RHBV isolates from Colombia in response to reports from the Tolima region that cv. Llanos 5 was more susceptible to RHBV than previously expected. The importance of variability in vector and virus populations affects both the selection methods for RHBV resistance and potentially the regional performance of resistant rice varieties.

These studies were started during 1994 and will continue in 1996. *T. orizicolus* and RHBV virus isolates from Tolima were collected and vector and nonvector colonies established in the greenhouse. Nine varieties and advanced rice breeding lines (Bluebonnet 50, O. Caribe 8, CICA 8, O. Llanos 5, Colombia 1, Orizyca 1, IRAT 124, CT8008 and CT8837) representing a range of resistance reactions were selected as tester varieties and screened for resistance to RHBV at four different plant ages (10, 15, 20, and 25 days post planting) using the CIAT and Tolima vector colonies.

Compared to the CIAT colony and RHBV vector isolate, the Tolima colony and RHBV vector isolate was more virulent. Averaged across varieties, plants exposed to the Tolima colony had a significantly higher average % RHBV infection level than those exposed to the CIAT colony. Also, as described above and in 1994, all the lines tested, including O. Llanos 5 and Colombia 1, were more susceptible to RHBV infection when they were 10 days old compared to those that were 15-25 days old. Likewise, averaged across dates, varieties exposed to the Tolima colony had a higher average % RHBV infection level compared to the CIAT colony. This effect was most evident for moderately resistant to resistant plants.

As was mentioned, these findings have practical implications for the management of *T. orizicolus* in the field. Given the fact that RHBV appears to be increasing in Colombia and that even resistant varieties such as Llanos 5 are susceptible to the virus at early growth stages (i.e. <10 days) if rice growers have high populations of *T. orizicolus* in their fields during this period the utility of such varieties should be augmented by the judicious use of a single insecticide application during this critical period.

Subproject 2. Survey of Prevalence of plants and *T. orizicolus* Infected with RHBV in Colombia.

In response to a perceived increase of RHBV infected fields in Colombia, a collaborative project with FEDEARROZ and CORPOICA was begun in July to survey for the prevalence of plants and *T. orizicolus* infected with RHBV in various regions of Colombia. To date, samples have been obtained from the rice growing regions of Tolima (Tolima), Villavicencio (Meta), Cucuta (Norte de Santander) and Huila (Huila). Samples of *T. orizicolus* were collected either by Field or Research Assistants from FEDEARROZ and CORPOICA, or by members of the Rice Program, frozen and sent to CIAT for ELISA analysis. RHBV has been detected in all regions sampled, except Huila. However, few samples have been obtained from this department. The region of Villavicencio currently has the highest level of RHBV with the average percent of *T. orizicolus* infected with RHBV (actual vectors or virulifierous planthoppers) in rice fields being ca. 4.7%. In comparison, the percent of actual vectors averaged across all samples in the Tolima region is ca. 1.8%. The level of RHBV present in Villavicencio region should raise some concerns as this level is approaching the level of actual vectors in the field (10-15%) that is typically encountered during an epidemic of RHBV. During 1996, CIAT and

FEDEARROZ will continue to monitor the extent of RHBV present in Colombia, and, we will initiate studies focused on determining the number of potential vectors (i.e. planthoppers capable of transmitting the virus but not yet infected) present in the field during 1996.

Tropical Forages

The Virology Research Unit (VRU) continued activities in support of two main areas of tropical forages research: 1) the detection and characterization of viruses affecting tropical forages, and 2) the development of germplasm screening methods for genetic improvement purposes.

In the first area, the VRU has accepted the responsibility to index all of the tropical forage germplasm imported by CIAT, for the presence of viruses of quarantine significance. Following detection of viruses in tropical forage germplasm, the infectious agents are isolated and characterized to produce diagnostic tools that can be used for specific virus detection in conjunction with quarantine and plant improvement activities at CIAT and collaborating national programs.

In the area of disease screening, the VRU is conducting tests to determine the most reliable artificial inoculation procedures for the evaluation and selection of tropical forage germplasm possessing resistance to viral pathogens of economic importance.

Subproject 1. Characterization of a potyvirus infecting *Brachiaria* spp.

Last year, we reported on the molecular characterization on the potyvirus detected in *Brachiaria brizantha* at CIAT. This virus was shown to have a nucleotide sequence similarity of 97.3 % with the corresponding fragment analyzed of the coat protein of johnsongrass mosaic virus strain JG (JGMV-JG), previously described in Australia. This year, an antiserum prepared to JGMV-JG in Australia, was tested at CIAT with the *Brachiaria* potyvirus. The antiserum reacted positively, confirming the molecular characterization of the *Brachiaria* potyvirus as a strain of johnsongrass mosaic virus. However, the *Brachiaria* potyvirus has not been shown to infect johnsongrass under artificial or natural conditions at CIAT. So far, *Brachiaria* remains the main host of this virus in Colombia. A manuscript of this publication has been submitted to the Journal of Phytopathology.

Subproject 2. Characterization of a potyvirus infecting *Paspalum conjugatum*.

A potyvirus was isolated from a mosaic-affected plant of *Paspalum conjugatum* growing in the locality of Santander de Quilichao, Cauca, Colombia. At first, the virus seemed to be the same as the *Brachiaria* potyvirus described above. However, recent tests indicate that the two viruses are serologically related but not identical. The *Paspalum* virus has a different host range, including *Sorghum* differentials not infected by the *Brachiaria* virus, such as *S. bicolor* 'Rio' and 'Trudex'. The *Paspalum* virus also infected five accessions of *P. conjugatum* not previously infected by the *Brachiaria* potyvirus. However, the *Brachiaria* potyvirus infects *Panicum maximum* while the *Paspalum* virus does not.

Reverse transcription-polymerase chain reaction of total nucleic acid extracted from infected *P. conjugatum*, resulted in the amplification and subsequent cloning of a product that, once sequenced, revealed the presence of an open reading frame of 149 amino acid residues and an untranslated region of 233 nucleotides. Percentage nucleotide sequence similarities of 66.5 and 64.9% were observed between the *Paspalum* virus and the *Brachiaria* and JGMV-JG strains of JGMV, respectively. Analysis of the deduced amino acid sequence of these viruses, showed higher (85.9 and 86.6%) similarities. Nevertheless, it is apparent that the *Paspalum* potyvirus is not closely related to JGMV, and that it is different from the other members of the sugarcane mosaic virus, with whom it has even lower amino acid and nucleotide sequence similarities.

Subproject 3. Characterization of a sobemovirus infecting *Calopogonium mucunoides*:

The sobemovirus tentatively characterized last year as a strain of southern bean mosaic virus (SBMV) infecting calopo, was partially characterized at the molecular level in 1995. The following abstract summarizes the research conducted:

The cultivation of calopo, *Calopogonium mucunoides* Desv., as a promising forage legume for the lowland tropics, was hindered by its susceptibility to a severe yellow mosaic disease observed in the Eastern Plains of Colombia. An isometric virus ca. 28 nm in diameter was observed by electron microscopy in leaf extracts, purified preparations and in phloem cells of systemically infected calopo plants. The virus was transmitted by mechanical means and by the chrysomelid beetle *Diabrotica balteata*. The host range of the virus was restricted to the legumes *Phaseolus vulgaris, Vigna unguiculata, V. radiata, Centrosema* spp., and *Senna occidentalis*. The physical and chemical properties of the calopo virus were similar to those reported for the sobemovirus group, and the virus was antigenically related to the bean and cowpea strains of southern bean mosaic virus (SBMV). However, the host range of the calopo virus differed from the pathogenicity spectra of the bean, cowpea, Ghana and Mexican strains of SBMV. Nucleotide sequence analysis of a 609 bp fragment amplified from the coat protein region of the calopo

sobemovirus, revealed similarities of 81.8 and 66.1 % with the corresponding regions of the bean and cowpea strains of SBMV. The respective homologies increased to 83.7 and 67.8 % when the deduced amino acid sequences of these viruses were compared. It is concluded that the mosaic disease of calopo is caused by a previously undescribed sobemovirus for which the name calopo yellow mosaic virus is suggested.

PROJECT AREA No. 2: Control of economically important plant viruses affecting CIAT-mandated commodities.

Beans

Subproject 1. Screening for common mosaic resistance

The incorporation of bean common mosaic virus (BCMV) resistance in the improved germplasm produced by the Bean Program continues to be a critical objective of the Program. This year, different materials were screened for their reaction to BCMV. Among the germplasm screened were: parental materials (90), 1,400 plants for incorporation of monogenic dominant resistance, and 2,667 plant selections for incorporation of multiple resistance to the mosaic- and necrosis-inducing strains of BCMV.

A total of 2,880 plants were also inoculated for a study of gamete selection for multiple resistance. Finally, the VRU conducts BCMV screening tests for NARIs and other research institutions, world-wide. This year, the VRU screened 122 lines for Colorado State University in search of lines possessing monogenic dominant resistance.

Subproject 2. Screening for bean golden mosaic resistance

Bean golden mosaic virus (BGMV) is considered the most devastating viral pathogen of beans in the tropical lowlands of Latin America. The only sustainable method of control for BGMV has been the deployment of tolerant cultivars, the majority of which has been produced by CIAT in collaboration with NARIs.

The VRU has been screening bean germplasm for BGMV resistance, under controlled conditions at CIAT. This methodology has been shown to accurately reproduce symptom expression under field conditions. The artificial inoculation capacity developed at CIAT, has also led to the investigation of the various mechanisms of BGMV resistance detected in *Phaseolus vulgaris*. These mechanisms and the genes that condition them, are being characterized and combined in different genetic backgrounds.

In 1995, 290 breeding materials were screened for their reaction to BGMV. These results were used to further select lines possessing multiple resistance to other diseases and pests.

Cassava

Subproject 1. Screening the core collection for resistance to cassava frogskin virus.

During 1995, a field experiment was begun to analyze the 630 accession of the cassava core collection for tolerance or resistance to cassava frogskin virus. The entire collection will be screened over several years and the most promising clones will be tested in multilocational trials. Any resistant or tolerant accessions will be used as parents in development of resistant germplasm pools.

The major limitation on this experiment has been getting a sufficient quantities of a single source of the virus to inoculate the entire core collection. During the first year of the experiment over 200 accessions were inoculated by grafting with CFSV. During the first year, approximately 35% of the accessions showed the expression of root symptoms that are typical of CFSV. The remaining accessions are being tested to assure that they are infected with the virus. This was the first cycle and the symptoms tended to be mild. Root weights were taken and these will be compared with average data for the core collection. The first year data will also serve as the baseline data for yield data.

The source of inoculum and the remaining accessions of the core collection were propagated during 1995. The goal is to have most of the core collection assessions inoculated with a common source of the virus and planted in the field by March 1996. This is the beginning of a extensive search for resistance or tolerance to CFSV. This is a continuing activity that will take at least three to five years to get meaningful results.

Subproject 2. Quarantine Activities

Core collection: For three years, the VRU in conjunction with the GRU and Cassava Program have continued the certification of *in vitro* cassava germplasm. The priority during this time has been the core collection and some elite varieties. Once *in vitro* clones are tested and certified, they become available for international exchange.

During 1995, 245 accession of the core collection of CIAT were testing for cassava common mosaic virus, cassava X virus (CsXV), cassava Colombian symptomless virus (CCSpV), and CFSV. This year less than half of the accessions tested were free of

viruses. One third of the accessions tested in 1995 were infected with cassava frogskin disease. The percentage of plants in the field core collection infected with CFSV is increasing every year. When the testing program began only 15% of the accessions were positive for CFSV.

The total number of the core collection accession that have been certified as virus free is 437 out of a total of 630. More than 95% of the core collection has been tested, and approximately 20% are infected with CFSV, 8% with CCSpV, and 3% with CsXV. There are two challenges ahead. The first is to introduce the remaining 193 accessions as virus free into the core collection. The second is to maintain a field core collection that is virus free. CFSD affects the yield and starch quantity of the roots, and CFSV is rapidly disseminating throughout the core collection. This is affecting the utility of the core collection.

Rice

Subproject 1. Diversification of resistance to Rice hoja blanca virus

RHBV is present throughout much of tropical America and there have been documented RHBV epidemics since 1935. Epidemics of RHBV are cyclic with outbreaks occurring every 8-15 years. The last major outbreak of RHBV was during the early to mid 1980s. When an outbreaks of RHBV occur, yield losses have been estimated to be between 25% and 50%. As new epidemics of RHBV occur, similar losses can be expected throughout the affected areas. The vector of RHBV is the planthopper, *Tagosodes orizicolus* (Muir). The virus multiplies both in rice and in its planthopper vector. The virus also causes a disease in the insect and those insects which harbor the virus are less fit compared to those that do not. It is speculated that this phenomenon, coupled with a slow, progressive build-up of plants infected with virus in the field, is responsible for the cyclic nature of the RHBV epidemics.

Besides being the vector of RHBV, the planthopper insect *Tagosodes oryzicolus* (Muir) is a serious pest of rice that causes direct damage. The direct damage that is caused by the planthopper and the uncertainty of RHBV epidemics are the reasons farmers spray up to 5-6 times to control this planthopper.

The levels of RHBV detected in the field are currently increasing in Colombia. Venezuela and Ecuador recently experienced outbreaks of RHBV, and Costa Rica is currently experiencing an outbreak of RHBV. The increases in reports of the incidence of RHBV may portend a new cycle of a RHBV epidemic. Some of the objectives of this project include more complete monitoring of viruliferous planthoppers, surveys of disease

incidence, and a better evaluation of rice cultivars and breeding lines that are resistant to the planthopper and/or the virus. This should help mitigate losses, should an epidemic occur, and reduce the long term losses caused by recurring epidemics.

Subproject 2. Field and Greenhouse Evaluations for Resistance to RHBV.

The RHBV evaluations are conducted twice yearly in the field, and when requested in the greenhouse. Out of the total 3,467 lines evaluated in the field during the first semester of 1995, 16% were classified as highly resistant to RHBV (RHBV rating ≤1); 15% were classified as resistant (RHBV rating =3); 10% were classified as intermediate (RHBV rating =5). The remaining 59 % were considered susceptible (RHBV rating ≥7) (Table 1). To assure that advanced lines have a good level of resistance only those lines that have a rating of ≤3 were recommended for further evaluation.

In this evaluation, 270 advanced RHBV tolerant lines from the CIAT Irrigated Rice Breeding Program and, 99 lines from FEDEARROZ were tested. 95% of the CIAT Irrigated Rice Breeding lines exhibited a RHBV rating ≤3, and 22% of the FEDEARROZ lines exhibited a RHBV rating ≤3. The level of resistance in the FEDEARROZ lines was similar to the level of resistance in the total populations because these lines had not been previously screened for RHBV resistance. This shows the importance of selective screening to assure high levels of RHBV resistance in advanced lines.

It should be also noted that the same 270 CIAT RHBV tolerant lines planted in the second semester were evaluated during the first smester in the same plot, and a high degree of variation was noted between these two evaluations. The coefficient of determination (r^2) calculated for the two evaluations was 0.33 indicating that only 33% of the variation in RHBV could be accounted for by knowledge of the prior RHBV rating. The reasons for the high degree of variability are unclear. Semester by semester, insect population density, vector capacity, and random environmental variation are undoubtedly significant sources of error in these evaluations. The current selection method is being evaluated to determine how to minimize the variation between separate trials.

Tropical Forages

Subproject 1. Development of a reliable screening methodology to select *Brachiaria* genotypes resistant to potyviruses.

Artificial inoculation tests conducted in 1994, yielded infection rates of up to 35 and 80% when the *Brachiaria* potyvirus was manually inoculated onto vegetative propagules and seedlings of *Brachiaria* spp., respectively. The lower inoculation efficiency observed for vegetative propagules relative to seedlings, is not unusual for most virus-host systems,

where the phenomenon known as 'adult plant resistance' is responsible for low infection rates of viruses inoculated by artificial means.

This year, a different mechanical inoculation technique (the air-brush technique) used for other potyviruses of grasses, such as sugarcane mosaic virus, was tested using *Brachiaria* seedlings. The results from this test conducted at 30 and 50 p.s.i, yielded average transmission efficiencies of 11 and 6%. Consequently, this technique was considered inadequate for screening purposes.

The mechanical inoculation technique used at CIAT has yielded infection rates of 100 % for other grasses, such as sorghum, maize, and *Paspalum* spp. While the species of *Brachiaria* tested are more difficult to inoculate with the conventional manual inoculation technique, the current infection rates of 80-90% are adequate for screening purposes, as long as the *Brachiaria* test plants are inoculated as seedlings.

Subproject 2. Effect of johnsongrass mosaic virus on seed production of *Brachiaria* spp.

Last year, a preliminary test was conducted to determine the effect of the *Brachiaria* potyvirus on seed yield of *B. brizantha.* The average seed yield of 15 *B. brizantha* plants systemically infected by the virus was 92.4, and 4 out of the 15 plants tested did not yield any seed. The average yield of the virus-free plants was 213 seeds and all of the plants produced seed.

The above experiment was repeated this year, increasing the number of *B. brizantha* test plants to 50 per treatment (virus-infected and virus-free). A randomized block design with five reps of 10 plants each for the two treatments, has been chosen for this experiment. The test plants were inoculated in June and we are waiting to harvest the seed.

Subproject 3. Evaluation of the possible seed transmission of the *Brachiaria* strain of johnsongrass mosaic virus in *B. brizantha*.

The potential seed transmissibility of the *Brachiaria* strain of johnsongrass mosaic potyvirus was investigated. A total of 1,433 seeds collected from systemically infected *B. brizantha* plants were germinated, and the resulting seedlings assayed for the presence of the virus. None of the seedlings assayed was infected by the virus. These results reduce the probability of seed transmission of the virus to less than 0.07%. Therefore, there is no evidence of seed transmission of JGMV-Brac in seed of B. brizantha.

Subproject 4. Screening of *Brachiaria* germplasm for its reaction to the *Brachiaria* strain of johnsongrass mosaic virus.

A total of 131 *Brachiaria* sp. selections made in Caqueta and Carimagua, have been under continuous screening by frequent rejuvenation and manual inoculation of the vegetative propagules. To date, a total of 64 hybrids have proved susceptible to the virus. The list of the hybrids that have escaped infection under artificial conditions is the following:

BR93/0005	**BR93/0262**	**BR93/0271**	**BR93/0278**
0284	0370	0416	0497
0590	0601	0747	0839
0929	1079	1088	1245
1247	1256	1263	1264
1266	1282	1286	1301
1321	1336	1371	1417
1505	1649	1650	1651
1653	1656	1676	1677
2014	2053	2179	2382
2385	2400	2770	2772
2790	2795	3126	3179
BR93/3303	3305	3310	3312
3317	3687	3693	3697
3704	3705	3709	3781
3910	3916	3970	3975
3983	4016	4022	

The above hybrids should be further evaluated when seed becomes available, to increase the efficiency of inoculation and distinguish between virus resistance and 'adult plant resistance'.

Subproject 5. Screening of *Arachis* spp. germplasm for its reaction to peanut mottle virus.

The following Table shows the results of the screening of 18 *Arachis pintoi* and 4 *A. repens* accessions for their resistance to peanut mottle virus.

Accession No.	Reaction to PMoV	Acc. No.	React. PMoV
Arachis pintoi			
22148	mosaic	22157	mosaic
22149	mosaic	22158	symptomless
22150	mosaic	22159	symptomless
22151	mosaic	22160	mosaic
22153	mosaic	22172	mosaic
22154	mosaic	22173	symptomless
22155	mosaic	22174	symptomless
22155	mosaic	22175	symptomless
22156	mosaic	22176	symptomless
Arachis repens			
22161	symptomless	22162	symptomless
22163	symptomless	22164	symptomless

Subproject 6. Indexing of imported tropical forage germplasm for the presence of viruses of quarantine significance.

The VRU will be indexing all of the tropical forage germplasm imported into Colombia for the presence of viruses. This operation will be critical for the implementation of the new quarantine measures agreed upon with the Colombian Government specifically for tropical forages.

This operation was started in the second semester of the year and, so far, a total of 120 plant introductions of 26 different species have been indexed for the presence of viruses using electron microscopy and nucleic acid analyses. Of the 120 introductions, six were shown to contain virus-like nucleic acids and were kept under quarantine for further tests.

Indexing of Tropical Forage Germplasm

Genus	Species	Origin	E.M.	ds-RNA
Arachis	pintoi	Brazil	(-)	(-)
Arachis	repens	Brazil	(-)	(-)
Arachis	villosa	Brazil	(-)	(-)
Flemingia	macrophylla	Vietnam	(-)	(+)
Desmodium	heterocarpon	Vietnam	(-)	(+)
Abrus	precatorius	Vietnam	(-)	(-)
Desmodium	heterophyllum	Vietnam	(-)	(+)

Genus	Species	Origin	E.M.	ds-RNA
Desmodium	velutinum	Vietnam	(-)	(+)
Dendrolobium	triangulare	Vietnam	(-)	(-)
Dendrolobium	lanceolatum	Vietnam	(-)	(-)
Uraria	rufescens	Vietnam	(-)	(-)
Phyllodium	pulchellum	Vietnam	(-)	(-)
Desmodium	gangeticum	Vietnam	(-)	(-)
Dendrolobiu	mumbellatum	Vietnam	(-)	(-)
Christia	obcordata	Vietnam	(-)	(-)
Pueraria	phaseoloides	Vietnam	(-)	(+)
Centrosema	tapirapoanense	Brazil	(-)	(-)
Centrosema	angustifolim	Brazil	(-)	(-)
Stylosanthes	guianensis	Phillipines	(-)	(-)
Macrotyloma	axilare	Australia	(-)	(-)
Chamaecrista	rotundifolia	Australia	(-)	(-)
Mucuna	pruriens	Venezuela	(-)	(-)
Mucuna	pruriens	Brazil	(-)	(-)
Mucuna	pruriens	Honduras	(-)	(-)
Macrotyloma	axillare	Costa Rica	(-)	(-)
Macrotyloma	axillare	Kenya	(-)	(-)
Macrotyloma	axillare	Rwanda	(-)	(-)
Macrotyloma	axillare	Malawi	(-)	(-)
Pachyrrhizus	hybrid	Africa	(-)	(+)

CURRENT COOPERATIVE RESEARCH PROJECTS WITH ADVANCED RESEARCH INSTITUTIONS

Italian Government

TITLE: "CHARACTERIZATION OF CUCUMOVIRUSES INFECTING BEANS IN THE MAIN PRODUCTION AREAS OF THE WORLD".

Institutions: Instituto de Fitovirologia Applicata, Turin, Italy.

Principal Investigators: Dr. Vittoria Lisa and Dr. Francisco J. Morales.

"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)

UNDP

TITLE: "ECOLOGICALLY-SUSTAINABLE CASSAVA PLANT PROTECTION IN SOUTH AMERICA AND AFRICA: AN ENVIRONMENTALLY SOUND APPROACH"

Institutions: EMBRAPA, IITA, CIAT

Principal Investigators: A. Bellloti, L. Calvert, C. Lozano, S. Lapointe.

1995 PUBLICATIONS

Anderson, P.K. and Morales F.J. The Emergence of New Plant Disease: The Case of Insect-transmitted Plant Viruses. N.Y. Acad. Sci. 70: 181:194.

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Characterization of cassava vien mosaic virus: A distinct plant pararetrovirus. Journal of General Virology 76: 1271-1278.

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Calvert, L.A., Cuervo, M., Ospina, M.D., Fauquet, C., & Ramirez, B.C., 1995. Characterization of cassava common mosaic virus. Accepted for Publication in Journal of General Virology.

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