VIROLOGY

Activity 1. Resistant varieties for cassava frogskin disease.

Introduction

In the Amazon regions of Brazil and Colombia, it was observed that there where apparent difference in the reaction of varieties to cassava Frogskin disease (CFSD). Some varieties developed typical root symptoms, while other varieties that were planted in same fields did not develop symptoms. This led to the idea that some cassava landraces are resistant to CFSD. In 1995, it was decided to test the 640 accessions of the CIAT cassava core collection for resistance to cassava frogskin disease (CFSD). The results have shown that tolerance to CFSD is widespread in cassava germplasm. More than 100 tolerant lines have been identified and are potential sources of resistant to CFSD. During the three last cycles, around 50 lines have been evaluated for their agronomic characteristics and resistance to other pests. All these lines are rated as tolerant and have remained infested with CFSD at least seven growing cycles.

Evaluation of cassava for resistance to CFSD

The plants tested were from the core collection of 640 cassava lines that are representative of the CIAT cassava collection that consists of over 6000 lines. All plants in this trial were graft inoculated using stem cuttings of the cassava line CT5460-10. This line reacts like Secundina and when it is affected with CFSD, the plant develops mosaic leaf symptoms. This meant that it was easy to assure that the sources of inoculum were indeed affected with CFSD. Originally five plants from each line were inoculated by grafting with the CFSD affected stem cuttings of line 5460-10. In the last three years between 44 and 50 lines were grown in randomize block design of 4 repetitions with 10 plants per repetition and evaluated visually for root symptoms. Representative plants in these lines were assayed for CFSD by grafting stem cuttings (rootstock) to Secundina (scion), and the new leaves were examined for mosaic symptoms. All of the plants tested were positive for CFSD. The rating scale used was 1 for no symptoms, 2 for very mild symptoms, 3 for moderate symptoms, and 4 for severe symptoms. The ratings, of 30 lines and their yields during the last three years, are summarized in table 1. These are the best cassava lines in the CIAT cassava core collection for resistance to cassava frogskin disease and that yield well in the conditions at the CIAT experiment station at Santander de Quilichao, Cauca, Colombia. There are eight lines from Peru, four from Colombia, and only two from Brazil. Almost 50% of the lines selected came from Countries where CFSD is endemic. One odd result is the there are four lines from Malasia. It is suspected that they share common resistant parents.

There is ample resistance in the cassava germplasm for cassava frogskin disease. It is a form of tolerance because the plants remain infected and the disease is transmitted thought the infected stem cuttings. Under the condition of mid-altitude tropics, these lines have remained tolerant year after year. Some lines do have more disease in some years, but this is expected given that in cool condition, there is a tendency for greater expression of the root symptoms. After eight years of field trials, we have a solid base to state that the resistance is stable and holds up under the range of

climatic variation that occurs at the screening site. From just the core collection of CIAT, landraces or varieties have been identified for most of the countries where CFSD is endemic and an important production constraint.

What needs to be done

The 30 varieties reported in these trials are resistant to CFSD and many have adequate yields under the conditions tested. There is also data on 100 other lines with tolerance. This means that there is a wide range of germplasm options for cassava growing areas where CFSD is a problem. These resistant varieties can be tested using participatory selection, and this should give the farmers a method to reduce economic losses due to CFSD and select cassava that meets there criteria of agronomic and utilization traits.

Additional trials are needed to determine if the resistance will be effective at higher elevations. We are looking for participatory farmer groups to test these materials in the Department of Cauca.

In Countries where CFSD is endemic, screening local varieties that have little or no disease should be done. It is probable that many will be resistant varieties.

It is time to start a study to understand the genetics of resistance to CFSD. The same populations that can be used to determine the genetics of resistance can be used to develop molecular markers for the resistance. This will be useful for countries where the disease is not common, but there is demand for resistance materials.

Active breeding programs that incorporate CFSD resistance should be started in Colombia, Brazil, Costa Rica and other countries where the disease is endemic. Resistant varieties are needed to minimize losses due to CFSD.

Table 1. The best lines in the CIAT core collection for resistance to CFSD.

Variety	2000-2001		2001-2002		2002-2003		3 years
	Symptoms	Yield	Symptoms	Yield	Symptoms	Yield	Average Yield
M Per 183	1.00	3.95	1.02	5.50	1.00	3.07	4.17
M Per 438	1.00	3.95	1.00	2.69	1.00	1.79	2.81
M Chn 2	1.00	3.32	1.00	2.16	1.00	1.69	2.39
M Mex 95	1.03	2.79	1.04	2.35	1.00	2.00	2.38
M Per 213	1.00	2.70	1.00	2.16	1.00	2.11	2.32
M Bra 886	1.08	2.32	1.50	2.56	1.08	1.71	2.20
M Ecu 68	1.00	1.18	1.00	1.91	1.00	3.15	2.08
M Col 634	1.00	2.54	1.19	2.04	1.29	1.56	2.04
M Mal 50	1.00	3.13	1.00	1.58	1.08	1.38	2.03
M Per 431	1.00	2.12	1.00	1.86	1.00	1.98	1.99
M Gua 78	1.00	1.97	1.20	1.63	1.04	2.21	1.93
M Col 1468	1.03	2.21	1.30	1.83	1.33	1.52	1.85
HMC 1	1.00	1.72	1.23	1.62	1.25	1.69	1.68
M Bra 325	1.00	2.22	1.00	1.68	1.25	1.12	1.67
M Per 209	1.00	1.99	1.12	1.91	1.00	1.08	1.66

Variety	2000-2001		2001-2002		2002-2003		3 years
	Symptoms	Yield	Symptoms	Yield	Symptoms	Yield	Average Yield
M Cr 59	1.13	2.00	1.06	1.59	1.20	1.43	1.60
M Per 243	1.00	1.29	1.00	1.49	1.06	1.98	1.59
M Mal 24	1.00	1.91	1.04	1.66	1.08	1.65	1.55
M Gua 41	1.05	1.67	1.00	1.39	1.06	1.56	1.54
M Mex 80	1.00	1.40	1.07	1.82	1.06	1.04	1.42
M Per 184	1.72	1.28	1.54	1.87	1.24	0.86	1.34
M Mal 13	1.00	1.16	1.02	2.16	1.00	1.77	1.31
M Ind 26	1.04	1.96	1.11	1.14	1.00	0.35	1.15
M Cr 79	1.23	1.71	1.23	1.22	1.21	0.50	1.14
M Mal 38	1.03	0.95	1.07	1.38	1.14	1.06	1.13
M Col 2157	1.00	1.13	1.00	1.14	1.04	0.77	1.01
M Per 377	1.03	1.07	1.00	0.96	1.00	0.73	0.92
M Par 163	1.03	1.47	1.09	0.48	1.23	0.58	0.84
M Bol 1	1.00	0.89	1.00	0.77	1.03	0.63	0.76
M Mex 102	1.00	0.73	1.00	0.43	1.00	0.74	0.63

Activity 2. Further studies to associate a reolike virus in *Manihot esculenta* affected with cassava frogskin disease.

Detection of a Genomic Segment of Cassava Frogskin Virus

In last years report, we reviewed the evidence for a reolike virus in cassava. This included multiple double stranded RNA species, virus-like particles and cDNA clones that have homology with rice ragged stunt virus. This virus appear to be associated with CFSD but we asserted that additional research was needed before reaching a definite conclusion. This year, we have made a plant by plant analysis of the dsRNA products using both the cassava frogskin virus (CFSV) Segment (S)5 and the CFSV S1 cDNA clones. These are extractions that do not involve amplifying either RNA nor DNA. With the CFSV S5 clone, the results are consistent and nothing is detected in the healthy plants while a dsRNA product of approximately 3000 nucleotides is found in the CFSD affected plant. This product is specific and hybridizes with the CFSV S5 clone **(Figure 1)**. In these experiments, we used CFSD infected and healthy plants of the varieties Secundina and CMC40 (M Col 1468). These varieties demonstrate two distinct types of plant reactions. Secundina is highly susceptible and develops mosaic symptoms on the leaves as well as the typical root symptoms. CMC40 is a tolerant variety that never has leaf symptoms and the root symptoms are general mild.

This is approximately the size expected of the genomic S5 segment of a reo-virus. The rice ragged stunt virus (RRSV) S5 segment is 2682 nucleotides and shares amino acid homology with the CFSV S5 sequence. The detection of the CFSV S5 genomic segment is further evidence of the presence of a reolike virus in cassava. Since it could only be detected in the CFSD affected plants, this is additional evidence that the virus is associated with the disease.

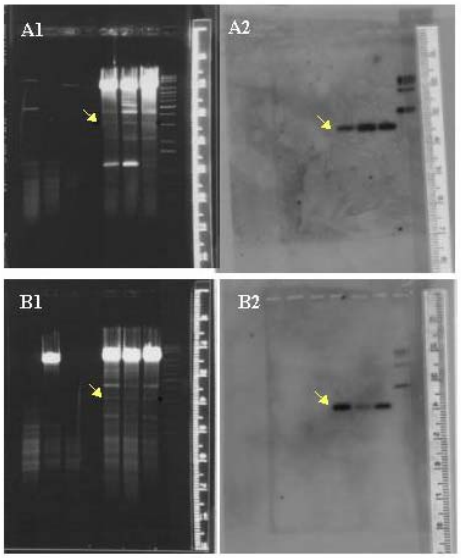

Figure 1. Double stranded RNA from healthy (lanes 1-3) and CFSD affected (lanes 5-7) cassava of the variety CMC40 (A1) and Secundina (B1). The hybridization of the dsRNA using the cDNA CFSV S5 clone for CMC40 (A2) and Secundina (B2). Lane 8 is a molecular weight marker. Each lane represents an individual plant.

Detection of the CFSV Segment 1

In the dsRNA extractions and hybridization, detection of the CFSV Segment 1 proved to be inconsistent. It was never detected in any health plants but was not consistently detected in the CFSD affected plants. When a band was detected, it appeared to be the same product that was detected by the CFSV S5 clone. It is known that the resolution of the dsRNA segments is fairly poor in agarose gels and that several "single bands" in agarose resolve into two or three products in polyacrylamide gels. In one experiment using polyacrylamide gel, the CFSV S5 segment was detected by hybridization but the CFSV S1 segment was not. The Segment 1 is detected from dsRNA extractions using specific primers designed from the CFSV S1 clone by reverse transcriptase PCR. PCR products of the expected size were amplified in the CFSD infected plants of Secundina and CMC40 but not in the healthy controls.

In one experiment, the primer CFSV S1 forward was used to prime the reverse transciptase reaction to produce cDNA. This was followed by PCR using the primers CFSV S1 forward and the CFSV S5 reverse. A PCR product of approximately 700 nucleotides was amplified and it hybridizes with the CFSV S1 clone, but does not hybridize with the CFSV S5 clone. This is evidence that the Segment 1 and Segment 5 are distinct genomic segments, and over 50 cDNA clones were produced and they are being analyzed. When the 700 nucleotide RT-PCT product is sequenced, it will be determine more information will be available on the genome of CFSV.

Diagnostic Method for the Detection of CFSD

Using dsRNA extraction followed by hybridization with the CFSV S5 is proposed as a diagnostic method. The dsRNA technique is too variable to be used for diagnostic purposes, but adding a specific hybridization greatly increases the confidence of this method. The clone CFSV S5 has proven to consistently detect CFSV and it is only detected in CFSD affected plants.

The limitations of this diagnostic technique are that the costs involved in the extraction and hybridization are moderately high per sample, and 5 grams of plant tissue are needed for each dsRNA extraction. The diagnosis can be done in two days, and this is a major advantage over the grafting with Secundina, which is the current standard detection method. Additional testing is underway to assure that all the different sources of CFSD are detected using this method.

Conclusions: Progress has been made in the characterization and detection of CFSV. This year, there has been additional characterization of the reo-like virus that is infecting cassava. The consistent detection of the CFSV using both specific hybridizations and RT-PCR S5 are steps forward in the association of the virus and the disease. Since plants produce few dsRNA, this type of extraction is appropriate for the partial purification of the genomic segments of the virus. Now that CFSV cDNA clones are available and can be used to identify specific products, the dsRNA technique has been a reliable detection method. This diagnostic method while relatively expensive will increase the confidence the testing required by other countries to receive CIAT cassava lines.

Until recently, CFSD was controlled by phyto-sanitary methods. With the extensive testing and identification of resistant varieties, we are prepared to recommend control strategies that are based on germplasm.

Contributor: Lee A. Calvert, Maritza Cuervo, Ivan Lozano, Natalia Villareal, and Jose Arroyabe.