Identification of a reolike virus infecting Manihot esculenta and associated with cassava frogskin disease

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Introduction

Cassava frogskin disease (CFSD) is a disorder of unknown etiology that affects cassava and was first reported in 1971 from southern Colombia (Hernández *et al.*, 1975). CFSD is endemic in the Amazon regions of Colombia, Peru and Brazil. There is evidence that the range of CFSD is increasing and it has spread to other cassava growing regions of Colombia, Brazil, Venezuela and Costa Rica.

In CFSD affected cassava, the root periderm and corky layers enlarge to form raised lip shaped fissures. Roots that are severely affected do not fill with starch. In areas where the disorder is endemic the yield losses can be 100% (Lozano & Nolt, 1989) **(Fig. 1)**

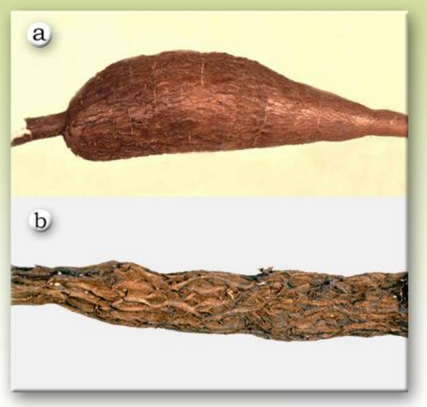

Figure 1. Cassava frogskin disease (CFSD) symptoms. a) Healthy cassava root, b) CFSD Affected cassava root

Isometric viruslike particles approximately 70 nm in diameter were found in thin sections of leaves, petioles, stems and roots of FSD affected cassava. These viruslike particles have an inner darker-stained core and an outer lighter coat, and were often found in groups or associated with inclusion bodies. **(Fig. 2)**. This report includes evidence on particles and cDNA clones that have viral homology that were associated with the disease.

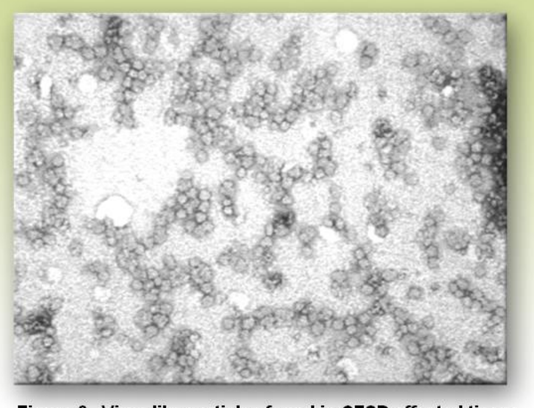

Figure 2. Virus like particles found in CFSD affected tissue

Materials and Methods

Source of host plants and isolates: The isolates of CFSD were collected both in the Andean and Amazonian regions of Colombia. Other varieties that had been tested as free of CFSD where also used as healthy controls. maintained in greenhouses by vegetative propagation.

Double-stranded RNA extraction and analysis. A modification of the method of Morris and Dodds was used to extract double-stranded RNA (dsRNA) from FSD affected plants. (Cuervo, 1989)

RT-dsRNA-cDNA-AFLPs:. cDNA was obtained from dsRNAs. The polymorphic products between healthy and CFSD plants were eluted from the gel, cloned and sequenced.

RT-PCR: The synthesis of cDNA was made from ds-RNA with primer down (5' TAA GGG TAT GGT GTG TGA TTT 3') derived from the S5 clone and with primer F10 (5' TGA GTA ACA TTT TAC CAG CAC CA 3') derived from the S1 clone. The PCR reaction was made with primers down and up (5' GTT AGC ATT ACC ATT CTC ACA T 3') of S5 and with primers F10 and Revers 218 (5' CAA TTC AAC TGG ATC TGC GA 3') of S1 . The PCR products were separated in agarose and capillary blotted into nylon membranes and hybridized with the S1 and S5 probes.

Results and Discussion

Double-stranded RNA extraction and analysis: Nine species of dsRNA were consistently present in plants affected with FSD. All nine species of nucleic acid were determined to be dsRNA, and compared with ds-RNA markers, the size of the species were estimated to be 4000, 3900, 3700, 2600, 1800, 1750, 1700, 1100, and 1000 bases. **(Fig. 3)**

A cDNA clone of 280 nucleotides was obtained from direct cloning, that contained an open reading frame of 93 bases and had 37% identity and 45% similarity with the P5 protein of rice ragged stunt virus. Because of the difficulties in generating additional cDNA clones that did not have homology with ribosomal RNAS, it was decided to use AFLP analysis to identify potential amplified regions associated with CFSD

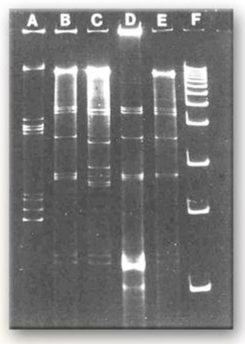

Fig 3. Profiles of dsRNA in affected plants.

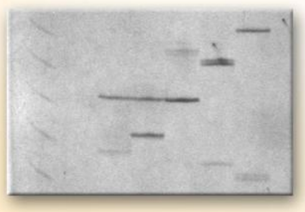

Fig. 4. Polymorphic products RT-dsRNA-cDNA-AFLPs. M: 123pb molecular marker, 1.at 3. CFSD affected varieties, 4. and 5. Healthy Secundina

RT-dsRNA-cDNA-AFLPs: In the seven restriction site-primer combinations used to amplify cDNA-AFLP products from the dsRNA from healthy and affected roots, a total of 37 polymorphic products were identified. Of these 37 products, the 21 were present in the CFSD affected plants and 16 were found in the healthy plants. These products range in size from ca. 190 to 1400 base pairs.

In five different cassava lines, one ALFP product consistently appeared in the CFSD affected plants but was absent in the healthy controls. (**Fig. 4**).

This AFLP product was cloned and two independent clones were sequenced. The cDNA clones were 228 nucleotides in length and contained an open reading frame of 72 amino-acids. The putative protein had 50% identity and 70% similarity with a portion of the P1 protein of rice ragged stunt virus.

dsRNA hybridizations: Neither of the probes showed reaction in the healthy control varieties.

The S5 clone hybridized with a dsRNA of 3000 bp. (**Fig. 5**) in 9 varieties affected with CFSD, but did not react with 2 affected varieties.

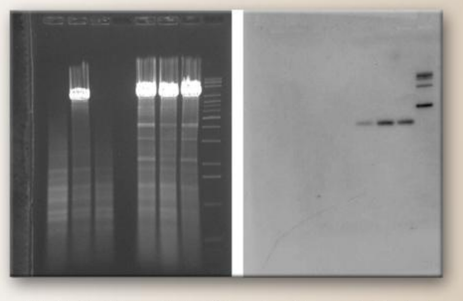

Fig 5. Profiles of dsRNA in healthy and affected plants and hybridization with S5 probe. 1. at 3. Healthy secundina, 4. at 6 affected secundina, M: 1Kb Molecular marker.

RT-PCR: PCR amplifications were found when the S1 and S5 primers were used. When the PCR products were hybridized with their respective probes, the S5 probe showed a consistentpattern between the healty and CFSD affected plants (**Fig. 6**). The S1 primers were unspecific.

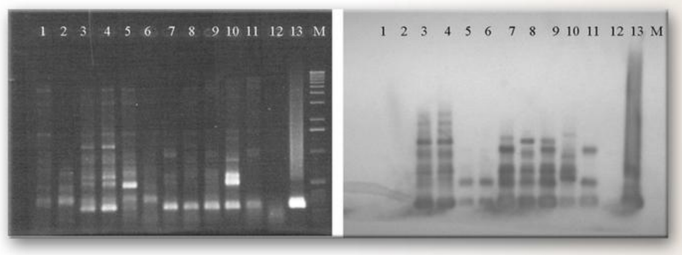

Fig. 6. RT-PCR derived from the S5 clone and the respective hybridization. 1-2 Healthy varieties, 3-11 affected varieties, 12. DNA free Reaction 13. Plasmid control, M: 1Kb Molecular marker.

Conclusion

- Two cDNA clones were derived from cassava infected from Cassava Frogskin disease. One of these clones was derived using reverse transcriptase cDNA AFLP.
- The putative proteins of these cDNA clones had homology with the segment 1 (S1) and S5 of Rice Ragged Stunt Virus.
- These clones were tested used RT-PCR and hybridization analysis and reacted with cassava that is was infected with CFSD but not with healthy plants.
- A virus in the Reoviridae family has been associated with cassava frogsking disease.

References

- Cuervo. I.M. (1989) Caracterizacion de los acidos ribonucleicos de doble cadena (ARN-cd asociados a enfermedades virales en yuca (Manihot esculenta, Crantz). Tesis Ing. Agr. Palmira. U. Nacional de Colombia. 96p.
- Hernández, A., Calderón, H., Zárate, R.D. and Lozano J.C. (1975) El cuero de sapo de la yuca (*Manihot esculenta*, Crantz). Noticias Fitopatológicas 4, 117-118.
- Lozano, J. C. & Nolt, B. L. 1989. Pest and pathogens of cassava. Pages 174-175 in: Plant Protection and Quarantine: Selected Pests and Pathogens of Quarantine Significance Vol. 2 R.P. Kahn, ed., CRC Press, Pr. Inc., Boca Raton FL.
- Morris, T.J., and Dodds, J.A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69: 854-858.