Development and implementation of a highly sensitive and reliable molecular test for the detection of cassava frogskin virus

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The problem

Cassava frogskin disease was first observed in the department of Cauca, Colombia, in 1971 (Hernández *et al*. 1975). Diseased cassava plants do not show visible symptoms above-ground, but their storage roots may completely fail to develop. The disease is currently present in the majority of regions in Colombia, and has now been reported from Brazil, Costa Rica, Panama, Peru and Venezuela. Cassava is vegetatively propagated, and, consequently, the primary transmission of the causal virus (CFSV) occurs through the use of infected stem cuttings. In areas where the disorder is endemic, yield losses can reach up to 100%[Figure 1] (Lozano & Nolt 1989). In a limited number of cassava genotypes, including Secundina (Col 2063), CFSV-infected plants are stunted, and the leaves develop mosaic symptoms.

Figure 1. Cassava roots affected with CFSD

The traditional way around

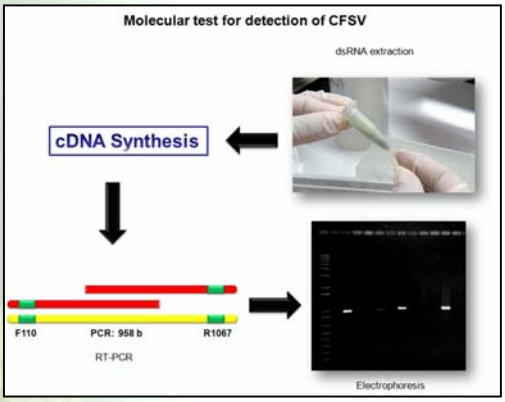
Due to the difficulty in isolating the causal agent of this disease in past decades, the detection of CFSV in symptomless but infected cassava plants had been achieved by grafting the highly susceptible 'Secundina' cultivar, which shows clear mosaic symptoms in the presence of the cassava frogskin disease agent. However, this is a slow process that requires 72 weeks for the final evaluation, even though an improvement allows a diagnosis in 21 weeks.

Once the most common plant pathogens (e.g. fungi, bacteria, phytoplasm) and abiotic factors had been discarded as possible causal agents, the etiological research focused on the possible presence of a plant virus. The Virology Unit (VRU) of CIAT first demonstrated the existence of double-stranded RNA of possible viral origin in cassava frogskin-affected plants around 1989. Since that date the VRU has been implementing the latest molecular techniques to clone the viral RNA present in diseased cassava plants, until considerable evidence was accumulated to postulate the existence of a plant virus belonging to the family *Reoviridae*.

The breakthrough

Based on continuous advances in the cloning of reovirus genomic components, a RT-PCR assay has been developed to detect the virus in infected, symptomless cassava plants [figure 2]. Initial experiments demonstrated that the virus could be detected in different clones and regions of Colombia, even though minor genetic variability was detected in the virus (Calvert *et al*. 2008).

Figure 2. Diagram for molecular method of CFSV



Having determined the consistent association of the suspected reovirus with the frogskin disease of cassava, the new molecular diagnostic test was used to evaluate 508 cassava accessions maintained by CIAT GRU, for the presence of the virus. In order to determine the reliability of the RT-PCR assay, this molecular technique was compared with the grafting technique on all 508 accessions [Table 1].

Table 1. Comparison of grafting and RT-PCR techniques in 508 cassava accessions

508 GRU Accession	#	Grafting	RT-PCR
Argentina	34	34 Negative	34 Negative
Brazil	91	88 Negative	88 Negative
		2 Positive	3 Positive
		1 Negative	
Colombia	91	91 Negative	89 Negative
			2 Positive
Ecuador	20	20 Negative	20 Negative
Indonesia	88	88 Negative	88 Negative
Paraguay	23	23 Negative	23 Negative
Peru	15	15 Negative	15 Negative
Guatemala	11	11 Negative	11 Negative
Venezuela	13	13 Negative	13 Negative
C. Rica, Mex., Pan., USA, Cub., Rep. Dom.	26	26 Negative	26 Negative
FJI., Mal., Viet., Phili., Tai., Nig.	17	17 Negative	17 Negative
Others	2	2 Positive	2 Positive
Wild species	43	43 Negative	43 Negative
Breeding material	34	34 Negative	34 Negative
Positive control	8	8 Positive	8 Positive
Negative control	8	8 Negative	8 Negative

The results obtained showed that the RT-PCR method was more sensitive and reliable that the more time-consuming grafting method, having detected the CFSV in seven accessions, as compared to only four of the seven accessions infected detected by the grafting (Secundina) technique [Table 2]. More importantly, the RT-PCR technique takes less than a week and no glasshouse space to deliver the expected results [Figure 3].

Figure 3. Comparison of different methodologies for CFSD detection

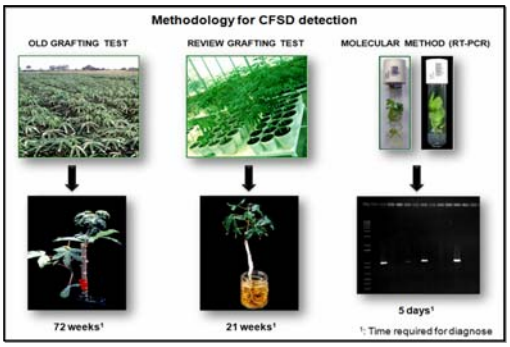


Table 2. Confidence level with Grafting and RT-PCR methods

Test	Number of tested plants	Confidence level
Grafting	505/508	99,40%
RT-PCR	508/508	100%

Impact

The implementation and utilization of the RT-PCR methodology has great relevance, not only for the production of material free of the cassava frog skin virus, but also for the conservation and distribution of genetic resources of cassava deposited in the genebank of CIAT.

Currently, almost the entire *in vitro* collection of *Manihot esculenta* maintained by GRU of CIAT, has been evaluated, and over 99.5% of the collection is now free of CFSV and ready for international distribution [Figure 4].

Figure 4. *In vitro* collection of *Manihot esculenta* in GRU of CIAT

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Literature cited

Hernandez A. Calderon H, Zarate RD, Lozano JC (1975) El Cuero de sapo de la yucca (Manihot esculenta Crantz). Not Fitopatolog. 4:117-118

Lozano JC, Nolt BL. Pest and pathogens of cassava. In: Kahn RP (ed.), Plant Protection and Quarantine: Selecto Pest and pathogens of quarantine significance, Vol 2, Boca Raton, FL, USA, CRC Press, 1989, pp 174-175

L. A. Calvert, M. Cuervo, I. Lozano, N. Villareal, and J. Arroyave. 2008. Identification of three strains of a virus associated with cassava plants affected by frogskin disease. Journal of Phytopathology 156, 647-653