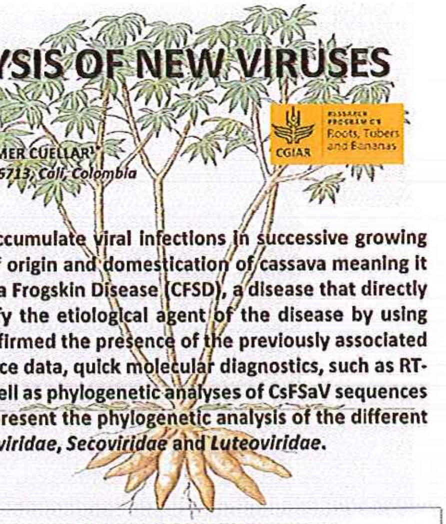
IDENTIFICATION, DETECTION AND PHYLOGENETIC ANALYSIS OF NEW VIRUSES INFECTING CASSAVA IN COLOMBIA



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ABSTRACT

Cassava (*Manihot esculenta* Crantz.) is a vegetatively propagated crop. This characteristic makes it highly prone to accumulate viral infections in successive growing cycles, causing seed degeneration and thus representing a threat to global food security. South America is the center of origin and domestication of cassava meaning it has coevolved with pathogens for longer. However, in the Americas one of the major constraints of production is Cassava Frogskin Disease (CFSD), a disease that directly affects root formation causing reduction of potential yields by half or more. A long search has been done to identify the etiological agent of the disease by using serological, biological and molecular methods. Our work in cassava plants affected by CFSD collected since 1980 has confirmed the presence of the previously associated reovirus (CsFSaV), but also revealed a diversity of novel virus species infecting cassava. Based on newly obtained sequence data, quick molecular diagnostics, such as RT-PCRs, have been developed to consistently detect these viruses. Restriction fragment length polymorphisms (RFLPs) as well as phylogenetic analyses of CsFSaV sequences isolated from Colombia, suggested the occurrence of mixed infections with different geographic distribution. Here, we present the phylogenetic analysis of the different CsFSaV isolates as well as the phylogenetic relation of the recently identified viruses belonging to the families: *Alphaflexiviridae*, *Secoviridae* and *Luteoviridae*.

Identification of new viral species infecting cassava plants

Cassava Frogskin Disease (CFSD) is the major challenge to cassava productivity in Latin America causing significant yield losses of up to 90% (Pineda et al., 1983). The disease is characterized by the failure of the storage roots to accumulate starch **(Fig. 1)**. Three novel viral species infecting cassava in Colombia were identified on CFSD-affected cassava plants by using high throughput deep sequencing of small RNA.

Phylogenic analysis using neighbor-joining phylogenetic tree from a.a. sequences of RdRp genes aligned with CLUSTALW and 1000 bootstraps replications; clustered the viruses to the genera *Polerovirus (*Cassava Polero-like virus- **CsPLV***)*, *Potexvirus (*Cassava New alphaflexivirus-**CsNAV***) and* *Torradovirus (*Cassava torrado-like virus-**CsTLV***)*, respectively. The previously reported Cassava frogskin associated virus (**CsFsaV**) (*Reoviridae*) was also found in this plants.

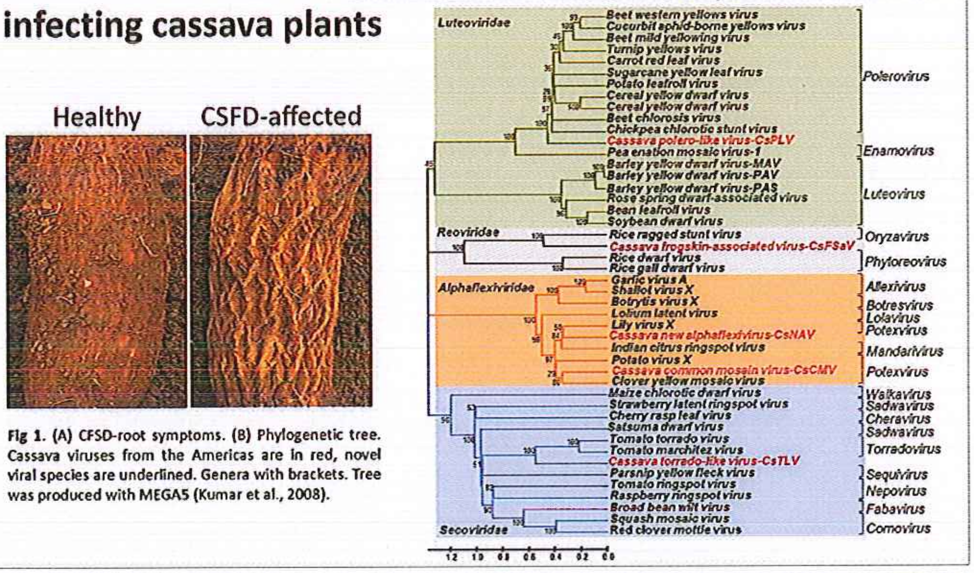

Fig 1. (A) CFSD-root symptoms. (B) Phylogenetic tree. Cassava viruses from the Americas are in red, novel viral species are underlined. Genera with brackets. Tree was produced with MEGA5 (Kumar et al., 2008).

Virus detection on field-collected cassava plants

CFSD-affected plants were collected in the Amazonas, south/central and northern Colombia since 1980 **(Fig. 2A)** and were maintained in a glasshouse at CIAT. Samples were tested by RT-PCR for the presence of the novel viruses: **CsPLV, CsNAV** and **CsTLV.** All samples with exception of SM3375-113 and BIPD-289-25 tested positive to CsFSaV [segment 4 (s4)]. Given the extensive germplasm exchange between production regions it is possible the unnoticed introduction of these viruses to other countries.

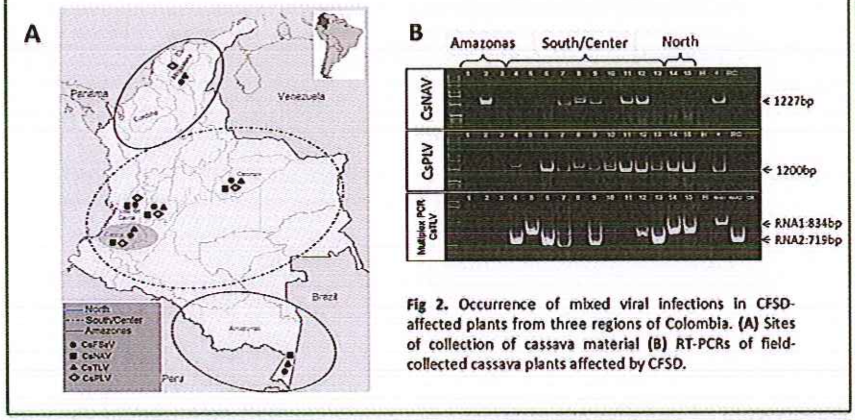

Fig 2. Occurrence of mixed viral infections in CFSD-affected plants from three regions of Colombia. **(A)** Sites of collection of cassava material **(B)** RT-PCRs of field-collected cassava plants affected by CFSD.

Phylogenetic analysis of CsFSaV

Sequence analysis of CsFSaV PCR products revealed the presence of two different **s4** sequences that could be distinguished by an *EcoRV* restriction site **(Fig. 3A)**. Phylogenetic analyses confirmed the presence of two main phylogenetic sub-groups. Amazonas is the site with higher genetic variability where both strains are present suggesting a possible origin of this virus.

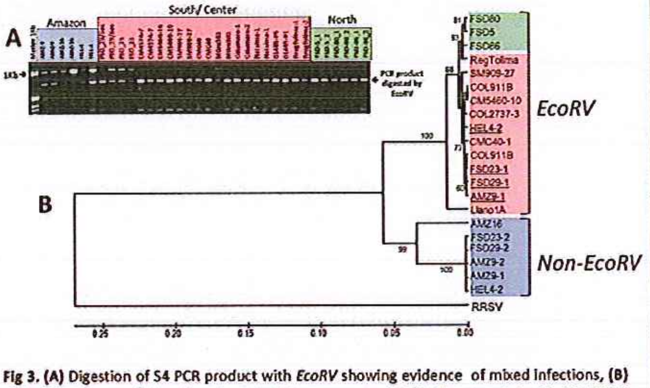

Fig 3. (A) Digestion of S4 PCR product with *EcoRV* showing evidence of mixed infections, **(B)** phylogenetic tree of the aligned nucleotide sequence of CsFSaV segment 4 with CLUSTALX and neighbor-joining tree constructed with MEGA5. Bootstrap 1000 and bootstrap values above 60% are indicated. Underlined names indicate isolates from samples collected in the Amazonas region.

CONCLUSIONS

- Novel viral species and CsFSaV are commonly found in mixed infections in CSFD-affected plants in Colombia.
- The genetic variability found in Amazonas on CsFSaV sequence analyses provided us a first hint on the epidemiology of the virus. Same studies are been carried out for novel viral species.
- There is an overlapping geographic distribution of these viruses.
- Studies to elucidate virus interactions will be done to better understand viral pathogenesis and evolution.

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