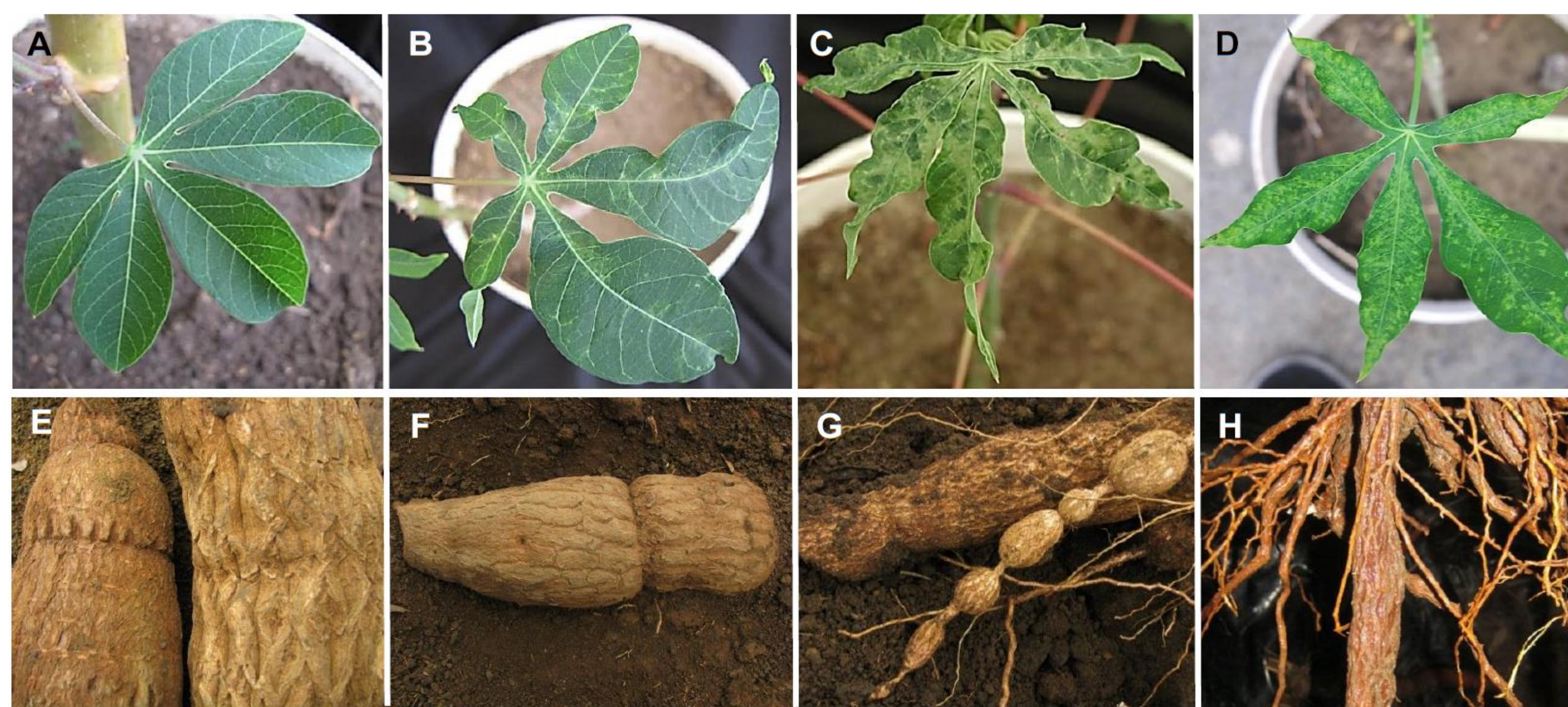


Cassava (*Manihot esculenta* Crantz.) is the third most important source of calories for human nutrition in the tropics. Viruses infecting cassava in Africa are associated with significant yield losses and epidemic outbreaks, e.g. Cassava Brown Streak Disease. In Latin America, viruses are associated with mild, temporal and/or localized disease outbreaks in cassava, relatively easy to control by positive selection as in the case of cassava Frogskin Disease (FSD). Nevertheless, yield reductions significantly limit farmer's incomes. We have detected, characterized and isolated new virus species found in cassava from Latin America affected by FSD. These viruses occur in mixed infections in severely diseased plants but they are not associated with disease symptoms in leaves when they occur in single infections. Genome characterization revealed the presence of novel virus species belonging to families *Alphaflexiviridae*, *Luteoviridae*, *Reoviridae* and *Secoviridae*. The low transmission of FSD and its efficient control by positive selection suggests an inefficient vector for this disease, which could be related to the requirement of mixed infection and the presence of different vectors for severe disease to occur. The symptomless nature of single-infections in cassava could explain temporal outbreaks due to distribution of undetected contaminated planting material.

**Fig. 1: A diversity of symptoms associated with Frogskin Disease**



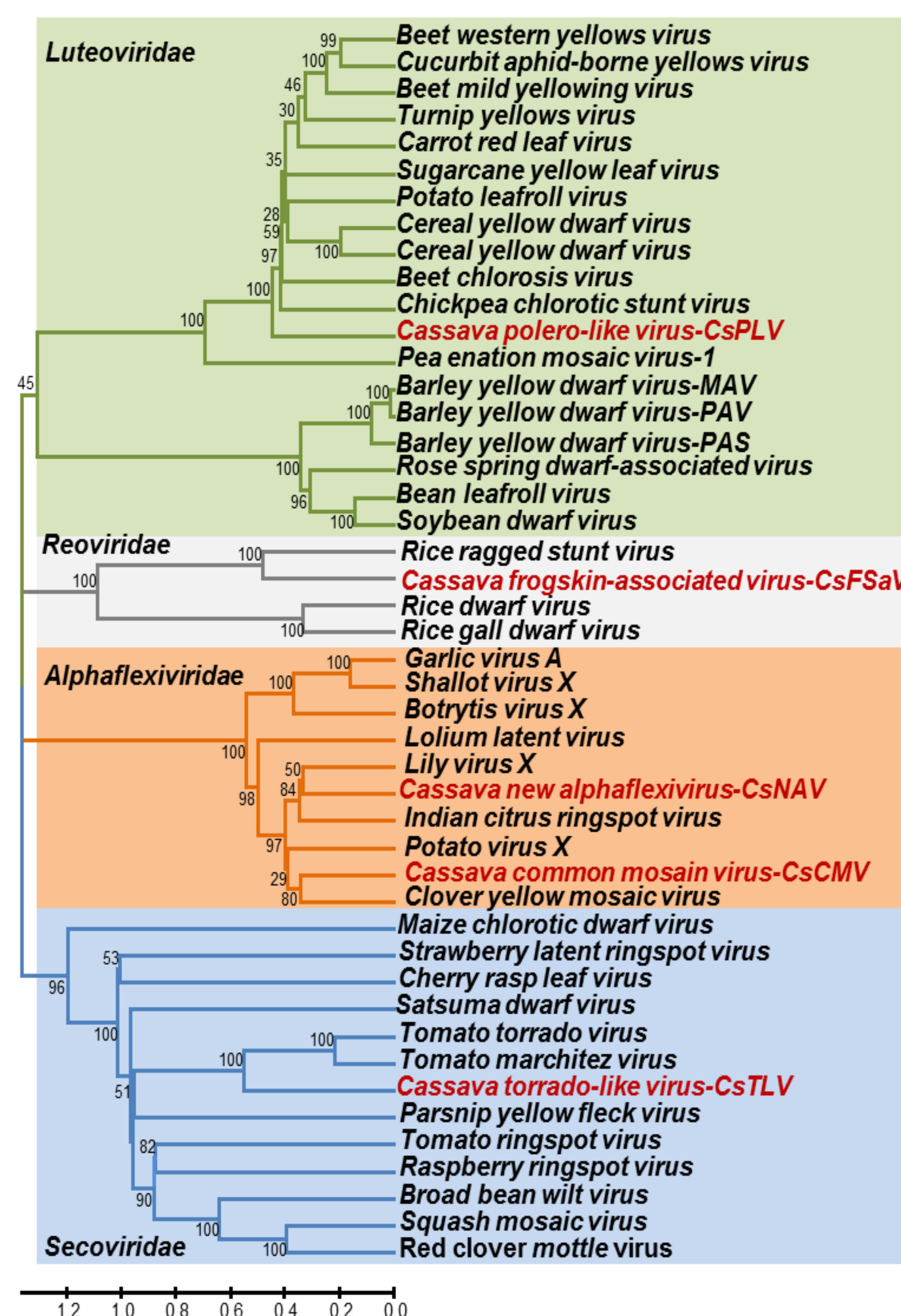
FSD is the major challenge to cassava production in Latin America. The disease is characterized by the failure of the storage roots to accumulate starch (E-F). Although most cassava landraces do not show symptoms of disease in the above-ground parts (stems and leaves) some CFSD-affected plants display distinct symptoms in leaves (A-D). We have tested a collection of CFSD-affected plants and detected a mix of reovirus strains infecting these plants. CFSD-affected plants were collected in the Amazonas, south/central and northern Colombia since 1980 and were maintained in a greenhouse at CIAT. When stems of these plants were used to graft-infect the indicator plant 'Secundina', different leaf symptoms were induced in all tested plants. Although all plants were infected by reoviruses, this did not explain the diversity of symptoms observed in the indicator plant.

**Fig. 2 and Table 1: A diversity of cassava viruses detected in mixed infections**

Samples were negative in ELISA or RT-PCR tests to the previously characterized *Cassava common mosaic virus*, *Cassava virus X* and *Cassava vein mosaic virus*. Failed hybridization tests suggested the presence of novel unrelated graft-transmissible viruses. Deep sequencing of small interfering RNA readily identified novel viral species on FSD-affected cassava plants. Using sequence information from assembled contigs we designed PCR primers and to test field-collected plants and a cassava germplasm collection maintained at CIAT.

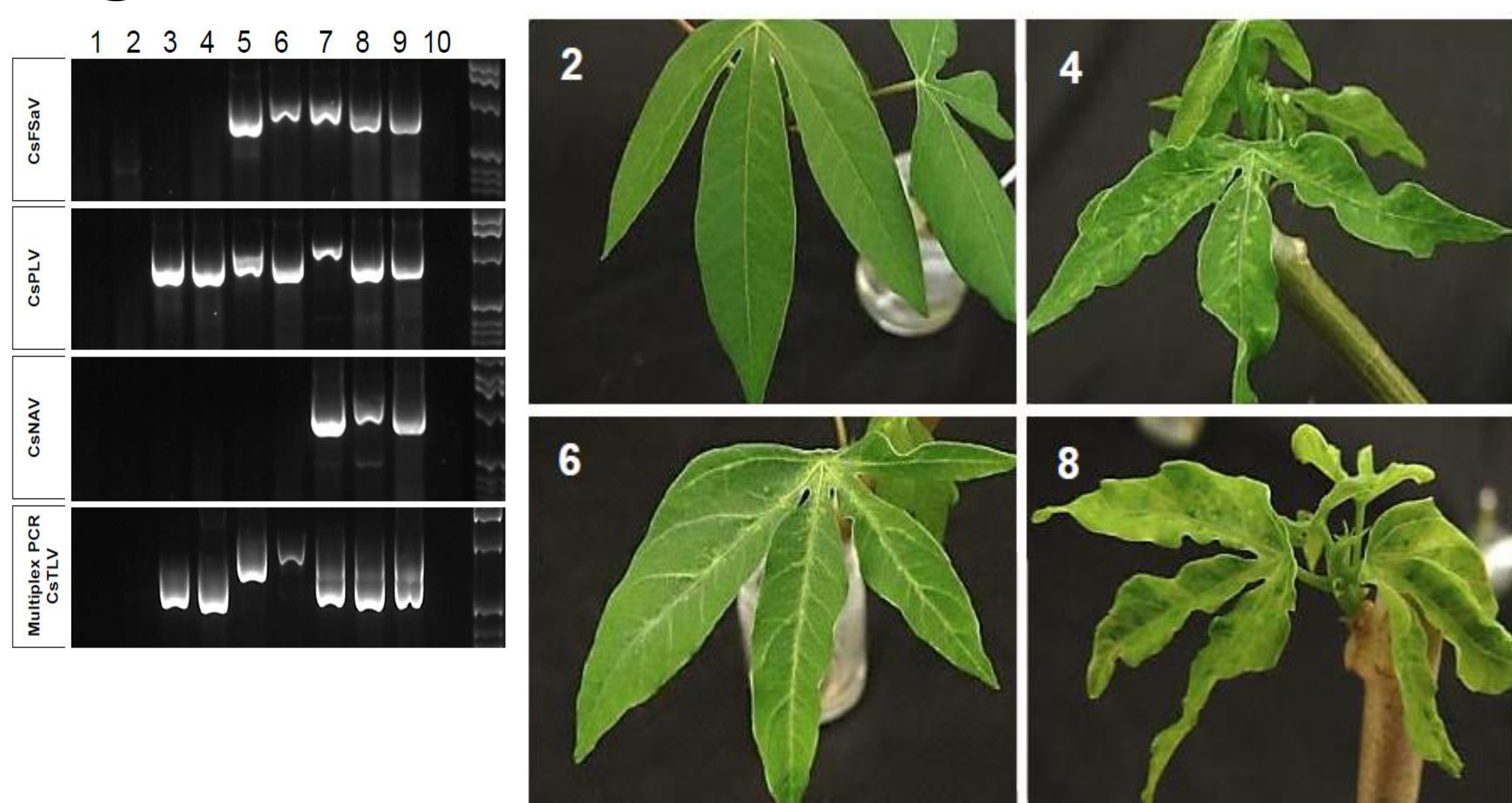
Apart from the previously reported reovirus *CsFsaV* (*Reoviridae*), phylogenetic analysis using neighbor-joining (MEGA3) and the amino acid sequences of RdRp genes clustered the new virus sequences closer to the genera *Polerovirus* (Cassava Polero-like virus; *CsPLV*), *Potexvirus* (Cassava New alphaflexivirus; *CsNAV*) and *Torradovirus* (Cassava torrado-like virus; *CsTLV*).

Interestingly mixed infected plants were associated with disease symptoms in the indicator landrace 'Secundina' while samples 'single' infected by reoviruses did not induce leaf symptoms in this indicator plant. Given the lack of reaction in 'Secundina' indicator plants it is possible that the distribution of these viruses to other countries could go unnoticed. The RT-PCR protocol developed during this work contributes to a safer cassava germplasm exchange.



Isolate	Host Cultivar	Collection data	Secundina graft	Genbank ID
BRA1299	BRA1299	Valle del Cauca, Colombia	(-)	KC924878
BRA383	BRA383	Brazil, 1982	(-)	KC924886
BRAS04	BRAS04	Minas Gerais, Brazil, 1982	Mosaic	KC924875
CM2772-3	CM2772-3	Valle del Cauca, Colombia	(-)	KC924884
CM3306-4	CM3306-4	Valle del Cauca, Colombia	(-)	KC924885
CM523-7	CM523-7	Valle del Cauca, Colombia	(-)	KC924880
CM4574-7	CM4574-7	Valle del Cauca, Colombia	(-)	KC924883
COL1185	COL1185	Santander, Colombia, 1970	(-)	KC924877
COL1287	COL1287	Boyaca, Colombia, 1970	(-)	KC924876
COL1505	COL1505	Venezuela, 1970	(-)	KC924869
COL2215	COL2215	Magdalena, Colombia, 1982	(-)	KC924868
COL9118	COL9118	Bolivar, Colombia, 1984	(-)	KC924870
CR29	CR29	Limon, Costa Rica, 1984	(-)	KC924874
CR158*	CR158	Costa Rica, 1961	ND	KC924866
CR169*	CR169	Panama, 1977	ND	KC924852
ECU43	ECU43	Manabi, Ecuador, 1970	(-)	KC924873
ECU72	ECU72	Zamora, Ecuador, 1970	(-)	KC924853
HMC1	HMC1	Valle del Cauca, Colombia	(-)	KC924882
PAN51	PAN51	Ocu, Panamá, 1970	(-)	KC924867
PAR36	PAR36	Amambay, Paraguay, 1983	(-)	KC924872
SG700-3	SG700-3	Valle del Cauca, Colombia	(-)	KC924879
VEN24	VEN24	Venezuela, 1971	(-)	KC924871
VEN77	VEN77	Venezuela, 1971	(-)	KC924881
AM216	ND	Amazonas, Colombia, 1990	Mosaic	KC924848
AM29-a	ND	Amazonas, Colombia, 1990	Mosaic	KC924863
AM29-a-P2	ND	Amazonas, Colombia, 1990	Mosaic	KC924864
AM29-b	ND	Amazonas, Colombia, 1990	Mosaic	KC924862
AM29-b-P2	ND	Amazonas, Colombia, 1990	Mosaic	KC924865
Cauca1B	COL2215	Cauca, Colombia, 2005	Mosaic	KC924886
CMC40-4	COL1468	Valle del Cauca, Colombia, 2012	Mosaic	KC924860
CMC40-5	COL1468	Valle del Cauca, Colombia, 2012	ND	KC924850
COL2737-3	COL2737	Yopal, Colombia, 2013	ND	KC924855
COL9118-b	COL9118	Bolivar, Colombia, 1984	ND	KC924849
FSD23-a	ND	Cauca, Colombia, 1984	Severe mosaic	KC924856
FSD23-b	ND	Cauca, Colombia, 1984	Severe mosaic	KC924859
FSD29-a	COL1684	Cauca, Colombia, 1990	Mosaic	KC924857
FSD29-b	COL1684	Cauca, Colombia, 1990	Mosaic	KC924858
FSD5	COL2063	Magdalena, Colombia, 1980	Mosaic	KC924890
FSD80	COL2063	Magdalena, Colombia, 1980	Mosaic	KC924891
FSD86	COL2063	Magdalena, Colombia, 1980	Mosaic	KC924851
HEL4-a	ND	Amazonas, Colombia, 1992	Mosaic	KC924861
HEL4-b	ND	Amazonas, Colombia, 1992	Mosaic	KC924887
Llan01A	COL2177	Meta, Colombia, 2005	Mosaic	KC924892
RegTolimaA	COL2063	Tolima, Colombia, 1992	Mosaic	KC924893
SM909A	SM909-25	Valle del Cauca, Colombia, 2001	Mosaic	KC924894
VEN-Costa	COL2215	Sucre, Colombia, 2005	Mosaic	KC924889

**Fig. 3: Different mixed virus infections induce distinct symptoms**



In order to study the interactions of the different viruses detected in this work and their role in disease, we are isolating them in single-infections and graft-infecting 'Secundina' plants with different virus combinations. On the left we show the infection of rootstocks (odd numbers) and 'Secundina' grafts (even numbers) by RT-PCR. Our results indicate that different mixed infections induce distinct leaf symptoms such as leaf deformation (4), vein clearing (6) and severe leaf mosaic as shown in picture (8). No symptoms were observed in 'single' infections. These viruses have been detected in samples collected since the 1970's and they can also be detected in other countries of the region such as Costa Rica, Ecuador and Argentina. Currently we have developed real-time PCR protocols to detect all viruses and measure changes in virus titers in single and mixed infections. Most importantly the same experimental set up is being used to study the effect of these viruses on root symptoms in cassava under greenhouse and field conditions.

## Conclusions and on going work

- Plants affected by Frogskin Disease are infected by *CsFsaV* in mixed infection with other viruses.
- Mixed virus infections with *CsFsaV* in FSD-affected plants are commonly found in Colombia. All these viruses show geographic variation among sequence isolates.
- In single infections these viruses are not associated with disease symptoms in leaves of cassava therefore indexing protocols by indicator plants are being modified.
- Virus interactions are currently under investigation to better understand viral pathogenesis and identify Frogskin Disease causing agent(s).
- Complete genome characterization of one isolate of each virus indicates they are novel viral species infecting cassava.
- Transmission studies are underway to test transmission of *CsFsaV* and *CsTLV* by whiteflies and *CsPLV* by aphids.