SP04

Variability in P1 gene redefines phylogenetic relationships among Cassava brown streak viruses (*Ipomovirus*; *Potyviradae*)

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SUMMARY

Forty-five nucleotide sequences from the P1 gene of Cassava brown streak virus isolates from major cassava producing areas of east and central-southern Africa were analysed. We isolated and sequenced (Sanger method) 33 isolates (from Malawi and Tanzania) and analyzed them with previously reported 12 representative sequences from Uganda, Kenya, Tanzania, Malawi and Mozambique. Multiple alignments revealed three distinct regions of insertions and deletions to classify CBSVs. Phylogenetic analyses revealed three major clades associated with CBSVs *viz* CBSV, UCBSV and a novel distinct clade, closely related to CBSV, which has been tentatively called *CBSV-Tanzania*. Isolates belonging to clade CBSV shared 89.32 – 99.90% nucleotide sequence identity, while those from CBSV-Tanzania and UCBSV were 70.86 – 88.39% and 83.40 – 97.71% identical respectively. Members of the distinctive sub-group CBSV-Tanzania as shared as low 62.30 and 62.98% nucleotide identity with members of CBSV and UCBSV main groups. Our study proposes that the species CBSV has distinct sub-populations, and that CBSV-Tanzania is a major sub-group belonging to the species CBSV. High genetic variation among Malawi and Tanzania isolates may have some novel insights on virus evolution in the region.

INTRODUCTION

Cassava brown streak disease (CBSD) is caused by two distinct species of *Ipomoviruses*, *Cassava brown streak virus* (Monger et al., 2001) and *Ugandan cassava brown streak virus* (Mbanzibwa, et al., 2009; Winter et al., 2010), belonging to the family *Potyviridae*. Here we report on the genetic diversity of the CBSVs' P1 gene and presents substantial evidence for the presence of a novel and distinct *Cassava brown streak virus-Tanzania* (CBSV-Tanzania) sub-species. This is a major sub-group of viruses closely related to the species CBSV capable of causing cassava brown streak disease.

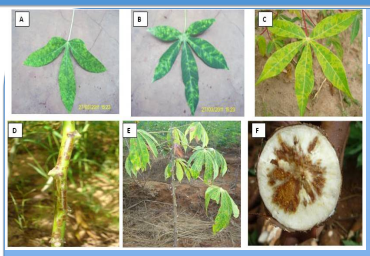

Figure 1: CBSD symptoms

A, B, and C are CBSD infected leaves. D is stem infection, E shows the whole plant infected, while F is tuberous root infection, showing the typical dry necrotic rot.

Foliar symptoms in CBSD-infected plants include feathery chlorosis along veins. This starts along the margins of secondary veins expanding to cover the tertiary veins. It may finally produce chlorotic blotches. Chlorosis may occur in roughly circular patches between veins. More recently other symptoms associated with CBSD infection include spotty chlorosis, chalky white necrosis in roots; as well as poor leaf retention and irregular senescence of leaves. The most destructive of all the symptoms associated with CBSD infection is necrosis in the storage root cortex because this may render 70% of the tuberous root inedible. These symptoms usually develop after foliage symptoms.

MATERIALS AND METHODS

National surveys were conducted in Malawi and Tanzania in 2013 where cassava stems were collected and maintained in screen house at DSMZ Plant virus department, Germany. Amplification and sequence assembly was done at DSMZ. SimPlot was used to determine nucleotide sequence similarities and differences, while diversity was determined by DNA sequence polymorphism (DnaSP v 5.10). Sequence Demarcation Tool (SDT v 1) was used classify nucleotide sequences basing on pairwise identities. Sequences were aligned by ClustalW and edited manually. MEGA 6.06 was used to construct the phylogenetic trees, and editing was done by Fig Tree v 1.4.2.

Figure 2: Cassava plants maintained in screen house.

RESULTS

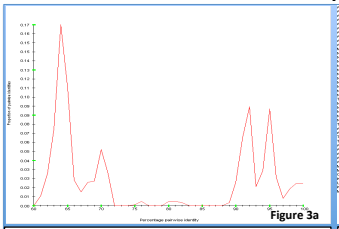

Figure 3a

Figure 3a: Pairwise identify frequency distribution plot for CBSVs partial P1 sequences. The horizontal axis indicates percentage pairwise identities, and the vertical axis indicates proportions of the identities within the distribution.

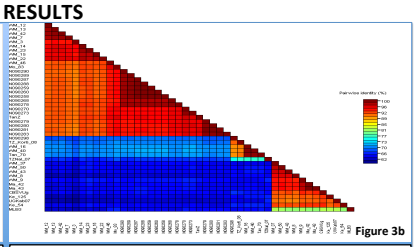

Figure 3b

Figure 3b: Pairwise identity matrix generated from CBSV partial P1 gene sequences. Each coloured key represents a percentage to the identity score between two sequences (One presented horizontally to the left and other vertically at the bottom).

Analysis of nucleotide diversity revealed greater diversity for the distinct CBSV-Tanzania (π = 0.20) as compared to CBSV (π = 0.05) and UCBSV (π = 0.09) (Table 1). The average nucleotide difference for CBSV-Tanzania was much higher (K = 102.40) than that of CBSV and UCBSV, which were 24.01 and 47.82 respectively. When put together, the species CBSV show much greater and wider genetic variation at nucleotide level than UCBSV (Figure 4).

Table 1: Analysis of nucleotide sequence diversity among the CBSV phylogenetic clades

Groups	*Nucleotide diversity (π)*	*Average nucleotide difference (K)*
CBSV	0.05	24.01
CBSV-Tanzania	0.20	102.40
UCBSV	0.09	47.82

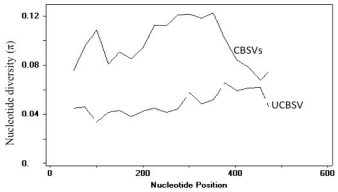

Figure 4: Nucleotide diversity for CBSVs' P1 gene. The analysis shows variation at species level (CBSV and UCBSV)

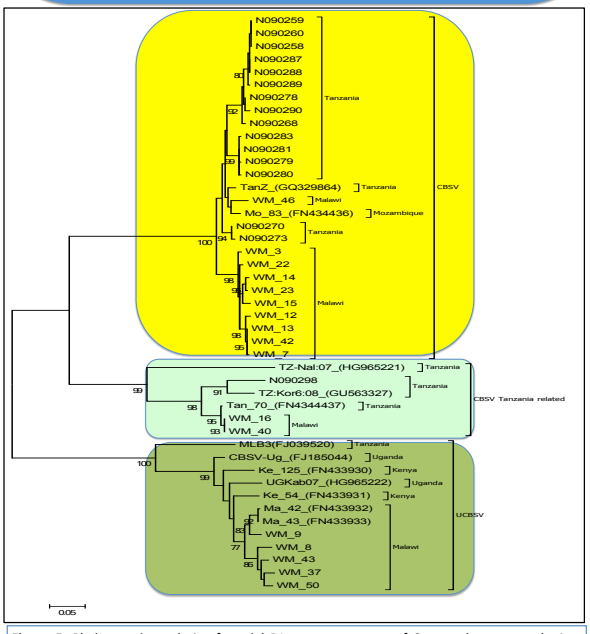

Figure 5: Phylogenetic analysis of partial P1 gene sequences of Cassava brown streak virus isolates. Other sequences previously characterized from Tanzania, Mozambique, Kenya, Uganda and Malawi (available from the GenBank) were added to the analysis. Bootstrap analysis was done with 1000 replications. Only bootstrap values higher than 70% are shown here. The tree was constructed using Maximum Likelihood statistical method, performed in MEGA6.06.

CONCLUSION

Much as CBSV has greater genetic diversity, distribution-wise, UCBSV is more geographically widespread than CBSV. UCBSV is found in Tanzania, Uganda, Kenya, Malawi while CBSV and CBSV-Tanzania are currently more prevalent, though not limited to, Tanzania, Malawi and Mozambique. Our in-depth look at CBSVs from Malawi and Tanzania revealed previously hidden diversity, including a novel major clade. While we do not have evidence from the P1 region that this clade is the product of recombination between UCBSV and CBSV, further characterization of full genome sequences may clarify the origin of this intermediate clade, and ascertain whether this clade represents a novel strain or species of CBSV. Regardless of the eventual designation of this clade, identifying this informal subgroup will ease characterization and discussion of further isolates, and may aid in diagnosis and plant breeding.

Acknowledgment: We greatly acknowledge full financial support from the project "Disease Diagnostics for sustainable Cassava Productivity in Africa" funded by BMGF/UK DFID through Mikocheni Agriculture Research Institute.