New Disease Reports

First report of *Soybean chlorotic blotch virus* and *West African Asystasia virus 1* infecting cassava and a wild cassava relative in Cameroon and Togo

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Cassava mosaic disease (CMD), caused by begomoviruses (family *Geminiviridae*), is a major constraint to cassava (*Manihot esculenta*) production in Africa (Fargette *et al*., 1993). CMD-associated begomoviruses group either in the *African cassava mosaic virus* (ACMV) or *East African cassava mosaic virus* (EACMV) clusters (Bull *et al*., 2006). To determine the suspected begomoviruses associated with CMD symptoms, leaf samples were collected from five symptomatic cassava plants in Cameroon, and from three symptomatic plants in Togo in August 2014 (Table 1). Total DNA was isolated as described by Shepherd *et al*. (2008). Circular, single-stranded DNA was enriched by rolling circle amplification (RCA). The RCA products were pooled by country of origin and sequenced using the Illumina HiSeq 2500 next-generation sequencing (NGS) platform. Sequence reads were assembled into full-length viral sequences (c. 2.7 kb) using SeqManNGen software v.3 (DNASTAR Inc., Madison, WI) and subjected to BLASTn analysis. In addition to the ACMV DNA-A and B components as expected, the DNA-A and B components of *Soybean chlorotic blotch virus* (SbCBV) (Olufemi *et al*., 2010) and *West African Asystasia virus 1* (WAAV1) (Wyant *et al*., 2015) were also identified. For SbCBV, sequence identities ranged from 97-99% and 95-96% for the DNA-A and B components, respectively, whereas for WAAV1, sequence identities ranged from 92-95% and 93-96% for the DNA-A and B components, respectively. The sequences have been deposited in the GenBank as the DNA-A components SbCBV-CM-Cas (Accession No. KT444613), SbCBV-TG-Cas (KT454813), WAAV1-CM-Cas (KT444603), and WAAV1-CM-wildcas (KT444605), and as the DNA-B components SbCBV-CM-Cas (KT444614) SbCBV-TG-Cas (KT454814), WAAV1-CM-Cas (KT4446040, and WAAV1-CM-Cas (KT444606).

To confirm the presence of SbCBV and WAAV1 in the cassava samples, PCR was carried out using specific primers designed to the *CP* and *BV1* gene, located on the DNA-A and B components of SbCBV and WAAV1, respectively. The DNA sequence for each cloned amplicon was determined by Sanger sequencing. Amongst the five samples collected from Cameroon, three were infected only with ACMV, one was infected with ACMV and WAAV1, and one was infected with ACMV, SbCBV and WAAV1. The three Togolese samples were all infected with ACMV and one was also infected with SbCMV. BLASTn analysis of the *CP* and *BV1* genes indicated that they shared 99-100% sequence identity with the NGS-determined sequences. To our knowledge, this is the first report that cassava may be infected by SbCBV or WAAV1 in Africa. The isolates shared a close phylogenetic relatedness with previously described SbCBV and WAAV1 isolates, respectively (Fig. 1). This indicated that cassava-associated isolates are strains or isolates of SbCBV and WAAV1 based on the guidelines of the ICTV *Geminiviridae* Study Group for strain and species demarcation (Brown *et al*., 2015). The grouping of EACMV, SbCBV, WAAV1, and *Madagascar Asystasia virus* (MAAV) together in a separate clade (Fig. 1), suggests that they have probably evolved from a common ancestor, despite perhaps recent adaptation to host plants representing different plant families. Whether symptoms caused by SbCBV and WAAV1 are masked by the presence of ACMV in cassava remains to be determined. Giving the small number of samples analysed, further work is required to understand the contribution of these two viruses in cassava mosaic disease epidemiology in Africa.

Acknowledgements

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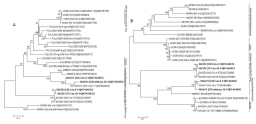

Figure 1

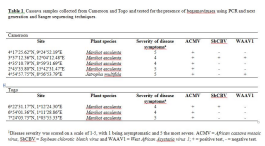

Figure 2

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