New Disease Reports

First report of maize lethal necrosis disease in Rwanda

I.P. Adams [1]*, V.A Harju [1], T. Hodges [1,2], U. Hany [1], A. Skelton [1], S. Rai [1,2], M.K. Deka [1,3], J. Smith [1], A. Fox [1], B. Uzayisenga [4], C. Ngaboyisonga [4], B. Uwumukiza [5], A. Rutikanga [6], M. Rutherford [7], B. Ricthis [7], N. Phiri [8] and N. Boonham [1]

[1] *The Crop Protection Centre, The Food and Environment Research Agency, Sand Hutton, York, YO41 1LZ, UK*; [2] *University of York, York, YO10 5DD, UK*; [3] *Department of Entomology, Assam Agricultural University, Assam, India. 785013*; [4] *Rwanda Agriculture Board, P.O. Box 5016 Kigali, Rwanda*; [5] *Ministry of Agriculture and Animal Resources, P.O. Box 621 Kigali, Rwanda.*; [6] *High Institute of Agriculture and Animal Husbandry, P.O. Box 210 Musanze, Rwanda*; [7] *CAB International, Egham, UK*; [8] *CAB International Africa Regional Centre, Nairobi, Kenya*

*E-mail: Ian.Adams@Fera.gsi.gov.uk

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Maize lethal necrosis (MLN) is a disease of maize caused by the combination of *Maize chlorotic mottle virus* (MCMV) and a potyvirus (Uyemote *et al*., 1981). MLN caused by MCMV and the potyvirus *Sugarcane mosaic virus* (SCMV) was identified in Kenya in 2012 (Adams *et al*., 2013) and associated with significant damage. Subsequent to the outbreak in Kenya, MLN symptoms have been associated with MCMV and SCMV in Tanzania and Uganda and, based on our initial findings, MCMV but not SCMV in Rwanda (ASARECA, 2013). The control of MLN is a priority for the East Africa region. We report here a fuller investigation of the causal agents of MLN from Rwanda.

In March and June 2013 samples of maize showing signs of MLN symptoms were received from the Northern Province of Rwanda for MCMV and SCMV testing. The samples were tested by real-time PCR as described in Adams *et al*. (2013). By this method all samples tested positive for MCMV and negative for SCMV. In order to determine if MCMV was responsible for the symptoms, or if another potyvirus was present, deep sequencing of total RNA extracted from four of the maize samples was performed. Indexed ScriptSeq libraries (Epibio, UK) were produced and sequenced on a MiSeq sequencer (Illumina, UK) using 500 cycle V2 reagents. The resulting 10 million 250 bp paired-end reads were split by index, quality filtered to a score of above Q20 and assembled using Trinity (Grabherr *et al*., 2011). Contigs over 200 bp were then compared to the GenBank nr database using BLASTX and the resulting data analysed using MEGAN as previously reported to generate draft genomes (Adams *et al*., 2013).

Complete genomes of MCMV were recovered from all four maize samples from Rwanda (GenBank Accession Nos. KF744393-KF744396) and shown to have a very close relatedness with MCMV from Kenya and China (99% homology), and with some separation from MCMV from the United States (97-96% homology) (Fig. 1). Complete SCMV genomes were found in three of the four samples (KF744390- KF744392). Fig. 2 shows the relationship between these and previous isolates. The SCMV isolated from Rwandan samples is distinct from that isolated in Kenya (87% identity) and most closely related (95% identity) to strain SCMV-DMB (BA00797). A closer examination of the sequence at the primer binding regions of the respective SCMV genomes shows a high degree of divergence and explain the negative results following real-time PCR testing using the assay designed to the SCMV sequence from Kenya (Adams *et al*., 2013).

These data would support a shared origin for MCMV in Rwanda and Kenya, but different sources for SCMV. This is not unexpected as SCMV has been observed for many years in East Africa (Louie, 1980), whereas MCMV is believed to be new to the region (Adams *et al*., 2013). MCMV would now appear to be spreading across the East Africa region, potentially in seed, and causative of MLN where populations of SCMV are already endemic. These findings have implications for the spread of MLN throughout Africa and for the testing for MCMV and SCMV. Control of the disease may prove more effective by initiating quarantine measures to counter the movement of MCMV, rather than attempting to control the endemic SCMV. This report confirms that MLN, caused by MCMV and SCMV, is now present in Rwanda and that this is most probably due to the recent introduction of MCMV.

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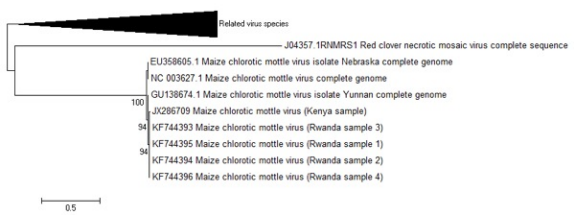

Figure 1

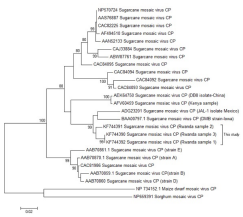

Figure 2

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