First report of a *Turnip yellows virus* in association with the Brassica stunting disorder in South Africa

S-A. New[1], S.W. van Heerden[2], G. Pietersen[3], L.L. Esterhuizen[1]

[1] Department of Biochemistry, University of Johannesburg, South Africa
[2] Sakata Vegenetics RSA Pty (Ltd), South Africa
[3] ARC-Plant Protection Research Institute and University of Pretoria, South Africa.

Over the last four years, Brassica farmers across diverse regions of South Africa (SA) have observed a new disease called 'Brassica stunting disorder'. The disease affects various *Brassica* species, but cabbages (*Brassica oleracea* var *capitata*) appear to be the most affected. The disease is characterized by stunted plants, flattening and sometimes purpling of the leaves, side shoot development, vascular discoloration in the stem and/or midrib of leaves, poor root development, low yield and low quality of the final product, thereby reducing the market value. In order to develop effective disease management practices and ensure continued sustainable production of *Brassica* crops in SA, the disease causal agent(s) needed to be identified. To this end, healthy and diseased cabbages were collected in Brits (North West) in July 2014. Nucleic acid (DNA and RNA) was isolated from two healthy control plants and six symptomatic plants. Total RNA was converted to cDNA using the TransPlex whole transcriptome amplification kit (Sigma, USA) according to the manufacturer's instructions. Both DNA and cDNA were prepared using the Nextera DNA sample preparation kit (Illumina) and sequenced using an Illumina HiSeq 2500, using 4 Truseq SBS chemistry with read lengths of paired-ends (2 x 125 bp). *De novo* sequence assembly and analysis was performed using the CLC Bio Genomics Workbench (version 5.5.1) and the assembled contigs were analyzed against the genome database in Genbank by BLASTn. A single large contig (5465 bp) of viral origin was found in all the symptomatic plants. Nucleotide blast searches showed that the contig has a maximum identity of 91% (with 100% coverage) to *Turnip yellows virus* (TuYV) (Genbank Accession No. X13063), a single stranded RNA virus of the genus *Polerovirus* (family *Luteoviridae)*. The assembled near full-length sequence of the South African isolate of TuYV was deposited in Genbank under the accession number KU198395. To confirm the identity of the TuYV obtained by NGS, reverse transcription-polymerase chain reaction (RT-PCR) using the TuYV specific primers (TuYVUp 5'- GCA ATA GAC TTA CCA TCG ACC

TCA ACC - 3’ and TuYVDo 5’- TTT GGG CTA AAA GGG TAC CAT CAC TC - 3’) was used to amplify 114 bp of the viral polymerase RdRp, followed by Sanger sequencing (Lotos *et al*., 2014). Following the identification of TuYV in diseased *Brassica* plants, the above mentioned RT-PCR was utilized to screen for the presence of TuYV in diseased *Brassica* plants collected from various regions (Gauteng, Mpumalanga, Kwazulu-Natal, Free State, North West) across SA between 2013 and 2015 (total of 50 samples). RdRp-amplicons of the expected size were obtained from all symptomatic cabbage plants and also in symptomatic broccoli (*Brassica oleracea* var. italica), cauliflower (*Brassica oleracea* var. botrytis), kale (*Brassica oleracea* var. sabellica*)* and canola (*Brassica napus*), but were absent in symptomless plants collected in the same fields. The RdRp amplicons from 15 of these samples were sequenced and the identify of TuYV confirmed by phylogenetic analysis. This is the first report of TuYV in South Africa on a crop. While TuYV is clearly associated with *Brassica* stunting disorder, further studies are required to prove that it is the etiological agent of this disease.

References:

Lotos et al., Journal of Virological Methods. 198: 1–11