Fragments of about 200 nucleotides were amplified and cloned in vector pCR8/GW/TOPO® (Invitrogen). After sequencing, the fragments were transferred by LR recombination to the viral vector PotatoVirus X (PVX). This vector was transferred transformation to Agrobacterium tumefaciens \'GV3101\', and inoculated in plants. PVX empty vector (pGR107) and PVXGFP was used as controls and to analyze suitable host plant for the virus infection. Six plants species were initially tested: Nicotiana tabacum, Datura stramonium, Solanum melongena, Abelmoschus esculentus, Capsicum annum and Brassica oleracea. In Nicotiana tabacum cv. TNN and Datura stramonium the virus was detected by RT-PCR, 28 days after inoculation, in noninoculated leaves, confirming the systemic infection in the plants. N. tabacum cv. TNN and D. stramonium are known hosts for the whitefly and were shown to be also good hosts for PVX and therefore, were chosen for further experiments. Studies of vATPase and RPL9 gene silencing in the whitefly using PVXmediated VIGS are in progress.

PIV82 - THE COMPLETE GENOME SEQUENCE OF MELON YELLOWINGASSOCIATED VIRUS DETERMINED BY NEXTGENERATION SEQUENCING

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The Northeast region of Brazil is the major melon producing zone in the country, being responsible for 95% of the total national production. A devastating disease has been reported in melon plants since 1999. It is known as "yellowing of melon plants" (Amarelão do meloeiro), which is associated to a viral agent, Melon yellowing associated virus (MYaV). This virus belongs to genus Carlavirus in the family Betaflexiviridae, formed by a linear ssRNA (+) genome of ca. 9 kb. The genome contains six ORFs. The complete genome sequence of MYaV is still not available, thus the 'Next Generation Sequencing' (NGS) strategy was applied to unravel the genome sequence of an isolate of MYaV. Melon samples used in this study were collected from Jaguaribe-Açu agricultural center (CE/RN), which produces ca. 81% of the total national production, and where the virus incidence is frequently high. Plants exhibiting yellowing symptoms were subjected to viral semi-purification according to Cali Moyer (1991) protocol, with modifications. Total RNA was extracted from semi-purified virus preparations with Trizol LS Reagent (Invitrogen), and dried using RNAstable (Biomatrica). The ribosomal RNA molecules were removed from the extract and the remaining RNA was sequenced at Macrogen, Inc. (South Korea) by Illumina 2000 Hiseq with 100 bp pairedend. Mapping and assembly of viral quasicomplete genome sequence were done with the Software Geneious 8.1. The genome, lacking its 5' and 3' ends, was approximately 9 kblong, in a typical carlaviral genomic organization with six ORFs. Pairwise nucleotide comparison and phylogenetic analysis confirmed that this virus belongs to the genus Carlavirus. Based on the species demarcation criteria of this genus, viruses sharing nucleotide (nt) sequence identity of CP or polymerase genes lower than 72% or amino acid sequence identity lower than 80% are classified as distinct species. The nt sequence of the CP of this study shared 97% identity with the MYaV CP gene available in GenBank and isolated in 2010 (ID: AB510477), and 57.9% with Sweet potato yellow mottle virus (SPYMV), the closest member among the carlaviruses. The nt sequence of the RdRp gene (ORF1) shared the highest identity of 57.2% with SPYMV. In conclusion, the complete genome sequence of MYaV showed a typical carlaviral genomic organization, as well as its genome nt identity with other viruses low enough to be considered as a distinct viral species in the genus Carlavirus.