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Characterization of an isolate of *Lettuce big-vein associated virus* (LBVaV) detected in naturally infected tomato (*Solanum lycopersicum* L.) in Slovakia

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Abstract: A tomato plant (*Solanum lycopersicum* Linnaeus, labelled KVE) displaying virus-like symptoms, tested negative for common tomato viruses, was subjected to high-throughput sequencing (HTS) on the Illumina MiSeq platform using ribosomal RNA-depleted total RNA as a template. The analysis has revealed the contigs mapping to *Lettuce big-vein associated virus* (LBVaV). The near complete LBVaV-KVE sequence of RNA1 and RNA2 revealed 95.0 and 94.9% identity with the reference sequence, the same length of translated products and a typical varicosavirus genome organisation. After initial long-term maintenance of LBVaV-KVE in the original plant, the virus could be detected by RT-PCR or nanoLC-ESI-Q-TOF in new plants generated from lateral shoot cuttings or inoculated by stem chips, although not uniformly. So far, LBVaV was reported to infect lettuce and related species. Our study expands the natural host range of the LBVaV to tomato.

Keywords: genome; next generation sequencing; *Solanaceae*; *varicosavirus*

Application of high-throughput sequencing (HTS) in plant virology has provided new possibilities for the identification of new pathogens in many agricultural crops and for understanding disease etiology. Moreover, as an unbiased tool for plant virome determination, this approach revealed much broader host ranges of some plant viruses compared to the knowledge based on conventional techniques (Maree et al. 2018; Maclot et al. 2020).

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MATERIAL AND METHODS

In May 2016, about 30% of the tomato plants (*Solanum lycopersicum* Linnaeus) displaying virus-like symptoms were observed in a greenhouse in western Slovakia (locality Kvetoslavov). Although the shoestrings and vein banding symptoms on leaves resembled to those caused by *Cucumber mosaic virus* (CMV) infection (Figure 1), the DAS-ELISA did not reveal the presence of the suspected virus. Similarly, the DAS-ELISA tests resulted also negative for *Tomato mosaic virus*, *Potato virus Y*, *Potato virus S* and *Potato virus M*. Experimental mechanical inoculation of *Nicotiana benthamiana* with the sap from symptomatic tomato plant did not produce visible symptoms on tested plants, thus, the etiology of the virus-like disease remained undetermined.

An original symptomatic plant (coded as KVE) was then placed in pot under insect-proof greenhouse with controlled conditions (10/14 h light/dark photoperiod, 22/20 °C day/night temperature) and maintained for 20 months by occasional pruning, transplanting and fertilisation. During this period, the shoestring-like symptoms on newly developed shoots disappeared and the plant displayed only mild vein clearing or remained symptomless.

To elucidate the potential infection by other viruses, the leaf sample from the actively growing upper part of tomato plant was harvested in March 2018 and the total RNA was extracted with Nucleo Spin RNA Plant kit (Macherey-Nagel, Germany) and ribosomal RNA-depleted nucleic acid preparation was subsequently used as a template for high-throughput sequencing (HTS, 300-bp paired-end sequencing) on the Illumina MiSeq platform (Illumina, USA) as previously described (Tomašechová et al. 2020). A total of ca 1.25 millions of high-quality reads were trimmed and used for *de novo* assembly with a CLC Genomics Workbench (version 7.5). Seven out of ca. 11 500 contigs, with length over 300 bp, mapped to RNAs 1 and 2 of *Lettuce big-vein associated virus* (LBVaV), covering 71% of the genomes. Remaining genome sequences of the Slovak LBVaV isolate KVE (LBVaV-KVE) were generated by Sanger sequencing of RT-PCR products amplified with specific primers designed from HTS contigs. Subsequent remapping of original HTS reads to nearly complete LBVaV genome sequences using the Geneious (version 8.1.9) identified a total of 212 reads mapping to RNA1 (sequence depth 5.2×) and 699 reads mapping to RNA2 (sequence depth 18.7×). The resulting, coding-complete, LBVaV-KVE sequences of RNA1 (GenBank accession number MW595188) and RNA2 (MW595189) are 6 622 and 5 972 nucleotides long, respectively, missing 44–124 terminal nucleotides as compared to the reference genomes (NC_011558 and NC_011568). Besides LBVaV, the HTS analysis revealed the coinfection with Ranunculus white mottle virus (data not shown).

Figure 1. Symptoms on the original KVE tomato plant

RESULTS AND DISCUSSION

LBVaV is a soil-borne pathogen belonging to the *Varicosavirus* genus (family *Rhabdoviridae*) vectored by some *Olpidium* sp. (Maccaron 2013). LBVaV has a bipartite, negative-sense single stranded RNA genome (Sasaya et al. 2002; Sasaya et al. 2004). Originally named *Lettuce big vein virus* (LBVV), this virus was initially assumed to be a causal agent of Lettuce Big Vein Disease (LBVD), an economically important disorder characterised by vein clearing, big vein, ruffling of the edge of outer leaves, small or no head and characteristic leaf necrosis (Verbeek et al. 2013, Bernal-Vicente et al. 2018). However, further studies strongly suggested the primary role of an ophiovirus, *Mirafiori lettuce big-vein virus* (MiLBVV), and not LBVaV, in the etiology of LBVD (Sasaya et al. 2008).

Alignment of both LBVaV-KVE segments with the reference LBVaV sequence (NC_011558, NC_011568) revealed 95.0 and 94.9% identity, respectively, the same length of translated products and a typical varicosavirus genome organisation. LBVaV-KVE RNA1 contains antisense information for a long open reading frame (ORF) coding for an L protein with polymerase function (2040 aa) sharing 98.5% of identity with the ORF of NC_011558.

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LBVaV-KVE RNA2 contains five genes in the typical order 3'-N-2-3-4-5-5' (Walker et al. 2018) encoding N or capsid protein (ORF1) and four other products of unknown function. The aminoacid sequences deduced from ORF1-5 products had 99, 96.7, 99.3, 98.8 and 96.2% of identity, respectively, with the corresponding products of the reference LVBaV isolate. Phylogenetic analysis of the *CP* gene using available sequences in the GenBank showed LBVaV-KVE form a cluster with Spanish LBVaV isolates from lettuce (GenBank Accessions AY581691, AY366412) and from common sowthistle (GenBank AY839618) (Figure 2).

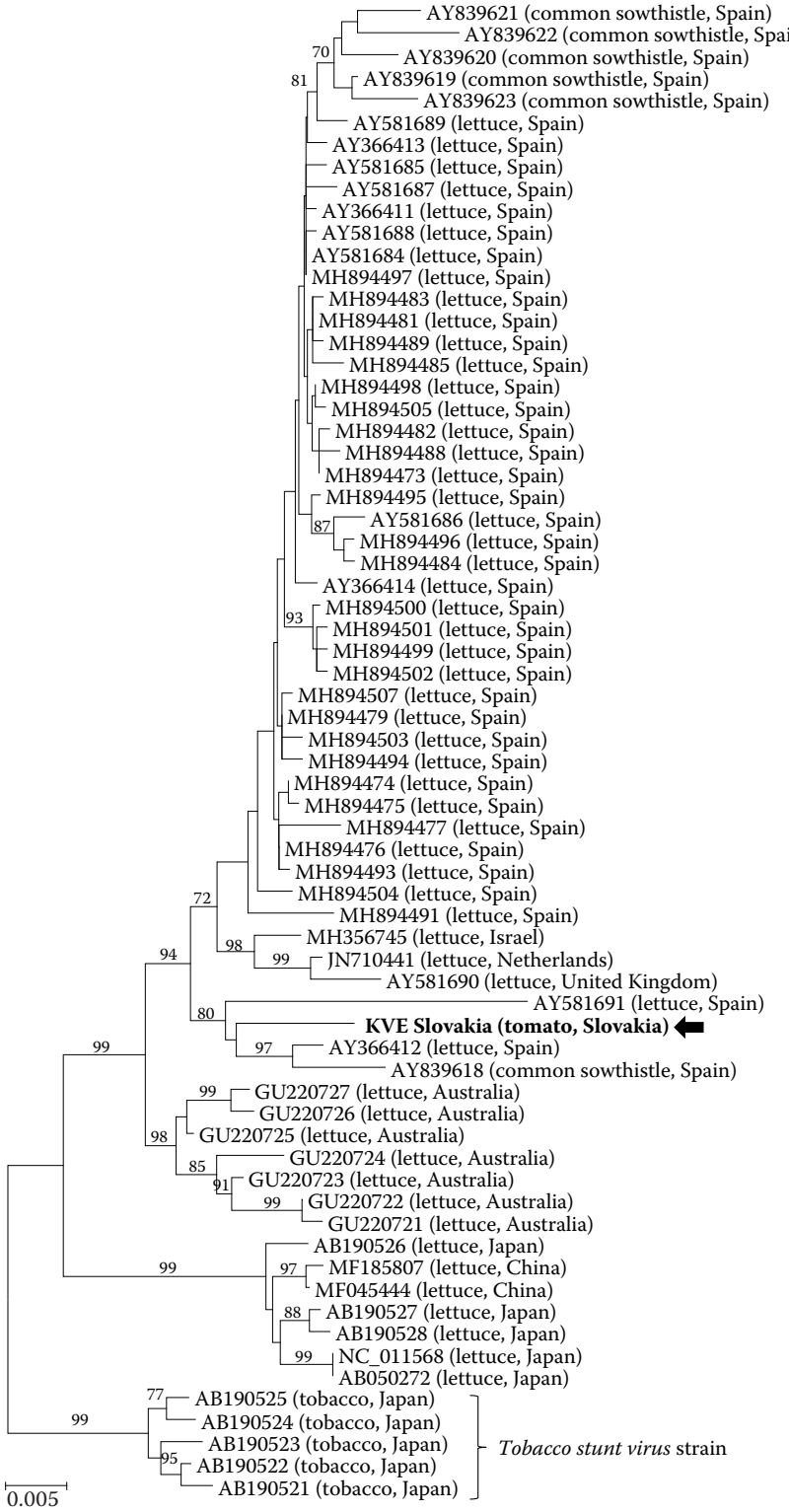

Figure 2. A neighbour-joining phylogenetic tree showing the relationship among *Lettuce big-vein associated virus* isolates based on the complete *N* gene (coat protein) sequences

The database isolates are identified by their GenBank accession number, country of origin and host

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To avoid the possibility that identification LBVaV in tomato was only a result of transient/local infection and in order to monitor the virus persistence in this host, we have maintained the original host under controlled conditions for more than 20 months and during this period, virus presence was demonstrated by RT-PCR using two primer pairs, designed in this work. Primer pair LB1_5870F (5'-AGCAACTGGAATGCTCTCGC-3') and LB1_6442R (5'-TCTATCCATCCACCGCTTGC-3') targets the 3' end of large RNA1 ORF (573 bp), while LB2_288F (5'-TCCTGGGATGATGAAAGCAC-3') and LB2_672R (5'-GAGTCCATCCAGTGAAGGAC-3') amplify a fragment from the *N* gene of RNA2 (385 bp).

After initial long-term maintenance of LBVaV-KVE in the original plant, lateral shoot cuttings from this plant were rooted to obtain a total of 45 new individual plants (Figure 3A). Two months after rooting, RT-PCR detection using LB1_5870F/LB1_6442R) and LB2_288F/LB2_672R has confirmed the LBVaV-KVE presence in 44% of tested plants.

Additionally, 20 tomato plants cv. Monalbo (2-months old) obtained from seeds, and kept under controlled conditions to avoid possibility of unwanted infections, were inoculated by 3cm-long chips from stem of the original KVE plant. 21 days post-inoculation, the presence of LBVaV RNA was detected in ca 30% of grafted tomato plants in RT-PCR using newly developed plant tissue (Figure 3B). Therefore, results of both experiments suggest erratic/irregular distribution of the virus in the original tomato plant.

To confirm the presence of LBVaV in the original sample by another independent method, the virus was successfully detected in leaf sample of tomato plant by a medium resolution nano-Liquid Chromatography-Electrospray Ionization-Quadrupole-Time of Flight (nanoLC-ESI-Q-TOF) as described by Cejnar et al. (2020). A proteomic search for viruses potentially present in the sample resulted in the identification of a specific peptide fragments with the unique sequence of 16 aminoacids characteristic for the portion of LBVaV L protein (polymerase).

In conclusion, LBVaV was detected in tomato by different methods (HTS, RT-PCR, nanoLC-ESI-Q-TOF). However, despite testing numerous additional tomato samples from different localities in Slovakia, no additional case of LBVaV infection has been recorded.

So far, LBVaV was reported to infect lettuce (*Lactuca sativa* Linnaeus) and related *Compositae* species, such as common sowthistle (Bernal-Vicente et al. 2018). Few divergent isolates were originally reported from tobacco *(*family *Solanaceae)* as *Tobacco stunt virus*, however, these isolates are now considered to form a divergent strain of LBVaV (Sasaya et al. 2005). Our study confirms that LBVaV can infect plants in the family *Solanaceae* and expands the natural host range of the virus to tomato. Other experiments, such as *Olpidium*-based transmission studies, can further clarify the significance of tomato as a host for this varicosavirus.

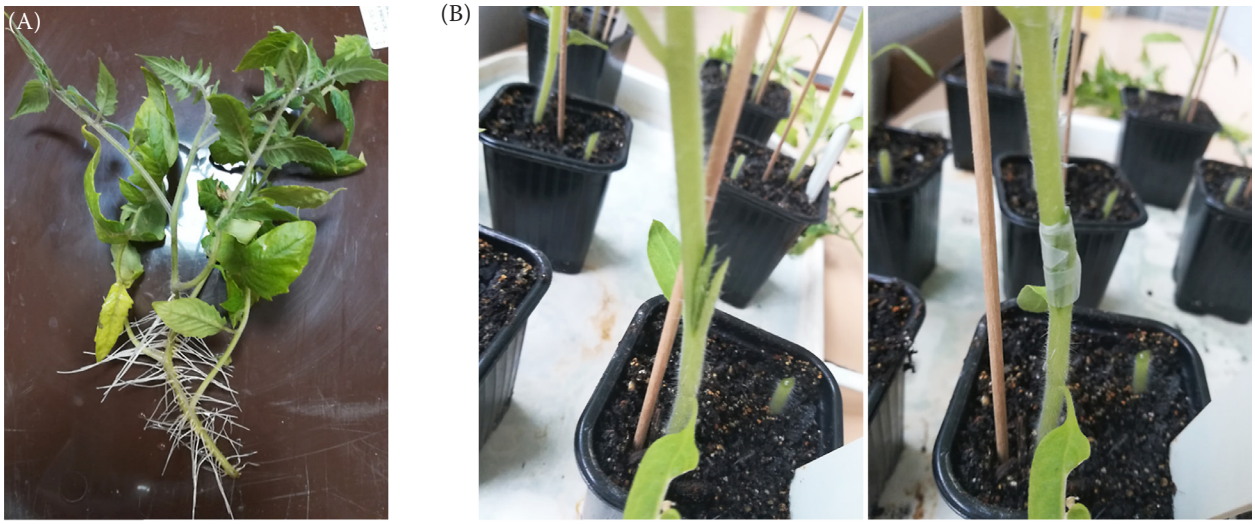

Figure 3. (A) Propagation of the original KVE plant by rooting of cuttings from lateral side shoots and (B) *Lettuce big-vein associated virus* transmission by grafting of stem chips

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