DETECTION AND CHARACTERISATION OF LITTLE CHERRY VIRUS-1 IN LOCAL SLOVAK CHERRY GERMPLASM

BENEDIKOVÁ Daniela¹, PREDAJŇA Lukáš², GLASA Miroslav²

¹National Agriculture and Food Centre, Research Institute of Plant Production, Piešťany, Slovakia
²Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovakia

Protection against harmful agents is an inseparable part of the intensive fruit-growing. Viruses present an important risk factor for the cherry production because of their hardly controlled dissemination and impossibility of direct treatment of viral diseases. Little cherry virus-1 (LChV-1) belongs to the Ampelovirus genus and its etiology is not yet completely understood. Typically, the fruits of infected trees are small, angular, failing to develop acceptable organoleptic properties and thus unmarketable. The information on the LChV genetic diversity in the European region is still scarce, therefore the absence of molecular data may complicate the development of polyvalent detection test [1-3]. The virus was recently detected in Czech Republic and Poland, which prompted the survey of its potential incidence in Slovakia.

The leaf samples were obtained in 2014 from sweet cherry trees in several regions of Slovakia (Brda, Nitra, Krakovany, Podolie, Dechtice, Piešťany, Bratislava), during the local cherry germplasm survey and characterisation.

Total RNAs were extracted from leaves using the Nucleospin RNA Plant kit (Macherey-Nagel). Random primer-synthesized cDNA was used for amplification of the partial capsid protein (CP) gene (456 bp) using proofreading Takara Ex Taq polymerase (Takara) and primers 1LC1277F (5’TCAAGAAGATCTGTGATGC3’ (sense)) and 1LC1322R (5’CGAACCTAGACTTTAGTATG3’ (antisense)). PCR amplification was performed under the following cycling conditions: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 53°C for 30 s and 72°C for 45 s; followed by a final extension step at 72°C for 10 min. PCR products generated from 9 sweet cherry LChV-1 isolates were directly sequenced. Sequence analyses were performed using MEGA v.5 or DnaSP v.5 programs.

Any specific symptom could be attributed to the LChV-1 infection (in most cases, the trees remained symptomless).

Neighbour-joining tree generated from the partial CP sequences obtained in this work and retrieved from GenBank showed important variability within LChV-1. Although most of Slovak isolates were phylogenetically related, they formed a distinct cluster and have been found divergent from previously characterised European LChV-1 isolates (nt identities with the reference German NC_001836 isolate reached 82.7 – 84.5%).

The isolate SK1044, phylogenetically related to the German JX669615 isolate, originated from a Prunus serrulata 'Kwanzan' tree grown in the botanical garden, thus probably introduced through the collection material. The presence of genetically distant LChV-1 isolates implies several introduction sources in Slovakia.

The variability observed within Slovak LChV-1 isolates analyzed in this study (4.2% nt divergence when considering also SK1044), the identification of these isolates in different localities and in old trees of local cherry genotypes suggest the efficient and long-term establishment of the LChV-1 in the environment.

Conclusions:

To our knowledge, this is the first report of Little cherry virus-1 in cherries in Slovakia.

Molecular characterisation revealed that most of Slovak LChV-1 isolates form a distinct cluster.

The newly designed primers allowed the sensitive and specific RT-PCR detection of LChV-1 and can be useful for further efforts to understand the prevalence, geographical distribution and pathogenicity of this virus.

References:
3. Candresse et al. (2013), Phytopathology 103: 293-298

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