Cherry virus A infecting cherries and plums in the Czech Republic – Short communication

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Abstract

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The presence of *Cherry virus A* (CVA) in the germplasm collections of sweet cherries and plums was studied. CVA was detected using the specific RT-PCR assay in six of eight sweet cherry and one of four plum cultivars. Specifity of amplicons and distant position of cherry and non-cherry isolates was verified by sequencing and phylogenetic analysis. Results indicate that the cherry landraces and cultivars could be infected by CVA more than it has been assumed.

Keywords: CVA; *Cerasus avium* L.; *Prunus domestica* L.; RT-PCR; sequencing

Cherry virus A (CVA), a member of genus *Capillovirus* in the family Betaflexiviridae, was first described infecting sweet cherry in southern Germany (Jelkmann 1995). It seems to be widespread, as it was found to infect different stone fruit tree species, mainly sweet and sour cherry, in different countries of North America (James, Jelkmann 1998; Sabanadzovic et al. 2005), Europe (Kirby et al. 2001; Komorowska, Cieslinska 2004; Mandic et al. 2007; Barone et al. 2008) and Asia (Isogai et al. 2004; Rao et al. 2009; Noorani et al. 2010). The sporadic CVA occurrence was noted in the Czech Republic too (Grimová et al. 2010).

CVA infection has not been connected with any specific symptoms or disease in cherry so far and seems to be latent although symptoms development on sensitive rootstocks or cultivars cannot be excluded. CVA usually occurs in mixed infections with viruses such as *Little cherry virus 1* or *2* (LChV1, LChV2) or *Prune dwarf virus* (PDV) in which case severe symptoms may be caused by the co-infecting virus(es), potentially damaging quality of fruits. Knowledge about CVA epidemiology is extremely limited and to date there is no evidence for the existence of vectors. The aim of the present work was to confirm the occurrence of CVA in germplasm collection and the identification of the isolates present in the Czech Republic.

MATERIALS AND METHODS

The sampling was done in the summer of 2008 and 2009. Leaf samples were randomly collected in the germplasm collection in Holovousy, from commercially propagated cultivars and landraces of sweet cherry (*Prunus avium* (L.) MOENCH) and plum (*Prunus domestica* L.) (Table 1). Total RNAs were extracted from ca. 100 mg fresh leaves using TRI-Reagent (Sigme Aldrich, St. Louis, USA) as rec-

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Table 1. Occurrence of CVA in different *C. avium* and *P. domestica* gene resources

Cultivar		No. of positive/tested trees
Cerasus avium (L.) MOENCH		
Pivovka	landrace, CZ	1/2
Pivka	landrace, CZ	1/2
Žalanka	landrace, CZ	1/2
Švestičková	landrace, CZ	1/2
Ladeho pozdní	cultivar, CZ	3/3
Early Lyons	cultivar, France	1/1
Sam	cultivar, Canada	0/2
Grolls Knorpelkirsche	cultivar, Germany	0/2
Prunus domestica L.		
Babče	landrace, CZ	0/2
Chrudimer	landrace, CZ	0/2
Černčická domácí	landrace, CZ	1/2
Meruňková renkloda	landrace, CZ	0/2

CZ – Czech Republic

ommended by the manufacturer. The samples were analysed by RT-PCR using the CVAfw1/CVArw1 CVA-specific polyvalent primers (Marais et al. 2012). The PCR products were purified using a Gel extraction kit (Qiagen) and cloned into the pGEM-T plasmid (Promega, Madison, USA). Three clones of each isolate were sequenced using a BigDye v. 3.1 Sequencing Terminator kit and an ABI PRISM 3730 Genetic analyzator (both Applied Biosystems, Foster City, USA). The fragments were assembled into the final contigs with the SeqMan program (Lasergene package, DNASTAR, Inc., Madison, USA). The sequences were deposited in the GenBank database under accession numbers JN676228 and JN676229. Sequences homologous to the cDNA sequences obtained were retrieved from the GenBank, using the BLAST program. All sequences were aligned with ClustalW as in MEGA 5.0 (Tamura et al. 2011). MEGA 5.0 was also used for the analysis of variability and the construction of phylogenetic tree using a neighbour-joining method.

RESULTS AND DISCUSSION

To confirm the presence or absence of *Cherry virus A* in the germplasm collections, the leaf samples were collected from sweet cherries and plums in Holovousy, Czech Republic. The isolated total RNAs were submitted to CVA-broad specific RT-PCR assay. CVA was successfully detected in both *Prunus* species and as a product of the expected size (302 bp) it was obtained in six of eight tested sweet cherry and in one of four tested plum cultivars. The high frequency of infection in cherry trees is in agreement with findings in other countries. James and Jelkmann (1998) identified a 40% infection rate in cherries in Canada and Germany, the same situation was described in Poland (Komorowska, Cieslinska 2004) and even an 85% rate in Serbia (Mandic et al. 2007). On the other hand, our results are in contrast with very low virus occurrence at the same locality reported earlier (Grimová et al. 2010). However, this discrepancy could be explained by the use of different PCR assays, i.e. the use of CVAfw1/CVArw1 primer pair in our study showed a greater reliability (Marais et al. 2012).

Detection of CVA infecting plum trees is more original and can be considered as an interesting result completing the knowledge about the ability of CVA to infect non-cherry hosts. This finding confirms *P. domestica* as a sporadic host of CVA as reported previously from France, Italy as well as the Czech Republic (Barone et al. 2008; Marais et al. 2012).

The specificity of all amplicons was verified sequencing the cloned cDNAs from three selected isolates, one from plum and two from sweet cherries. The sequences from sweet cherry cvs Ladeho pozdní and Pivka were fully identical (the sequence of isolate 6/13 was deposited in the GenBank, JN676228). The sequence from the Černčická domácí plum landrace (isolate 7/29, JN676229) showed distant relationship with the sequences from cherry, with only 89% nucleotide identity. BLAST analysis confirmed their homology with CVA sequences available in the GenBank and the CVA interhost species variability, as lower nucleotide sequence identity was confirmed between plum and cherry CVA isolates. Phylogenetic analysis based on both nucleotide and deduced amino acid sequences of the sequences determined here and of the sequences available in the GenBank generated similarly shaped trees and allowed discrimination with good bootstrap support of a 'plum' group (together with isolate PF, a French isolate from plum) and of a 'cherry' group (shown only amino acid tree, Fig. 1). This grouping of CVA isolates according to the host plant of origin confirms the phylogenetic relationships identified based on the analysis of a replicase short fragment by Marais et al. (2012).

Our results indicate that landrace cherry genotypes and cultivars in the Czech Republic are infect-

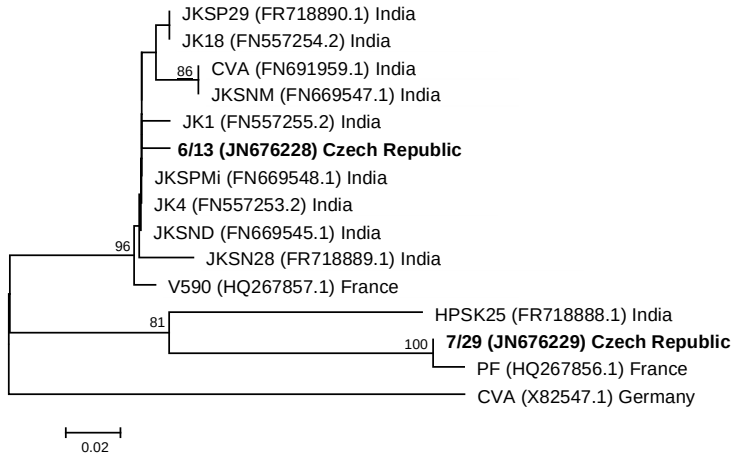

Fig. 1. Phylogenetic tree of CVA isolates constructed using the neighbour-joining analysis on deduced amino-acid sequences

The isolates are marked by the name and GenBank Acc. No., the new Czech isolates are in bold. Scale represents the number of substitutions per site. Only bootstrap value ≥ 70 was shown

ed more than it was expected and could represent a potential source of virus spreading. Although CVA occurs as latent infection, it is necessary to give attention to its occurrence as it could possibly increase the severity of impact of co-infecting viruses.

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