

First occurrence of *Cherry virus a (cva)* in the Czech Republic

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Abstract

A preliminary survey on *Cherry virus A (CVA)* has been performed in the Czech Republic in 2008-2009, including a germplasm collection, various growing areas and nurseries. 200 sweet and sour cherry leaf samples (*Prunus avium*, *P. cerasus*) were collected and tested by optimized RT-PCR using a new set of primers CVAZR2/CVAZF2. The 405 bp CVA-specific amplicon was obtained from two sweet cherry trees, namely cv. H 15/31 from Holovousy germplasm collection (originally from Romania) and the seedling *P. avium* from the nursery SEMPRA Turnov. To confirm RT-PCR results, CVA amplification products were directly sequenced. To our knowledge, this is the first report of CVA in the Czech Republic.

Keywords: CVA, sweet and sour cherries, RT-PCR.

Introduction

Sweet and sour cherries are important and widely grown fruits in the Czech Republic, producing 16 701 and 13 818 tons of fruit per year, respectively (Anonymous, 2008). A wide range of viruses and virus-like diseases is known to occur in cherry trees (Gilmer et al., 1976; Németh, 1986). In this region, the most common viruses include *Prune dwarf virus (PDV)*, *Prunus necrotic ringspot virus (PNRSV)* and *Cherry leaf roll virus (CLRV)* (Polák, 2007). *Cherry virus A (CVA)*, a definitive species in the genus *Capillovirus*, family *Flexiviridae*, is another, recently discovered virus attacking stone fruit trees. CVA was first reported in *P. cerasus* in Germany (Jelkmann, 1995). CVA infection is not limited to cherry, the virus was also found in apricot and peach. The virus does not appear to cause any obvious symptoms in the plants, but when combined with other viruses it may affect the severity of symptoms, or it may have some influence on graft incompatibility in susceptible combinations of scion and rootstock (James and Jelkmann, 1998). CVA is widely distributed in Europe, North America and Japan (Eastwell & Bernady, 1998; James & Jelkmann, 1998; Isogai et al., 2004; Komorowska & Cieślińska, 2004). Despite the importance of cherry production, no information is available about the occurrence and the potential incidence of CVA in the Czech Republic. Thus, a survey was done during the growing seasons of 2008-2009 encompassing different regions and germplasm collections, where sweet and sour cherries were grown. The information obtained was needed as the first step toward the search for control strategies of virus diseases in cherry trees.

Materials and methods

RT-PCR detection: Sweet and sour cherry plant material, infected by CVA, was received from B. Komorowska, Research Institute of Pomology and Floriculture, Skierniewice, Poland. Three methods were used for RNA extraction, namely phenol-chloroform extraction (Robinson, 1992), isolation with Qiagen Rneasy Plant Minikit according to the manufacturer's instructions and nucleic acid extraction using the modified silica capture method (Rott and Jelkmann, 2001). RT-PCR tests were done using the new set of oligonucleotides,

CVAZR2 [5'-ACCTTTGGAACAAACGATGC-3'] and

CVAZF2 [5'-CAAGAATCCAGGGGCCTACT-3']

amplifying a fragment of the virus coat protein (CP) and 3'Non-coding region (6891 to 7295 nt, 405 bp). Cycling parameters were as follows: denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec., and extension at 72°C for 1 min., totalling 35 cycles, with a final extension at 72°C for 7 min. Healthy cherry trees were used as negative controls. Products of amplification were stained by silver after polyacrylamide gel electrophoresis.

CVA monitoring: Using optimized RT-PCR method, a preliminary survey of the CVA occurrence was done in cooperation with Research and Breeding Institute of Pomology Holovousy Ltd. and with cherry growers from various growing areas in the Czech Republic during summer 2008-2009. In order to check the presence and distribution of CVA, leaf samples from 200 cherry trees were collected randomly for RT-PCR analysis. Acquired positive PCR amplicons were directly sequenced in both orientations (Biogen), sequence data were assembled with the BioEdit 7.0.9 program (Ibis Biosciences, USA) and compared with database sequences using BLAST on the NCBI Web server.

Results

RT-PCR detection: From the three methods of RNA extractions the modified silica capture was only suitable technique. The PCR method was very reliable as there were no problems with the identification of positive samples according to the presence of a specific bands, which were completely absent in negative controls.

CVA monitoring: Of the 200 samples tested, 2 sweet cherries, namely *P. avium* 'H 15/31' from Holovously germplasm collection (originally from Romania) and seedling *P. avium* from nursery SEMPRA Turnov, were positive for CVA in RT-PCR (Fig. 1). These infections were not associated with any particular symptoms. Sequences of 407 bp of CP amplicons obtained from PCR analysis were deposited in the EBI website with the accession numbers FN547890 (sequence code 09HOLche) and FN547891 (sequence code 09TURche). The alignment indicated that the nucleotide sequence of cv. H 15/31 and *P. avium* isolate were closely related to the published sequences of CVA (EMB Accession No. X82547.1) and had 98 % and 92 %, homology to the corresponding region, respectively. To our knowledge, this is the first report of CVA in the Czech Republic.

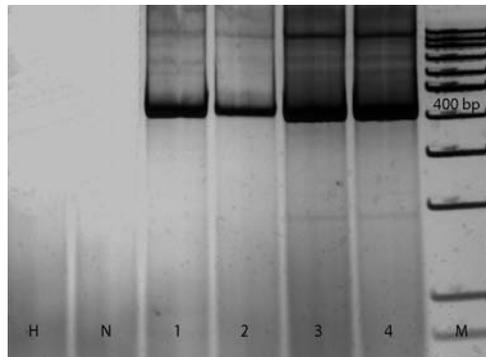


Fig. 1 CVA detection by RT-PCR in cherry trees. Lane M: DNA marker (Mass ruler low range, Fermentas); H: water control; N: healthy control; 1: cherry cultivar H 15/31, Holovously; 2: seedling *P. avium*, Turnov; 3, 4: CVA positive control (Poland). Polyacryl-amid gel stained by silver.

Discussion

A wide distribution of CVA in cherry sources was reported from Germany (James & Jelkmann, 1998) and Poland (Komorowska & Cieślińska, 2004), the neighbouring countries of the Czech Republic. Therefore, the occurrence of CVA was in all likelihood expected in the region and this assumption was partially confirmed by our studies. However, the low incidence of the virus did not correspond to the situation in other European countries. One of the potential explanations of such a small amount of acquired positive samples can be an utilization of unsuitable sets of primers. CVA diversity based on the sequence of an internal fragment of the RdRp (the PDO fragment) have shown the existence of five phylogenetic groups, with up to 19% genetic divergence (Marais et al., 2008). Nevertheless, the primer set used in the present work was designed in the N-terminal portion of the gene, comprising the relatively conserved 3' end of the CP gene and a part of the 3'NTR, allowing the detection of all the CVA isolates tested in previous studies (Svanella-Dumas et al. 2009).

In order to investigate whether this situation stems from a low incidence of the pathogen in the region or from the use of unsuitable diagnostic methods, further work should be done using other polyvalent assays for the efficient detection of all isolates of CVA.

Acknowledgements

This work was supported by the Czech Ministry of Agriculture (grant QG60123) and by the Czech Ministry of Education (MSM 6046070901). We are grateful to Dr. Komorowska for providing CVA infected leaf tissues from Poland.

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