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The variability of Hosta virus X isolates in the Czech Republic

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Summary. – The coat protein (CP) gene DNA sequences of nine isolates of Hosta virus X (HVX) from different regions of the Czech Republic were determined and compared with sequences available in GenBank. The sequences were almost uniform, the pairwise nucleotide identities among the Czech HVX isolates were 99-100%. The respective range was 98-100% when sequences from the GenBank were included. Therefore, phylogenetic analyses including Maximum parsimony and Bayesian analyses of either, DNA and deduced amino acid sequences, showed close relationship among isolates. Only the group of two isolates, HVXCR1 and HVXCR8 showed significant sequence divergence in phylogenetic trees. The HVXCR1-HVXCR8 group differs from the others by the substitution of glutamine (Q) by arginine (R). Moreover, these isolates showed different symptoms on infected hosta leaves – deformation on the leaves without a mosaic or mottling. This amino acid change may, therefore, have a biological significance.

Keywords: Hosta virus X; coat protein; phylogenetic analysis

Introduction

Hostas (*Hosta* spp.) are popular herbaceous perennial plants represented by over 7000 varieties (De la Torre, 2009), and widely cultivated due to their diversity in leaf-shape and color pattern, shade tolerance, and pest resistance. In the Czech Republic, hostas are grown as outdoor ornamental plants. Over four viruses in hostas have been reported in the world, but Hosta virus X (HVX) is the most economically significant virus (Ryu *et al.*, 2006). Leaves infected with HVX show symptoms, which include mosaic, mottling, irregular blotchy patches, chlorotic spots and distortion (Ryu *et al.*, 2002). Infected plants often exhibit reduced growth and dieback. HVX can be transmitted from infected to healthy plants by cutting practices used for propagation and breeding, as well as by means of HVX-contaminated soils (Ryu *et al.*, 2006). HVX was first identified and described in Minnesota, USA in 1996 (Currier and Lockhart, 1996). Since then, HVX was reported in hosta in other parts of the USA such as Kansas, Tennessee and Ohio (Kennelly *et al.*, 2007; Fajolu *et al.*, 2009; De la Torre, 2009) and other parts of the world such as Korea (Ryu *et al.*, 2002). In 2007, HVX was first discovered in Poland on infected seedlings imported from the Netherlands in 2003 (Cajza and Zielińska, 2007). In the Czech Republic, the virus was confirmed in 2010 (Koláčková *et al.*, 2011).

HVX has been completely sequenced in 2009 (HVX-Kr, Acc. No. AJ620114). Phylogenetic analysis of the type specimen of each genus of the family *Alphaflexiviridae* confirmed that HVX is a member of a distinct species of the genus *Potexvirus* (Fajolu *et al.*, 2009). HVX-Kr has a single-positive-stranded RNA genome 6528 nucleotides in length, excluding the poly(A) tail and contains five ORFs (De la Torre, 2009). The 5′-proximal ORF encodes a replicase. The next three ORFs, known as triple gene block, encode proteins that are involved in virus movement. The 3′-proximal ORF encodes the CP gene. The viral CP is required for genome encapsidation and virus cell-to-cell movement (Verchot-Lubicz, 2005).

This study reports the sequence variability of CP gene among HVX isolates collected from different regions of the Czech Republic and the identification of their phylogenetical

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Abbreviations: CP = coat protein; HVX = Hosta virus X

relationships with other previously reported isolates available in GenBank.

Materials and Methods

Czech HVX isolates. During 2010–2011, twentyfive hosta plants showing various virus-like symptoms including mosaic, mottling, leaf chlorosis and leaf distortion were collected from regions throughout the Czech Republic. The plants were obtained from the collection of hosta cultivars at Lednice (collection of the Faculty of Horticulture, Mendel University Brno), from Brno (The Botanical Garden and Arboretum, Mendel University Brno), from Prague (a specialized garden center), and from various garden centers in South Moravia and central Bohemia. ELISA was performed to determine the presence/absence of Impatiens necrotic spot virus (INSV), Tomato ringspot virus (ToRSV), Tobacco rattle virus (TRV), Tomato spotted wilt virus (TSWV), Tobacco ringspot virus (TRSV), and Arabis mosaic virus (ArMV) using commercial kits according to the manufacturer´s recommendations (DSMZ, Germany). The ELISA results did not indicate the presence of any of these six viruses. Symptomatic plants were tested for infection with HVX by means of RT-PCR. Nine HVX isolates were chosen for sequence analysis of the CP gene.

RNA extraction and RT-PCR amplification. Total RNA was extracted from 100 mg of HVX-infected hosta leaves using the RNase Plant Mini Kit (Qiagen, Germany) and the extraction was performed according to the manufacturer´s instructions. The presence of HVX viral RNA was tested by One Step RT-PCR Kit (Qiagen, Germany). The specific primer pair PHVXCP5 and PHVXCP3 was used to amplify the CP gene (Park and Ryu, 2003). PCR products of the CP gene were purified using QIAquick®Gel extraction kit (Qiagen, Germany).

Nucleotide sequencing. Isolates were sequenced using a Big Dye v.3.1 sequencing termination kit and an ABI PRISM 3100-Avant Genetic Analyzer (both Applied Biosystems). The raw sequencing data were processed using DNA Sequencing Analysis Software Ver. 5.1.

Table 1. HVX isolates from different part of Czech Republic used for DNA sequencing of the CP gene

Isolate	Cultivar	GenBank Acc. No.	Location
HVXCR1	Unknown	JF301948	South Moravia
HVXCR2	Unknown	JF301949	South Moravia
HVXCR3	H. 'Paul´s Glory'	JF301950	Prague
HVXCR4	Hosta minor	JF301951	Brno
HVXCR5	H. 'Scooter'	JF301952	Prague
HVXCR6	H. 'Patriot'	JF301953	Lednice
HVXCR7	Unknown	JF301954	South Moravia
HVXCR8	Unknown	JX293717	South Moravia
HVXCR9	Unknown	JX293718	Central Bohemia

Phylogenetic analyses. DNA sequences of the Czech isolates were checked for homologous sequences in GenBank using the BLAST program (Altschul *et al.*, 1990). The alignments of nucleotides and deduced amino acid sequences were performed using the Clustal W-Multiple Sequence Alignment (Larkin *et al.*, 2007). The DNA and new amino acid sequences were completed with data from the GenBank. Phylogenies were estimated either in PAUP*4.0b.10 (Swofford, 2003) or in MrBayes version 3.2.2. (Ronquist and Huelsenbeck, 2003) using the sequence of Cactus virus X (CVX) (Genome Net no. NC_002815) as an outgroup. CVX was selected as a representative member of the genus *Potexvirus* (Fajolu *et al.*, 2009). In Bayesian analysis, MrModeltest 2.3 (Nylander, 2004) was used to estimate the nucleotide substitution model for the DNA data-set – the best-fit substitution model was the general time reversible model with a proportion of invariable sites (GTR+I). For amino acid data the mixed model was selected. For both DNA and amino acid datasets, Markov chains were initiated from a random tree and were run for 2 million generations; samples were taken every 100th generation. For each dataset, the first 2000 trees were discarded. Log likelihood values from each replicate run were plotted against generation number in MCMC Trace Analysis Tool Version v1.4 (Rambaut and Drummond, 2007) to reveal whether any problems occurred with convergence. Bayesian branch supports were assigned as posterior probabilities on the consensus trees. In addition, maximum parsimony analysis was conducted in PAUP (Phylogenetic Analysis Using Parsimony). Trees were generated by heuristic search with a random addition of sequences (1000 replicates). Tree bisection and reconnection branch swapping during each heuristic search was done using the default settings. All characters were of equal weight and unordered. Bootstrap analyses (1000 replicates) used the same settings as above, but with a simple stepwise addition of sequences to obtain confidence in branch nodes.

Results and Discussion

Symptomatic plants were tested for infection and occurrence of HVX was confirmed in 19 out of 25 analyzed hosta plants. The CP gene sequences of isolates from different parts of the Czech Republic were sequenced and these sequences were deposited in GenBank under Acc. Nos. JF301948-JF301954, JX293717-JX293718 (Table 1). The amplified region of the CP gene for these isolates was 663 bp in length. The pairwise nucleotide identities among the Czech HVX isolates were 99–100%. The respective range was 98–100% when sequences from the GenBank were included. HVX-Kr (AY181252), HVX-USA (AJ517352), other isolates from the USA – Tennessee (FJ903386-FJ903415), Ohio (FJ403380-FJ403389), and isolates from Poland (FJ821702-FJ821705) were used for these comparisons. Comparison of the deduced amino acid sequence of the CP gene with all CP sequences showed

identities from 98 to 100%. The DNA sequences of HVXCR5, HVXCR6, and HVXCR9 were identical. Two or more nucleotide substitutions were observed in other sequences. Four nucleotide substitutions were observed in HVXCR3. This hosta plant was imported together with others into the Czech Republic in 2002. However, these nucleotide changes did not lead to any changes in the amino acid sequence. Among all nine isolates, there were only four single nucleotide changes that resulted in amino acid changes.

Among sequences of HVX, no relationship between genetic variability and geographical origin or the host cultivars was found (Fig. 1). However, no such relationship was found in either the isolates from Tennessee (Fajolu *et al.*, 2007) or from Ohio (De La Torre, 2009). The low genetic variability of CP gene in HVX isolates suggests a probable common origin, but it is likely to be maintained by selection pressure. The dispersal of HVX is mainly human-mediated via international trade. The hosta plants are distributed round the world, mainly from the

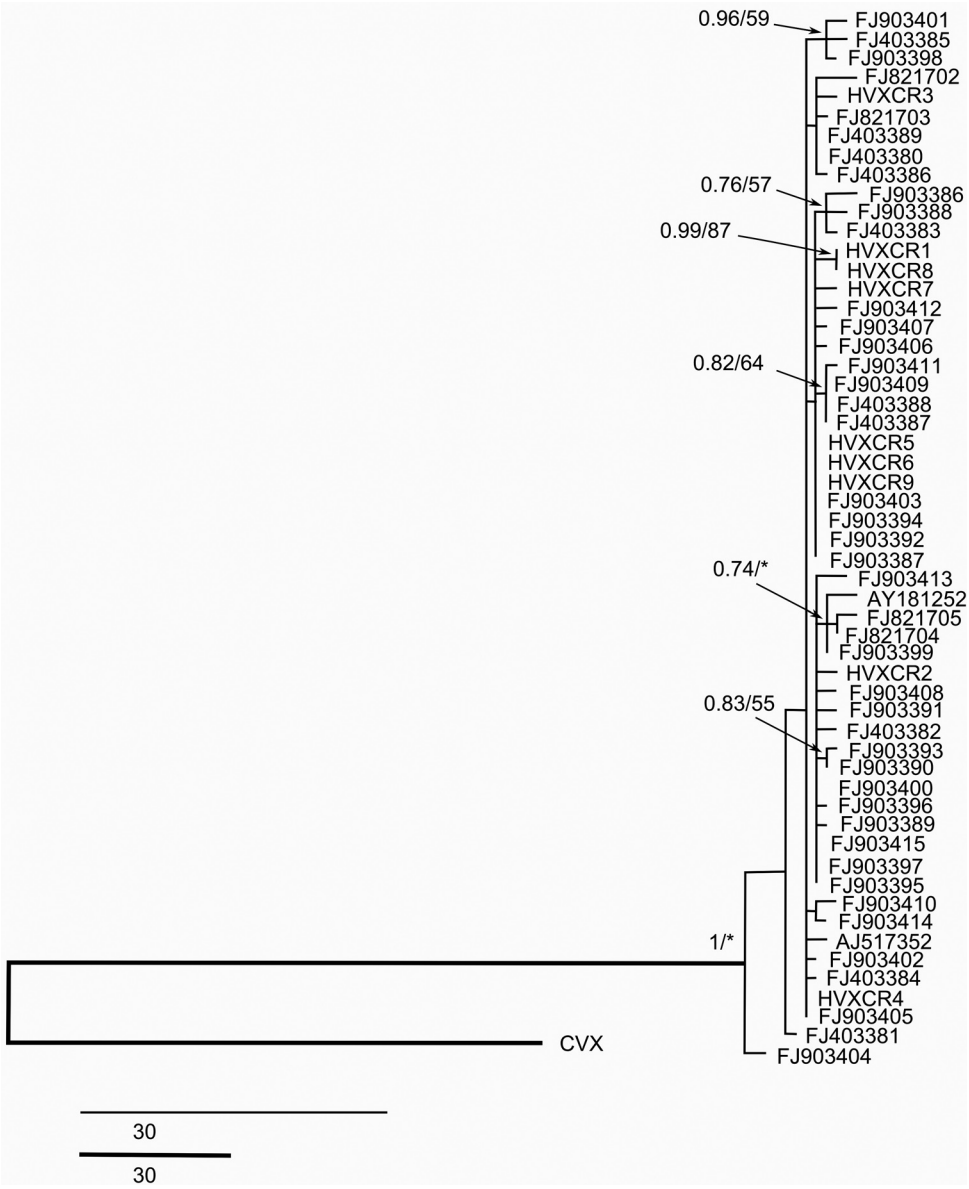

Fig. 1

Phylogenetic tree of CP-gene DNA sequences of HVX isolates

The Genbank Acc. Nos. refer to previously published sequences. The first number refers to Bayesian posterior probabilities, the second one to bootstrap proportion of Maximum parsimony analysis. Values smaller than 50% are not shown. The bars indicate number of substitutions per position.

USA and the Netherlands. The infected plants were imported from the Netherlands to Poland in 2003 (Cajza and Zielińska, 2007) and to the Czech Republic in 2002 (unpublished data). The long-term vegetative propagation is then responsible for the spread of HVX within the country. The virus is distributed mainly during the harvest process, not only through digging and cutting, but many of the overseas growers distribute virus by washing the plants in preparation for export. During this process, some of the outer layer of tissue is stripped off exposing sap that can infect other plants (Spece, 2010).

We detected only three amino acid substitutions among newly sequenced specimens. Thr26→Met (HVXCR7), Asp41→Glu (HVXCR7), and Gln158→Arg (HVXCR1, HVXCR8). Isolates with these substitutions showed different symptoms than the others – only deformations of the leaves were observed without mosaic or mottling. CP is important for virus movement and multiplication and plays an important role in symptom modulation (Meng *et al.*, 2006; Liu *et al.*, 2009). Changes between mild chlorotic and necrotic local lesions in tobacco are controlled by a single amino acid exchange in the CP of Alfalfa mosaic virus (AMV). When the Gln29 in the CP of AMV-strain 425 was mutated into the Arg present at this position in AMV-strain YSMV, the symptoms induced by the transcript on inoculated leaves changed from chlorosis to necrosis (Neeleman *et al.*, 1991). Single amino acid substitution at position 129 in the CP gene of Cucumber mosaic virus (CMV) is the determinant for local symptom expression, systemic movement and may induce resistant responses in plants (Kobori *et al.*, 2002). The CPs of some viruses are elicitors of gene-mediated resistance in plants (Verchot-Lubicz *et al.*, 2007; Mizumoto *et al.*, 2012). Two amino acid substitutions in the CP gene of Pepper mild mottle virus, Gln → Arg and Gly → Lys, were responsible for overcoming the resistance gene in *Capsicum* spp. Furthermore, the presence of either single amino acid substitution causes a deficiency in the systemic infection of *Capsicum chacoense* (Genda *et al.*, 2007). The detected amino acid changes can play a role in the virus biology, although the exact correlation between the changes and symptom manifestations requires further studies.

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