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OCCURRENCE OF POTATO VIRUS X ON HYBRID DOCK IN CZECH REPUBLIC

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Summary. – Hybrid dock of Uteush (*Rumex patientia* L. x *Rumex tianschanicus* A. Los., the family *Polygonaceae*) is a perspective high productive crop and in the last decade its farming area has continuously grown in Czech Republic. However, the introduction of this non-native perennial crop into a present plant production creates a new potential reservoir for some plant viruses. Also, the hybrid dock could become a host of currently uncommon or insignificant viruses. We screened two dock-farming localities situated in south-west and north-east part of the Czech Republic for the presence of potyviruses, potexviruses, and carlaviruses. In the south-west part of the country, we detected a high incidence of Potato virus X (PVX, the genus *Potexvirus*). In contrast, in the north-east part of the country we did not detect any dock plants infected with PVX. Next, two other viruses, Turnip yellow mosaic virus (TYMV) and Radish mosaic virus (RaMV) were mechanically inoculated and tested for their survival capacity and multiplication in the hybrid dock. Both viruses were detected 9 months after inoculation in the infected plants.

Keywords: hybrid dock; RT-PCR; Potato virus X; Radish mosaic virus; Turnip yellow mosaic virus

The genus *Rumex* L. (the family *Polygonaceae*) contains about 170 species of annual, biennial, and perennial herbs, which are very common in Northern hemisphere. The *Rumex crispus* (Curly dock) is regarded as one of the five most prolific plants in the world. More than 20 species and subspecies and dozens more hybrids and varieties are found in the territory of the Czech Republic (Kubát, 1990). Many of them are nuisance weed and the others are occasionally grown for culinary purposes. In the last decade, the fast growing plants were tested as a source for bio-fuel production. Since 1992 the hybrid of Uteush (*Rumex patientia* L. x *Rumex tianschanicus* A. Los.) has been grown in Czech Republic and the area of producing fields reached thousands of hectares in 2006. However, an introduction of this non-native perennial plant into current production increases a risk of harboring some plant viruses and their vectors. In addition, the plant could become a host of currently uncommon or insignificant viruses or could be a source of new viruses as well.

The Dock mottling mosaic virus (the genus *Potyvirus*) is the only virus naturally infecting *Rumex* spp. Infected plants with chlorotic mottling leaves were observed in Hungary and New Zealand (Brunt *et al*., 1996). However, this virus has not been completely characterized so far. Different dock species are also alternate hosts for a number of viruses as Tobacco vein mottling virus and Carnation vein mottle virus (the genus *Potyvirus*), Peach rosette mosaic virus and Cherry leaf roll virus (the genus *Nepovirus*), Wound tumor virus (the genus *Phytoreovirus*), Cucumber mosaic virus (the genus *Cucumovirus*), Red clover necrotic mosaic virus (the genus *Dianthovirus*), and Carnation mottle virus (the genus *Carmovirus*) (Brunt *et al*., 1996). Also, Alfalfa mosaic virus (the genus *Alfamovirus*) was detected by ELISA in the clustered dock (*Rumex conglomeratus*) in Australia (McKirdy and Jones, 1997), Pepino mosaic virus (the genus

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Abbreviations: CT = threshold cycle; PVX = Potato virus X; RaMV = Radish mosaic virus; TYMV = Turnip yellow mosaic virus; WClMV = White clover mosaic virus

Potexvirus) was detected in the naturally infected *Rumex* spp. (Anonymous, 2007), and Lettuce infectious yellows virus (the genus *Crinivirus*) was supposed to infect *Rumex crispus* (Brown *et al*., 1990). In addition to these accidental infections, there are also examples of the dock as a potential reservoir of other important viruses. Tomato spotted wilt virus (the genus *Tospovirus)* was detected by DAS-ELISA in about 30% of randomly collected samples of *Rumex* spp. in Greece (Chatzivassiliou *et al*., 2001) and less than 1% of infected dock plants was detected in Tasmania (Wilson, 1998). In South America, *Rumex obtusifolium* was identified as a potential reservoir of Potato yellow vein virus (the genus *Crinivirus*) (Salazar *et al*., 2000). The hybrid dock of Uteush grown in the Czech Republic was thoroughly tested for stand conditions, fertilization, mass production, energy features, and ash behavior (Petříková, 2006). On the other hand, it has not been examined for the occurrence of various viruses. This is the first report describing a natural viral infection detected on this plant.

In spring 2007, we screened two localities in the south-west and north-east of the Czech Republic and collected samples of sprouting leaves from 2–3 year-old wintered plants. The leaves were often asymptomatic or with slight discoloration on the margins or on the leaf top. For virus screening was used a highly sensitive RT-PCR method with the genus-specific primers for detection of potyviruses, potexviruses, and carlaviruses. The RNA was isolated from 0.1 g of leaves with the RNeasy Plant mini Kit (Qiagen) according to the manufacturer's recommendations and eluted with 30 µl of water. Complementary DNA was synthesized from 7 µl of RNA with iScript cDNA synthesis kit (Bio-Rad) in 10 µl volume. cDNA in volume of 2 µl was amplified with the genus-specific primers. For potyviruses, CPUP primer 5'-TGAGGATCCTGGTGYATHGARAAYGG-3' and P9505 primer 5'-GATCCTTTTTTTTTTTTTTTT-3' (Revers *et al*., 1999) was used. These primers amplify the 750 bp region encompassing capsid protein gene and the 3'non-coding region of most potyviruses. Primer pair for detection of potexviruses was designed from the conserved region of the RNA-polymerase gene. The primer pair pxFLK 5'-TTYCTNAARTCNCARTGGGTCA-3'and pxADF 5'-GCATCAAAAGTGGGRCCTTC-3' gave PCR product of about 430 bp. We tested this primer pair with PVX and White clover mosaic virus (WClMV, both viruses belong to the genus *Potexvirus*). Both tests resulted in a product of expected size (430 bp) and the amplification program of 30 cycles with 30 secs denaturation at 94°C, 20 secs annealing at 54°C, and 30 secs synthesis at 72°C was performed (Fig. 1). Further, we tested the dock samples and obtained amplification product of the expected size with primers amplifying potexviruses in samples collected in south-west of the country only (Fig. 2). The primer pair crlTFG 5'-CNTTTGGNGARAGCACNGG-3' and crlKLW 5'-YTTNARCCANGGRTCNCC-3' was designed to amplify segment of the RNA polymerase gene of about 330 bp occurring in most carlaviruses. However, the RT-PCR of samples with primers specific for potyviruses and carlaviruses were negative (results not shown).

Potexvirus-specific amplification products of samples 1, 4, 8, and 9 (Fig. 2) were cut from the gel, extracted, and sequenced with PCR primers and BigDye cycle sequencing kit (Amersham). Nucleotide sequence alignment of about 420 nt long segments showed identity of all four samples, what indicated that the infecting viruses were identical. Comparison of infecting virus sequences with virus sequences deposited currently in GenBank revealed the identity of the examined sequences with PVX. Phylogenetic analysis classified the identified PVX isolate from the dock as very similar to Eurasian isolates, but not to the South American ones. The nucleotide sequence of PVX isolate from the dock of Uteush was deposited in GenBank under the Acc. No. EU669005.

We found the high incidence of PVX in the dock fields in the south-west of the country, where nearly 90% of the dock plants were infected. On the contrary, the dock plants growing in the north-east of the country were completely negative for PVX. Both examined localities were supplied with seeds coming from the same producer**.** In spite of the fact that PVX is not transmissible by seeds or pollen, we examined the distributed seeds and we did not detect the presence of PVX. It is possible that the dock plants became infected from different plant sources by mechanical devices

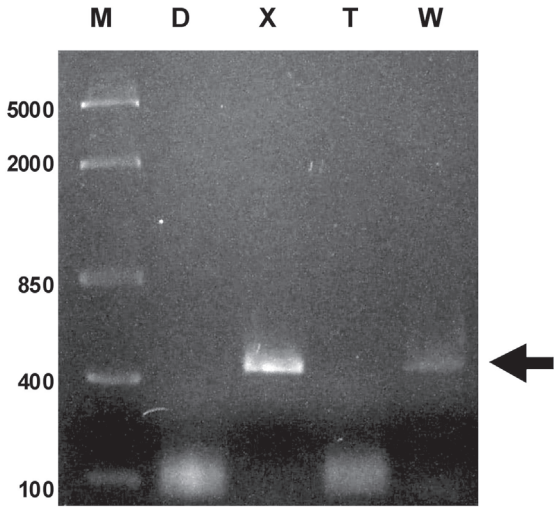

Fig. 1

RT-PCR products with potexvirus primers from healthy dock (D), PVX (X), healthy trefoil (T), and WClMV (W)

DNA size marker (M). Arrow indicates the product of 430 bp.

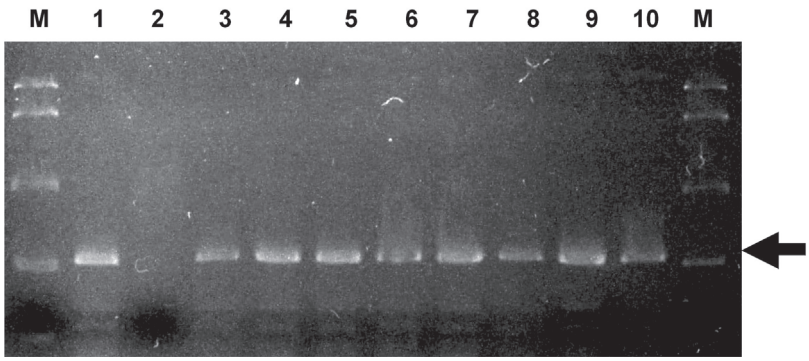

Fig. 2

RT-PCR products with potexvirus primers from 10 dock samples

DNA size marker (M). Arrow indicates the product of 430 bp.

used for cultivation of the fields or by wind, animals, or by contact between plants.

PVX is considered as a mild virus causing a faint mosaic on potato plants and the yield losses make about 10 to 20%. Nevertheless, the severity of symptoms and the yield drop greatly increases in mixed infections with other viruses, e.g. Potato virus Y (the genus *Potyvirus*) (Koenig and Lesemann, 1989). Although PVX is distributed worldwide, it could be controlled by rotation of crops and growing virus-free seed. If perennial dock became an important new source of PVX, it would adversely affect the effort of farmers to produce a high quality potato crop. However, the occurrence of mixed viral infection in the hybrid dock and its effect on the potato crop remains unknown. So far, no additional viruses besides PVX have been found in the wild-growing hybrid dock plants.

To test the ability of the hybrid dock to host other plant viruses, we examined the mechanical inoculation of RaMV (the genus *Comovirus*) and TYMV (the genus *Tymovirus*) on the dock seedlings. These viruses are common in oilseed rape and are easily transmitted by aphids and by flea-beetles. None of them was detected previously on any *Rumex* sp. plants. The producing areas of oilseed rape and hybrid dock have expanded substantially in the last years and moreover, the crops are grown in adjacent regions. Leaves of young hybrid dock plants sprouting from seeds were inoculated with RaMV and TYMV, cultivated for 9 months, and tested for the presence of the infecting viruses by real time PCR. The samples were prepared as above and primers TYup 5'-CCGGCCCATCACCTCTCACC-3' and TYre 5'-TGG TCGGGAAAGCTGGGGC-3' were used for amplification of 198 bp long segment of TYMV. Primers RMup 5'-TTGG TATGCTGGAAACCGAG-3' and RMre 5'-CACTCTTC AACTTCTTCCGTAGC-3' were used for amplification of 353 nt long segment of RaMV. PCR products of expected size (198 and 353 bp) were obtained and sequencing of the amplified fragments confirmed the presence of both viruses in the infected dock seedlings (results not shown).

To compare an amount of the three viruses (PVX, TYMV, and RaMV) in infected dock plants, we used simultaneous RT-PCR and evaluated their concentrations. The RNA was isolated from the identical amount of PVX-naturally infected leaves and from asymptomatic leaves of TYMV- and RaMV-artificially infected plants 3 months after inoculation. cDNA was prepared as above and the PCR reaction mixture was supplied with SYBR green stain. Surprisingly, the highest threshold number CT (the number of amplification cycle, where the signal starts its exponential increase) demarcating the lowest virus concentration was revealed for PVX-naturally infected plants (CT ranged between 27 to 30 in four samples). The concentration of RaMV was 4 to 500 times higher and CT of RaMV ranged from 18 to 25. TYMV multiplied in the hybrid dock to the highest concentration about 2,000 times higher than PVX and the CT of TYMV was about 16. These experiments demonstrated that the viruses common in unrelated oilseed rape crop are able to multiply efficiently in the artificially infected dock plants. Although the infected dock plants were symptomless, an unpredictable synergism could occur in combination with some other viruses. We did not evaluate the persistence of TYMV and RaMV in the hybrid dock for a longer time after infection. However, it is possible that these viruses can persist for a longer time than 9 months. Tomato spotted wilt virus (the genus *Tospovirus*) was able to persist in the infected *Rumex crispus* plants for two years (Groves *et al*., 2002).

The natural virus infection of wild growing dock species is very sporadic or completely absent. In 20 randomly collected samples of wild dock we did not find any positive sample for potex-, poty-, or carlaviruses (results not shown).

We performed screening of perennial hybrid dock for the presence of filamentous viruses belonging to three most abundant virus genera. High incidence of natural infection with PVX was detected, but not an infection with potyviruses or carlaviruses. Furthermore, we have shown that at least one tymovirus and one comovirus could efficiently replicate on this plant. Still, this hybrid plant has not been adequately characterized as a host for important plant viruses.

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