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Variability of Bean yellow mosaic virus isolates in the Czech Republic

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Summary. – Three isolates of Bean yellow mosaic virus (BYMV) from the Czech Republic originating from gladiolus plants were examined according to their biological and molecular characteristics. Partial sequence of coat protein-nuclear inclusion protein b (CP-NIb) coding region (768 bp) of these isolates were determined and compared with the corresponding sequences of different BYMV isolates obtained from GeneBank. Phylogenetic analysis showed that the Czech BYMV isolates were distributed across the three groups of phylogenetic tree. Their placement was not dependent on the geography or host plant.

Keywords: Bean yellow mosaic virus; phylogenetic analysis; sequencing; gladiolus

Introduction

Gladiolus is an important plant of the world floriculture industry and ranks amongst the top six flowers demanded on the market (Anonymous, 1997). In recent years, a decline in the production of gladiolus has been observed and the major contributing factor is a wide array of diseases affecting the gladiolus. Many viruses have been reported to infect gladiolus cultivars and the BYMV is the most prevalent one (Zaidi *et al.,* 1993). This virus causes mosaic and white-break symptoms on leaves and flowers, corm rot, and reduced flower production (Magie and Poe, 1972; Mokrá and Gotzová, 1994). BYMV belongs to the family *Potyviridae,* the genus *Potyvirus* that is the largest and the most economically important genus of plant viruses. The members of this genus cause severe disease in many leguminous and ornamental plants (Tsuchizaki *et al*., 1981; Kaneshige *et al*., 1991; Sasya *et al*., 1998). In 1925, BYMV was detected on French beans in USA and Netherlands and currently it is distributed worldwide (Mohammad and Angela, 2004). In comparison with other potyviruses, BYMV has a relatively wide host range and many BYMV isolates differ in pathogenicity and serological properties (Wada *et al*., 2000). So far, the variability of BYMV in the Czech Republic has not been studied in spite of the different pathogenicity of detected BYMV isolates (unpublished results). For taxonomic analysis the most useful region of potyviral genome is the gene encoding coat protein (CP). Its 3'-terminal region is used as a reliable criterion in distinguishing BYMV isolates from the other potyviruses (Pappu *et al*., 1993; Shukla *et al*., 1998) and the characteristic sequences in CP region are useful for the discrimination and reconstruction of the phylogenetic relationships among different strains of BYMV (Rybicki and Shukla, 1992). In contrast to CP, NIb exhibits the highest amino acid sequence homology among the potyviral gene products and acts as an RNA-dependent polymerase (Allison *et al.,* 1986). Therefore, this region is commonly used for designing of BYMV specific primers (Castro *et al*., 1993; Rosner *et al*., 1994; Hiroyuki Uga, 2005).

This study reports sequencing of CP-NIb region of the three Czech BYMV isolates obtained from gladiolus plants and identification of their phylogenetical relationships with other BYMV isolates published in GeneBank.

Materials and Methods

Virus isolates. Two BYMV isolates were obtained from Jestřabí (J95 BYMV) and Nedvědice (N4 BYMV), and third isolate (M71 BYMV) was obtained from a gladiolus plant grown from corm purchased on the market during season 2006–2007. The symptoms

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Abbreviations: BYMV = Bean yellow mosaic virus; CP = coat protein; NIb = nuclear inclusion protein b

of BYMV infection on leaves of gladiolus plants was confirmed by DAS-ELISA (Clark and Adams, 1977). These isolates were further tested by a biological method with *Pisum sativum* cultivars as an indicator plant by mechanical inoculation and the symptoms were observed after 3 weeks.

RNA extraction. Total RNA was extracted from 100 mg of gladiolus leaves by using RNase Plant Mini Kit (Qiagen) and extraction was done according to the manufacturer's instructions.

One step RT-PCR. The viral RNA was diluted and tested for the presence of BYMV by one step RT-PCR method (One step RT-PCR kit, Qiagen). Two overlapping fragments covering the 5' NIb-CP 3' region of 777 bp to 780 bp, respectively, were obtained with two different primer pair combinations. The first fragment was amplified using the primer pair BYMV1/2 (Hiroyuki Uga, 2005). For the amplification of the second one, the newly designed primer S1CF/S1CR spanning a part of the NIb-CP region was used for sequencing (S1CF: 5'-GATGAGGRGCTTGTGTGTCG-3'; S1CR: 5'-TCACCTGTTCCTCTCCATCC-3').

The procedure for one step RT-PCR consisted of reverse transcription at 50°C for 30 mins, the first denaturation step at 4°C for 5 mins, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min and terminated at 72°C for 10 mins. The 25 µl of one step RT-PCR reaction product was analyzed on 1.5% agarose gel. Electrophoresis was done with TBE buffer and the gel was stained with ethidium bromide. DNA was visualized using an UV transilluminator and 100 bp ladder was used as a size standard.

Sequencing. The PCR products were purified using QIAquick® Gel extraction kit (Qiagen). Each isolate was 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.

Phylogenetic analysis. Sequences of the Czech isolates (769 positions in the final dataset) were checked for homologous sequences in GeneBank using the BLAST program (Altschul *et al*., 1990) and were aligned with corresponding sequences of other BYMV isolates. The evolutionary history was inferred using neighbor-joining method (Tamura *et al*., 2007). The optimal tree with the sum of branch length = 1.22188256 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) was shown next to the branches (Felsenstein, 1985). The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages (Takezaki *et al*., 2004). The tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al*., 2007).

Acc. Nos. of BYMV isolates are: D28819, D83749, AY192568, AF185960, AF185962, FJ492961, FJ618532, X63358, AB079886, AB079782, AM884180, AY845012, AY845011, AJ311371, AB097090, AB097089, U47033, D89545, EU144223, FM180011, AF185961, DQ060521, DQ641248, AB079887, AB439730, AB439729, AY520092 and AB373203

Results and Discussion

The 3 studied BYMV isolates were selected according to the DAS-ELISA results. The occurrence of BYMV on the gladiolus plants was detected in two locations of Jestřabí field (isolate J95 BYMV), Nedvědice garden (isolate N4 BYMV) and in the samples from the market (isolate M71 BYMV) in the Czech Republic. The infected gladiolus plants showed usually mild symptoms. The infection of BYMV was confirmed by DAS-ELISA in all tested samples. In the subsequent biological test with pea plants, the isolate J95 BYMV produced mild mosaic symptoms, isolate M71 BYMV vein clearing symptoms, and isolate N4 BYMV severe mosaic symptoms.

The presence of specific viral RNA was verified in gladiolus plants by one step RT-PCR with virus-specific primers BYMV1/2. In the majority of cases, we detected only one specific product with the exception of the isolate J95 BYMV that produced two different products. The size of the first product was around 600–700 bp, while the size of the second one was 700–800 bp. We supposed that this result was probably due to the co-infection by different BYMV strains. On the other hand, we could not exclude the presence of some non-specific products or PCR artifacts. In reaction with primers BYMV1/2, the presence of PCR products of bigger size in these isolates could indicate the presence of some mutations (probably insertion) specifically in this part of virus genome. Variability in CP area is often associated with the important aspects of the virus life cycles and functions such as virus movement in the plants, aphid transmissibility, and virus accumulation in the plant cells (Andrejeva *et al*., 1999; Andresen and Johanin, 1999).

Variability of CP and NIb sequences is often used for phylogenetic analyses of many species of the genus *Potyvirus* (Handley *et al*., 1998; Alegria *et al*., 2003; Gadiou *et al*., 2008). The sequencing was started from the BYMV1/2 primers, which are covering mainly NIb gene (Oertel *et al*., 1997; Lecoq *et al*., 2000). In case of J95 BYMV isolate that gave two different products, we were able to sequence only product that was located at the same position as with other two isolates. Later, we continued the sequencing with successive part of virus genome that overlapped a part of NIb-CP region. For this amplification, the newly designed primers (S1CPF/S1CPR) were used in order to recognize the other part of CP gene. The sequences were amplified by one step RT-PCR method that gave the expected product of 765–775 bp for all 3 isolates. The virus sequences containing part of NIb-CP were obtained for each isolate and their identification as the BYMV was confirmed by the comparison with BYMV GeneBank sequences using the BLAST algorithm. The sequences of the 3 Czech isolates were compared with the sequences of 28 tested BYMV isolates deposited in GeneBank.

Phylogenetic analysis based on the 768 bp long nucleotide sequences part of 5' NIb-CP 3' region corresponding to 7986–8764 bp of AB439729 showed a distribution of the Czech isolates into 3 significantly distinct groups (Fig. 1).

The isolate M71 BYMV grouped with isolates AB439730 from gladiolus, D288191 and D837491 from *Nicotiana benthamiana* (Japan). The isolate J95 BYMV fell into group with isolates AB439729 from gladiolus (Japan) and AY 192568 BYMV from USA. The isolate N4 BYMV was grouped with isolates AB079782, AB097089, AB097090 from dwarf gentian, AB079887, AB079886 (all of them Japan), AJ311371 from vegetable (China), AM884180 from *Lisianthus* (Taiwan), AY845012, and AY845011 from *Vanilla fragrans* (India).

According to the phylogenetic analysis, the relationship between the isolates M71 BYMV and J95 BYMV was closer than with the isolate N4 BYMV. We did not find any relationships between BYMV isolates originating from different regions or host plants.

Based on these results, we confirmed that BYMV viral group was diversified mainly in some specific parts of the genome, especially in the CP region that is the most variable part of the viral genome (Shukla *et al*., 1988), whereas the NIb gene is very conservative. Therefore, the NIb gene is very useful in detecting a wide spectrum of BYMV isolates (Ward *et al*., 1992). Designing primers for the CP region is useful for finding differences between isolates, but it is not suitable in case of regular virus detection**.** It must be elucidated in following experiments, if the molecular analysis of 5'NIb-CP3' region of Czech BYMV isolates, which demonstrated their distribution into three groups, corresponds to the observed differences in pathogenicity symptoms. According to our knowledge, the sequence classification of the 3 Czech BYMV isolates into 3 groups represents the first description of these isolates on the molecular level.

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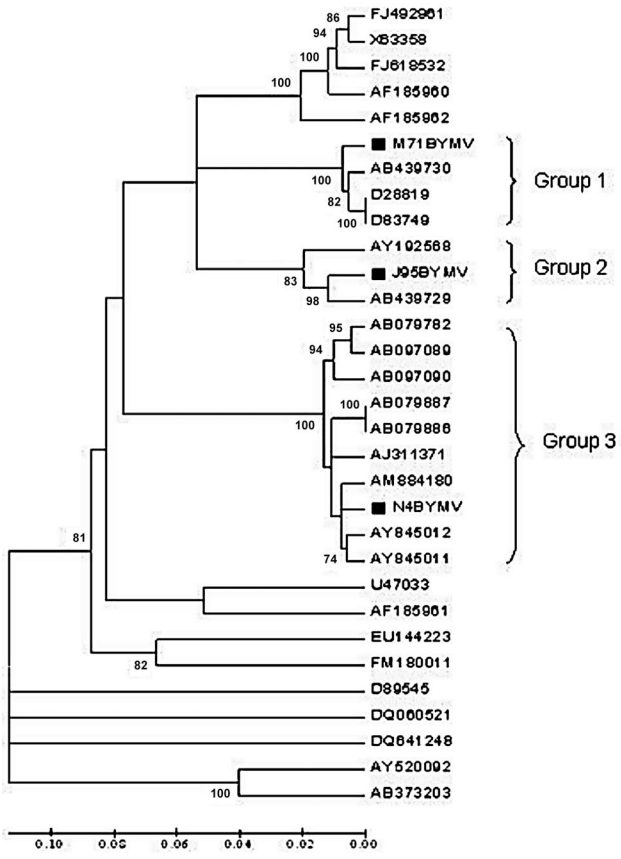

Fig. 1

Phylogenetic tree of BYMV isolates was constructed with 769 bp NIb-CP sequence and characterizes relationships between 3 Czech isolates (marked with black squares) and different isolates (GeneBank)

Bootstrap values >70% are shown next to the branches.

References

Alegria OM, Royer M, Bousalem M, Chatenet M, Peterschmitt M, Girard JC, Rott P (2003): Genetic diversity in the coat protein coding region of eighty-six sugarcane mosaic virus isolates from eight countries, particularly from Cameroon and Congo. Arch. Virol.148, 357–372. doi:10.1007/s00705-002-0916-1

Allison RF, Johnston RE, Dougherty WG (1986): The nucleotide sequence of the coding region of tobacco etch virus genomic RNA: evidence for the synthesis of a single polyprotein. Virology 154, 9–20 doi:10.1016/0042-6822(86)90425-3

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990): Basic local alignment search tool. J. Mol. Biol. 215, 403-410.

Andersen K, Johansen IE (1999): A single conserved amino acid in the coat protein gene of pea seed-borne mosaic potyvirus modulates the ability of the virus to move systematically in Chenopodium quinoa. Virology.241, 304–311. doi:10.1006/viro.1997.8967

Andrejeva J, Puurand U, Merits A, Rabenstein L, Jarvekulg L, Valkonen JP (1999): Potyvirus helper component-proteinase and coat protein (CP) have coordinated functions in virus -host interactions and the same CP motifs affects virus transmission and accumulation. J. Gen. Virol. 80, 1133–1139.

Anonymous (1997): Gene banks for 150 Gladiolus varieties developed. Agricultural News, Vol. III, No. 3, May–June, p. 139.

Clark MF, Adams AN (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34, 475-483 doi:10.1099/0022-1317-34-3-475 Castro S, Carazo G, Saiz M, Romero J, Blas CDe (1993): Use of enzymatic

cDNA amplification as a method of detection of bean yellow mosaic virus. Neth. J. Plant Pathol. 99, 97–100. doi:10.1007/BF01998477

Felsenstein J (1985): Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39, 783–791. doi:10.2307/2408678

Gadiou S, Dana Š, Milan N, (2008): Genetic variability of Plum pox virus isolates in the Czech Republic. Eur J Plant Pathol.121, 513–517. doi:10.1007/s10658-008-9272-7

Handley JA, Smith GR, Dale JL, Harding RM (1998): Sugarcane diversity in the coat protein coding region of twelve sugarcane mosaic potyvirus isolates from Australia, USA and South Africa. Arch. Virol. 143, 1145–1153. doi:10.1007/s007050050362

Hiroyuki Uga (2005): Use of crude sap for one-step RT-PCR based assays of Bean yellow mosaic virus and the utility of this protocol for various plant virus combinations. J. Gen. Plant Pathol. 71, 86–89. doi:10.1007/s10327-004-0166-z

Kaneshige H, Maeda T, Inouye N (1991): Host range and properties of bean yellow mosaic virus infecting crocus and serological relationships among three strains of BYMV. Nogaku Kennkyu 62, 225–240.

Lecoq H, Desbiez C, Delecolle B, Cohen S, Mansour A (2000): Cytological and molecular evidence that the whitefly-transmitted Cucumber vein yellowing virus is a tentative member of the family Potyviridae. J. Gen. Virol. 81, 2289–2293.

Magie RO, Poe SL (1972): Disease and pest associates of bulbs and plants In Koenig R, Crowley W (Eds): The World of Gladiolus. North American Gladiolus Council Inc., Edgewood Press, Maryland, pp. 155–167.

Mohammad A, Angela H (2004): A revised medium for rapid growth and bioassay with tissue cultures. Physiol. Plant 15, 257–332.

Mokra V, Gotzová B (1994): Collection of ornamental plants. Acta Horticulture 568, 193–199.

Oertel U, Schubert J, Fuchs E (1997): Sequence comparison of the 3'-terminal parts of the RNA of four German isolates of sugarcane mosaic potyvirus (SCMV). Arch. Virol. 142, 675–687. doi:10.1007/s007050050110

Pappu SS, Brand R,Pappu HR,Rybicki EP, Gough KH, Frenkel MJ, Niblett CL (1993). A polymerase chain reaction method adapted for selective amplification and cloning of 3' sequences of potyviral genome: application to Dasheen mosaic virus. J. Virol. Methods 41, 9–20. doi:10.1016/0166-0934(93)90158-N

Rosner A, Stein A, Levy S, Lilien-Kipni H (1994): Evaluation of linked PCR-tran scription amplification procedure for bean yellow mosaic virus detection in gladioli. J. Virol. Methods 47, 227–235. doi:10.1016/0166-0934(94)90080-9

Rybicki EP, Shukla DD (1992): Coat protein phylogeny and systematic of potyviruses. Arch. Virol. 5, 139–170.

Sasya T, Nozu Y, Koganezawa H (1998): Biological and serological comparisons of Bean yellow mosaic virus isolates in Japan. Phytopathol. Soc. Jpn. 64, 24–33.

Shukla DD, Strike PM, Tracy SL, Gough KH, Ward CW (1988): The N and C terminal of the coat protein Potyviruses are surface located and the N-terminus contains major virus specific epitopes. J. Gen. Virol. 69, 1497-1508. doi:10.1099/0022-1317-69-7-1497

Shukla DD, Strike PM, Tracy SL, Gough KH, Ward CW (1998): The N-and C-termini of the coat proteins of potyviruses are surface located and the N-terminus contains the major virus specific epitopes. J. Gen. Virol. 69, 1497–1508. doi:10.1099/0022-1317-69-7-1497

Tamura K, Dudley J, Nei M, Kumar S (2007): Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599. doi:10.1093/molbev/msm092

Takezaki N, Rzhetsky A, Nei M (2004): Phylogenetic test of the molecular clock and linearized trees. Mol. Biol. Evol. 12, 823–833.

Tscuhzaki T, Goto T, Fujisawa I, Yoshida K (1981): Virus disease occurring on legumes and vegetables in Hokkaido. Res. Bull. Hokkaido Natl. Agric Exp. Stn. 131, 71–93.

Ward CW, McKern NM, Frenkel MJ, Shukla DD (1992): Sequences data as the major criterion for potyvirus classification. Arch. Virol. (Suppl.) 5, 283–297.

Wada Y, Iwai H, Ogawa Y, Arai K (2000): Pathogencity and serological properties of Bean yellow mosaic virus isolates from gladiolus in Kagoshima prefecture. Jpn. J. Phytopathol. 66, 44–48.

Zaidi AA, Ram R, Zaidi SNH, Mukherjee D (1993): Diagnosis of viruses in some ornamental plants with special reference to serological methods: new developments. Indian Rev. Life Sci.13, 157–174.