First Report on Pea Seed-borne Mosaic Virus in Hungary

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One of the most important diseases of pea is caused by *Pea seed-borne mosaic virus* (PSbMV), which has a relatively wide host range. Since there are few varieties with resistance against the virus, and spraying insecticides is not very effective, the determination of the disease and the pathogen in the seeds is very important. Inoculum prepared from pea seeds showing typical virus symptoms caused very mild symptoms on *Chenopodium amaranticolor* and *C. quinoa*, but several chlorotic/necrotic lesions on bean (*Phaseolus vulgaris*) cv. Scarlet, and systemic symptoms with mosaic and curling of top leaves on bean cv. Maxidor. The detection of the virus was carried out by PCR using universal primers and virus sequence analysis. According to the phylogenetic analysis the PSbMV isolate identified in Hungary belongs to the pathotype P1 and associated with the cluster 2 isolates.

Keywords: Pea, bean, test plants, PCR, universal primer, virus sequence analysis.

PSbMV is a worldwide distributed, economically important seed-borne potyvirus, which infects pea and many other legume crops (Makkouk et al., 2012). In addition to infected pea seeds the virus is spread by aphid vectors in a non-persistent manner, but can infect also by mechanical inoculation (wind). Although PSbMV can cause significant yield and quality losses in pea, the effect on crop growth and yield is often missed as leaf symptoms are difficult to detect. Leaf symptoms include faint mottle and downward rolling and affected plants show mild stunting but the leaves sometimes remain completely asymptomatic. The discoloration and the reduced size of the seed are also characteristic features of PSbMV infection (Congdon et al., 2017).

The control of the disease raises some questions as well. Although there are efforts for breeding resistant genotypes worldwide, currently there are only few resistant varieties available (Van Leur et al., 2013a; Congdon et al. 2016). In addition, spraying insecticides is unlikely to be of real benefit because insecticides do not act fast enough to prevent the rapid spread of the virus by aphids. An important point in the control of disease is the use of healthy seed from virus-tested seed stock (Smýkal et al., 2010). Therefore a first step in the effective control of the disease is the determination of the disease and the pathogen in the seeds (Giakountis et al., 2015; Gheshlaghi et al., 2019; Van Leur et al., 2013b).

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In the Vegetable Research Institute, Kecskemét on pea breeding fields diseased plants were observed with symptoms suggesting PSbMV infection. In our study we have proved the infection of PSbMV, and to our best knowledge this is the first report on the identification of PSbMV in Hungary.

Materials and Methods

Plant material, virus source and germination of seeds

Visibly healthy and virus infected pea seeds (*Pisum sativum*, genotypes CS7, CS8 and AS8) were provided by Kinga Hütter (Vegetable Research Institute, Kecskemét) and were planted in pots and kept in greenhouse under normal conditions. In another experiment pea seeds were germinated in Petri dishes on wet filter paper at room temperature.

As test plants *Chenopodium amaranticolor* and *C. quinoa*, as well as different bean (*Phaseolus vulgaris*) varieties were inoculated.

For mechanical inoculations, 0.1 g of leaf material from virus-infected pea plants was ground in 25 ml of 0.1 M phosphate buffer, pH 7.2, and the infective sap was rubbed onto leaves without abrasive. The symptoms were evaluated successively.

Detection of the virus with universal primers

Total nucleic acid was extracted from 50 mg fresh pea leaves with the method described by White and Kaper (1989). First strand cDNA was synthesized with RevertAid First Strand cDNA Synthesis Kit (ThermoScientific), with poly T2 reverse primer (5'-CG-GGGATCCTCGAGAAGCTTTTTTTTTTTTTTTT-3') and the PCR amplification of the C terminal region of the NIb, the complete coat protein and the complete 3'UTR region was carried out with universal potyvirus forward primer poty7941 (5'-GGAATCCCCG-CGGNAAYAAAYAGYGGNCARC C-3') and poly T2 primers according to Salamon and Palkovics (2005). The PCR product was detected in 1.5% agarose gel electrophoresis in standard manner. The PCR products about 1600 bp length were excised and purified with High Pure PCR Product Purification Kit (ROCHE) then cloned into pGEM®-T Easy vector (Promega). The nucleotide sequences were determined (BIOMI) and deposited in the GeneBank.

Virus sequence analysis

The 1658 bp nucleotide sequence of the 5' terminal (Nter) 5' NIb-CP-UTR3' region of the Hungarian isolate was aligned with 12 other PSbMV sequences available from the GenBank (Table 1) using the ClustalW algorithm. Phylogenetic tree was composed by Maximum Likelihood method with 1000 bootstrap replicates using Tamura-Nei model (Tamura and Nei, 1993) in MEGA X (Kumar et al., 2018).

Table 1

GenBank accession numbers of the PSbMV strains selected in this study for phylogenetic analysis

Strain	Origin	Pathotype	GenBank accession number
Kreis Stormarn_18	Germany	unknown	MN399737
PSB194CZ	Czech Republic	P1	MK116871
US	USA	P1	AF127768
DPD1	Denmark	P1	D10930
L1	Denmark	P2	AJ252242
CAN (PV0316)	Canada	P1	Z48508
PK9	Pakistan	P1	AF127769
NZ	New Zealand	P1	D10453
PSB178CZ	Czech Republic	P1	EU293761
GER	Germany	not published	Z48509
PSB117CZ	Czech Republic	P1	EU293758
PSB58CZ	Czech Republic	P4	EU293765

Results

Symptoms on seeds and plants

The supposedly virus infected seeds showed necrotic rings and line patterns on the seed coat, malformation, splitting and reduced seed size (Fig. 1). The infected seeds germinated less percent than the healthy ones, and produced somewhat smaller seedlings compared to the healthy seedlings (data not provided). Otherwise no or very mild symptoms were detected on the virus infected plants.

Symptoms on test plants

On *Chenopodium amaranticolor* and *C. quinoa* plants chlorotic lesions developed, but just one or two lesions per leaf were observed. On *Nicotiana tabacum* L. cv Xanthi and on *Nicotiana benthamiana* plants symptoms were not detected even three weeks after the inoculation.

Fig. 1. Symptoms on virus infected pea seeds as compared to the control seeds

However, on the leaves of bean cv. Scarlet large number of chlorotic/necrotic lesions developed (Fig. 2A.). On the other hand, on bean cv. Maxidor strong systemic symptoms with mosaic and curling of top leaves were detected (Fig. 2B).

Fig 2. Virus symptoms on different bean cultivars (A): chlorotic local lesions on leaves cv. Scarlet (B) systemic symptoms on cv. Maxidor

Detection of the virus with universal primers and virus sequence analysis

The 5' region of the NIb, the complete CP and the 3' UTR region of the analyzed isolate was amplified using RT-PCR. The amplification with universal primers described by Salamon and Palkovics (2005) was successful resulting an approximately 1700 nt long fragment. The fragment was cloned and sequenced successfully. The identity of the cloned potyvirus was determined as PSbMV according to the BLAST algorithm in GenBank sequences (Table 1). The highest identity, 99.22% was detected with an unpublished PSbMV strain (MN399737) collected in Germany in 2018 proving the identification of PSbMV in Hungary. The determined nucleotide sequence of the Hungarian isolate was deposited in GenBank (Acc. No.: MT951241).

Fig.3. Phylogenetic tree based on the nucleotide sequences of the 5' terminal of the Nib-CP-UTR3' region. Maximum likelihood tree with 1000 bootstrap replicates was composed of PSbMV strains retrieved from GenBank and the newly isolated Hungarian strain (PSBMV HUN)

Phylogenetic analysis based on the nucleotide sequences of the 5' terminal (Nter) encompass the 1100 bp 5' Nib-CP-UTR3' region was carried out (Fig. 3). This analysis involved the nucleotide sequences of pathotype 1 (P1) isolates including cluster 1 (PSB117CZ, NZ, DPD1, PK9) and cluster 2 (PSB178CZ, CAN, US), cluster 3 contains the pathotype 2 strain (L1) and cluster 4 involves pathotype 4 (PSB58CZ) isolates were also represented. According to the phylogenetic analysis the PSbMV isolate identified in Hungary belongs to the pathotype P1 and associated with the cluster 2 isolates. The origin of the isolates in this cluster is considerably diverse, originated from the USA, Canada and also from Europe namely from Czech Republic and Germany.

The isolates from Czech Republic show high diversity since they were located in three significantly distant cluster (1, 2 and 4) (Šafářová, et al., 2008) and even a recombinant isolate was detected (Navrátil and Šafářová, 2019). In this respect regarding the close geographical location further analysis of the PSbMV isolates in Hungary would be of primary interest especially in connection with resistance breeding (Wylie et al., 2011).

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