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Short communication

Characterization of *Plum pox virus* PPV-BT-H isolated from naturally infected blackthorn (*Prunus spinosa* L.) in Hungary

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Abstract

Plum pox virus (PPV) was found naturally infecting blackthorn (*Prunus spinosa* L.) plants in different regions in Hungary. The virus was identified on the basis of reactions with indicator plants, by DAS–ELISA tests and by RT–PCR. PPV isolated from blackthorn (PPV-BT-H) caused yellow lesions with a necrotic centre on *Chenopodium foetidum* L. indicating that it belongs to the intermediate pathotype. The coat protein gene of the blackthorn isolate was cloned, sequenced and compared with other PPV sequences. The BT-H isolate and the Hungarian plum isolate SK68 belong to different subclusters of the M group in contrast to the Hungarian almond isolate (PA) which belongs to the D group. Detecting PPV on blackthorn demonstrated that this plant may be an important source and reservoir for PPV in Hungary.

Plum pox virus (PPV) is an aphid-borne, filamentous plant virus belonging to the genus *Potyvirus* of the family *Potyviridae*. PPV is known as a devastating pathogen of stone fruit trees in Europe infecting plums, peaches and apricot (Németh, 1986). More recently PPV has been reported occurring on sour cherry (Kalashyan and Bilkely, 1989; Nemchinov et al., 1996), sweet cherry (Crescenzi et al., 1994), walnut (Baumgartnerova, 1996) and almond (Pribék and Gáborjányi, 1997). In the past ten years a global distribution of PPV has been established in stone fruits since the virus was reported in Africa (Wetzel et al., 1991), South and North America (Acuna, 1993; Candresse et al., 1998a; Milius, 1999; Damsteegt et al., 2001) and India (Thakur et al., 1994).

PPV is maintained mainly in infected trees of orchards and/or their residuals from which it is transmitted by flying aphids. Although PPV has a relatively wide experimental host range, wild plants have not yet been studied intensively as natural reservoirs and potential sources.

Natural infection of blackthorn (*Prunus spinosa* L.) by PPV was reported in Yugoslavia (Jordovic et al., 1971), and then in the Czech Republic (Polák, 1998), but these PPV strains were neither characterized serologically nor by nucleotide sequence analysis.

PPV has been grouped into a number of pathological strains and serotypes. Kerlan and Dunez (1979) described two major serological groups: PPV-M and PPV-D, and the M group was further divided into two subclusters (Myrta et al., 2001). Nine general and serotype D specific epitopes have been positioned on the *E. coli* expressed amino terminal part of PPV coat protein (Candresse et al., 1998b). Immuno assay with monoclonal antibodies, PCR tests and RFLP-based detection was also compared, and an excellent correlation was found between the different techniques (Candresse et al., 1998a). A simultaneous PCR detection method was also developed to differentiate between the major PPV types: D, M, EA and C (Szemes et al., 2001). Several Hungarian isolates were investigated by ELISA and the existence of both

serotypes in Hungary was demonstrated (López-Moya et al., 1997). The nucleotide sequence of two Hungarian isolates is known; the SK68 isolate from plum belongs to the M group (Palkovics et al., 1993), while the PA isolate from almond was classified as a member of D group (Pribék et al., 2001).

A number of *P. spinosa* bushes growing on roadside bush-forest along agricultural fields in different regions of Hungary were surveyed for the symptoms of virus infection. About 10% of the blackthorn plants were found to be affected by mosaic and/or chlorotic ringspot symptoms (Figure 1A). Leaves of *P. spinosa* showing mosaic symptoms were collected. For pathological studies PPV was mechanically transmitted from selected blackthorns to test plants. Inocula were prepared by grinding the leaves of blackthorn in 1/15 M phosphate buffer (pH = 7.0) containing 2% PEG 6000 and 1 mg/ml active charcoal.

PPV was detected by DAS–ELISA tests using Bioreba PPV diagnostic kits. PPV was detected in each leaf sample of diseased *P. spinosa*. The absorbance values varied greatly. In some cases the virus concentrations in blackthorn leaves were similar to the leaves of *N. clevelandii* test plants infected by reference isolates (data not shown). Polák (1998) also showed that PPV could reach high concentrations in blackthorn flowers. PPV was successfully transmitted from selected blackthorn bushes to test plants. It is known that PPV isolates induce different symptoms on *Chenopodium foetidum* L. i.e.: yellow, necrotic, and yellow lesions with a necrotic centre, the latter called an intermediate pathotype (Németh, 1986). PPV-BT-H from blackthorn belongs to the intermediate pathotype based on the symptoms induced on *C. foetidum* (Figure 1B).

The presence of PPV in diseased *P. spinosa* was further confirmed by RT–PCR. Twenty-five milligrams (two small leaf disc) of blackthorn leaf tissue was homogenized in 600 µl 1× RT buffer (Amersham). After a brief spin in an Eppendorf centrifuge 1 µl supernatant was used for reverse transcription in a 10 µl RT reaction mix using universal PolyT primer. PCR amplification of cDNA was carried out using 2 µl out of 10 µl RT reaction mix with Unipoty and PolyT primers according to Deborré et al. (1995). PCR products were ligated into pBSK+ vector (Stratagene), *E. coli* DH5α cells were transformed and the cloned plasmids with inserts sequenced.

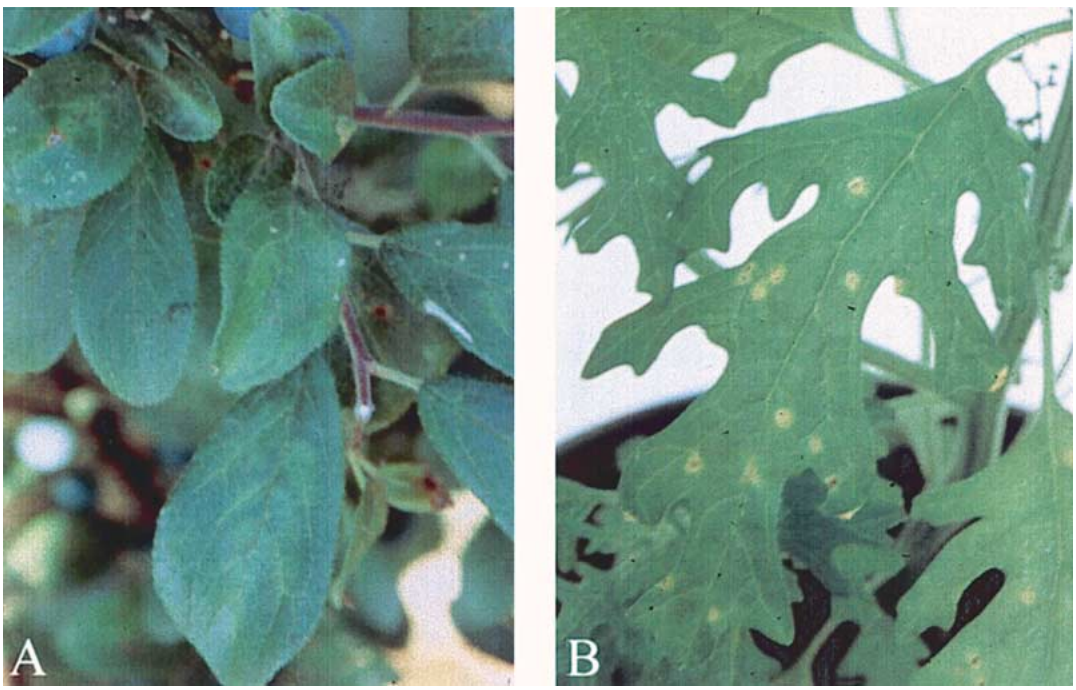

Figure 1. A. Mosaic and/or chlorotic ringspot symptoms on blackthorn (*Prunus spinosa* L.). B. BT-H isolate of *Plum pox virus* caused yellow local lesions with necrotic centre on *Chenopodium foetidum* L.

Data on a blackthorn isolate of PPV (PPV-BT-H, Acc. No.: AJ306420) was compared with other characterized Hungarian PPV isolates and PPV sequences available from the data bank. The nucleotide sequence data of the CP gene demonstrate that PPV-BT-H can be classified as a member of the M group of PPV. Even though a limited number of nucleotide sequence data is available for the M group, according to a phylogenetic analysis based on the amino acid sequence of the coat protein, it can be divided into two subclusters (Figure 2). This result is in good agreement with the serological results of Myrta et al. (2001) who recently described the two distinct subclusters called PPV-M1 and PPV-M2. Among other strains, 34 Hungarian isolates were examined from which 12 isolates were determined as members of the D group, while 22 belonged to the M2 group, among them strain SK68 (Myrta pers. comm.): not one was identified as the member of the M1 group. The Hungarian isolates BT-H and SK68, based on our sequence data, belong to two different subclusters of M group, while PA isolate is classified as a member of D group (Figure 2). The main differences between these isolates were found in the amino-terminal region of the coat protein. Focusing on the members of the M group, seven amino acid positions were found which could differentiate between the different M subclusters. In the case of the BT-H and the SK68 isolates these were amino acids 14, 38, 42, 58, 62, 68 and 70. Among these seven amino acids, two are able to differentiate between the M subclusters and the D serotype too (aa: 58, 68) (Figure 3). Using infectious recombinant clones it should be possible to identify the exact amino acid positions of the epitopes targeted by the MAbs that differentiate between M1 and M2 subclusters.

Natural infection of blackthorn by PPV is important from an epidemiological point of view. *P. spinosa*, as a woody perennial, is a reservoir host of the virus. It is also known to be an over-wintering host of the aphids *Phorodon humuli* and *Brachycaudus helicrysi* which are known vectors of PPV (Németh, 1986). Therefore, blackthorn has the potential to act as an important primary source of PPV. Although PPV-BT-H differed from all of the Hungarian reference strains of the virus, its CP nucleotide sequence is very close to an apricot isolate found in Slovakia (Glasa et al., 2001). The molecular variability of PPV occurring on blackthorn however needs further characterization.

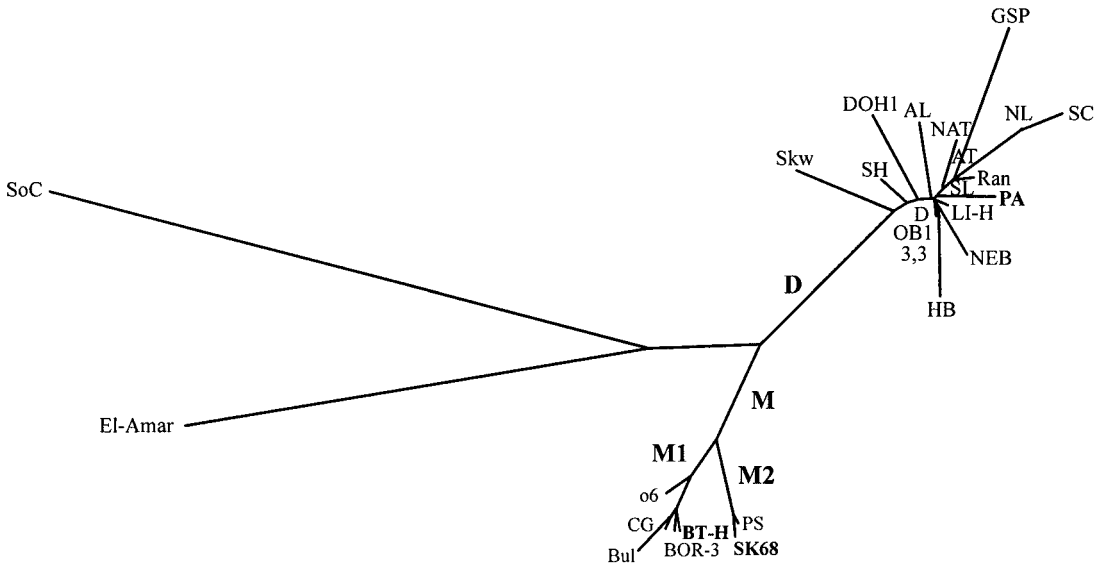

Figure 2. Phylogenetic tree of PPV isolates derived using the Wisconsin Package Version 9.1., Genetics Computer Group (GCG), Madison, Wisc. from the amino acid sequence of coat protein. The following isolates were analysed: 3,3 (AF172346); AL (X81078); AT (X57975); BOR-3 (AY028309); **BT-H** (AJ306420); Bul (X57976); CG (X81082); D (X16415); DOH1 (X81084); El-Amar (X56258); GSP (X81080); HB (X81076); LI-H (X81081); NAT (D13751); NEB (X81075); NL (X81074); OB1 (X81077); **PA** (AJ000340); PS (S57405); Ran (M21847); SC (X81083); SH (X81073); **SK68** (M92280); Skw (U27652); SL (X81079); SoC (X97398); o6 (S57404). Bold letters indicate Hungarian isolates.

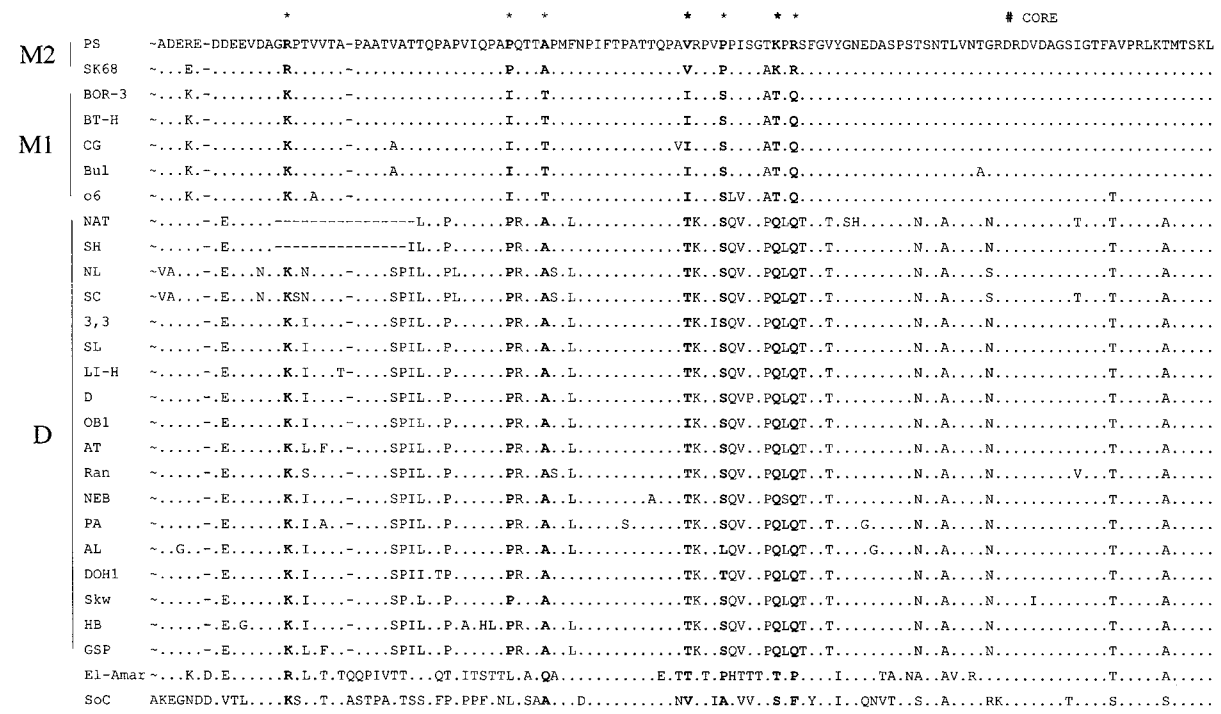

Figure 3. Alignment of the amino-terminal part of the coat protein sequences showing differences, generated by BoxShade Program Version 3.21. The asterix and amino acids with bold letters shows the seven amino acid positions which could differentiate between the different M subclusters, and two of them labelled bold asterix are able to differentiate between the M subclusters and the D serotype too. Double cross indicates the first amino acid of the core region of the coat protein.

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