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PHYLOGENETIC ANALYSIS OF PDV MOVEMENT PROTEIN COMPARED TO BROMOVIRIDAE MEMBERS AS JUSTIFICATION OF POSSIBLE INTERCELLULAR MOVEMENT

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Prune dwarf virus (PDV) is a member of the Ilarvirus genus which is widely spread all over the world and causes considerable economic losses in nurseries and orchards. The virus is transmitted via seeds and pollen and through vegetative reproduction. However, the mechanisms of cell-to-cell and systemic transport of the virus are still not known. For the first time this study presents phylogenetic characterization of the movement protein (MP) of PDV isolates from the GenBank database in the context of geographic origin. The prepared analyses were based on a comparison of the whole amino acid sequence of the MP-PDV, the RNA- binding domain (RBD) in MP of PDV and MPs of four viruses from the Bromoviridae family with known transport mechanisms. Two different bioinformatic programs ClustalW and Jalview were used, and MP sequence variability up to 8% at the amino acid level among PDV isolates was confirmed. In the constructed phylogenetic trees the isolate sequences clustered in three conserved groups. Further analyses revealed similarity of the MP amino acid sequence of PDV and Alfalfa mosaic virus (AMV) of up to 34% and a 40% similarity of RBD between these viruses which suggested that the PDV transport mechanism may be on some level the same as that for AMV.

Key words: PDV, movement protein, amino acid sequence, phylogenetic analyses

INTRODUCTION

Prune dwarf virus (PDV) belongs to the family *Bromoviridae* and to subgroup 4 of the genus *Ilarvirus* (Bujarski et al., 2011). This pathogen infects plum, apricot, peach and sweet cherry trees (Brunt et al.,1996; Sala-Rejczak and Paduch-Cichal, 2005; Milusheva and Borisova, 2005). It is widely known in European countries and also it is found in Australia, Canada, Egypt, New Zealand, USA and Turkey (Segdgley and Collins, 2002;

Sala-Rejczak and Paduch-Cichal, 2005; Ulubas-Serce et al., 2009; Pallas et al., 2012). Fulton (1971) classified PDV into a group of plant viruses with a global range. PDV strains and isolates are highly diversified when it comes to infectivity, antigenic properties, and the RNA3 nucleotide sequence, as well as the amino acid sequence of the capsid protein and the movement protein (Topachiiska, 1983; Paduch-Cichal et al., 2011). Nemeth (1972, 1986) reported that individual strains of the virus cause various diseases, e.g. cherry chlorotic ringspot, cherry chlorotic necrotic ringspot, cherry ring mosaic, cherry ring mottle, cherry yellow mosaic, chlorotic-necrotic ringspot and apricot gum flow.

The genome of the virus is divided into three (+) ssRNA single strands, referred to as: RNA1 (3.4 kb), RNA2 (3.1 kb), and RNA3 (2.2 kb) and a subgenomic molecule known as RNA4 (1.0 kb) synthesized during the viral replication process (Bujarski et al., 2011; Fulton, 1983; Kawakami et al., 2004). RNA1 is a monocistronic molecule of nucleic acid that has a single open reading frame (ORF), marked as ORF1a, which encodes protein P1. The P1 protein has two domains. The first one is a methyltransferase-like domain.

(Rozanov et al., 1992; Pallas et al., 2012), the other domain has the activity of helicase (Korolev et al., 1997; Ramptish and Estwell, 1997). P1 protein is implicated in viral RNA recruitment and anchoring to the vacuolar membrane where replication complexes are assembled (Bol, 2005). RNA2 is a nucleic acid monocistronic molecule (Bujarski et al., 2011). It has one open reading frame, marked as

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ORF2. The ORF 2 encodes the P2 protein. The P2 protein is an RNA-dependent RNA polymerase, which – together with the P1 protein encoded by RNA1 – participates in the synthesis of the viral RNA (Codoner et al., 2005). P1 and P2 proteins are considered the subunits of the viral replicase complex (Bol, 2005). RNA3 is a polycistronic nucleic acid molecule. It contains two open reading frames separated by an intergenic region. Located closer to the 5' end of RNA3 is ORF3a, which encodes the movement protein (MP), belongs to the "30K superfamily" (Kasteel et al., 1997; Mekuria et al., 2003; Pallas et al., 2012) and supports the viral cell-to-cell movement The MPs of this family are structurally characterized by the presence of a hydrophobic motif (HR) (Melcher, 2000). This motif is highly conserved in the family *Bromoviridae* (Codoner et al., 2005, Sanchez-Navarro and Pallas, 1997) and is proceeded by a conserved RNA-binding domain (RBD) (Herranz and Pallas, 2004), which is located between 56 to 85 residues (Fiore et al., 2008; Herranz et al., 2005). This domain may support viral RNA transport. MP translation proceeds directly from RNA3. The other open reading frame, ORF3b, is more proximal to the 3' end of RNA3 and it encodes the coat protein (CP). CP translation takes place with participation of the nucleic acid subgenomic molecule marked as RNA4 or sgRNA4 (subgenomic ribonucleic acid 4) (Ramptish and Estewell, 1997).

MP sequence similarity to the "30K superfamily" indicates its involvement in virus cell-to-cell transport (Codoner et al., 2005). Researchers are still not able to explain the mechanism of PDV transport. Several different mechanisms of viral transport are known in the *Bromoviridae* family (Van der Vossen et al., 1994; Flasiński et al., 1995). Sanchez-Navarro et al. (2005) showed that movement protein of *Prunus necrotic ring spot virus* (PNRSV, genus *Ilarvirus*), related to MP PDV can support cell-to-cell transport of modified *Alfalfa mosaic virus* (AMV). AMV particles with RNA3, which had inserted sequence of MP PNRSV were able to support the cell-to-cell transport. In addition, the C-terminus region of PNRSV MP is required for the interaction with the cognate CP and is implicated in its targeting to the plasmodesmata (Aparicio et al., 2010). Because of such a correlation between PNRSV and AMV, the aim of this study was to find a pattern by use of the bioinformatic analyses of MP sequences and RBD domains in PDV with comparison to the *Bromoviridae* family represented by the following viruses: *Alfalfa mosaic virus* (AMV), *Brome mosaic virus* (BMV), *Cucumber mosaic virus* (CMV) and *Cowpea chlorotic mottle virus* (CCMV). Our investigation considered that the pattern of MP amino acid sequences similarity indicates possible cell-to-cell transport in correlation with the MP function.

MATERIAL AND METHODS

The reference material comprised 18 isolates of the *Prune dwarf virus* (PDV), where a complete amino acid of the MP was published in the GenBank in 2013 (Table 1), and the sequences of the strains of: members of *Bromoviridae* with known viral transport mechanism: AMV, BMV, CMV and CCMV (http://www.ncbi.nml.nih.gov/genbank/).

The amino acid sequences of the MP and RBD (residues 56–85 in sequence) of these virus strains were compared with Clustal W software (http://www.ebi.ac.uk/Tools/clustalw/) using the Clustal W Multiple Sequence Alignment method (Larkin et al., 2007) with default settings. At a later stage, these sequence isolates were subjected to further analyses using Jalview (http://www.ebi.ac.uk) software and were presented in the form of phylogenetic trees based on the % identity Neighbor Joining method (Troshin et al., 2011).

RESULTS

COMPARATIVE ANALYSIS OF PDV ISOLATES REGARDING AMINO ACID SEQUENCES OF THE MOVEMENT PROTEIN

Sampled from the NCBI database the amino acids sequences of the MP of the PDV isolates had identical lengths: 293 amino acid (aa). The analyses comparing the isolates of the *Prune dwarf virus* made it possible to establish the homology of the amino acid sequences, which ranged from 92% to 100% (the highest: 100% – strains: PDV-PA78 and PDV-SWM1, PDV-SOF17P17 and PDV-SOF15P11 and also PDV-SW63 and PDV-PA63, the lowest: 92.15% – strains: PDV-SWRegina and PDV-SW6-1, PDV-SWRegina and PDV-SOF17P17 and also PDV-SWRegina and PDV-SWI-35). All the studied sequences of the movement protein of PDV isolates differed by at least 0–23 aa but no more than 23 aa.

The phylogenetic analysis covering the sequence of the MP of the PDV isolates made it possible to classify them into three groups (Fig. 1).

Group I included five viral strains: PDV-PE247, PDV-0917, PDV-SO40, PDV-0599 and PDV-SWRegina. The homology between sequences of the MP was over 95% for these isolates. The highest homology of the amino acid sequence within this group is exhibited by isolates PDV-PE247 and PDV-0917 (100%), and the lowest (95.24%) – by isolates PDV-SO40 and PDV-SWRegina.

Group II included four strains of the virus: PDV-137, PDV-SW9-1, PDV-SW6-1 and PDV-SWI-35. The homology of the nucleotide sequences of the MP-encoding gene between those strains ranged from 96% to over 99% (the highest (99.32%) for iso-

TABLE 1. List of isolates of PDV deposited in NCBI GenBank

No.	PDV isolates	Host plant (species, cultivar)	Origin	Accession number (MP amino acid)	Deposited in GeneBank by
1	PDV-137	Pumpkin cv. unknown	USA	AAA46818.1	Bachman E.J., Scott S.W., Xin,G., Vance V.B.
2	PDV-0599	Plum cv. unknown	Poland	ADG65215.1	Malinowski T.
3	PDV-0917	Sweet cherry cv. unknown	Poland	ADG65214.1	Malinowski T.
4	PDV-SW9-1	Sweet cherry cv. unknown	USA*	ADG56786.1	Mroczkowska K., Malinowski T., Sala-Rejczak K., Paduch-Cichal E., Eastwell K.
5	PDV-SW6-1	Sweet cherry cv. unknown	Italy*	ADG56785.1	Mroczkowska K., Malinowski T., Sala-Rejczak K., Paduch-Cichal E., Myrta A.
6	PDV-SWRegina	Sweet cherry cv. Regina	Poland*	ADG56784.1	Mroczkowska K., Malinowski T., Sala-Rejczak K., Paduch-Cichal E.
7	PDV-SW63	Wild cherry F12/1 clone	Poland *	ADG56783.1	Mroczkowska K., Malinowski T., Sala-Rejczak K., Paduch-Cichal E.
8	PDV-SO14	Sour cherry cv. Kormed	Poland *	ADG56782.1	Mroczkowska K., Malinowski T., Sala-Rejczak K., Paduch-Cichal E.
9	PDV-SWI-35	Sweet cherry cv. unknown	Poland*	AGD94947.1	Mroczkowska K. and Paduch-Cichal E.
10	PDV-PE-15-28	Peach cv. unknown	Poland*	AGD94946.1	Mroczkowska K. and Paduch-Cichal E.
11	PDV-SO20SZ3	Sour cherry cv. Royal Burgundy	Poland*	AGD94944.1	Mroczkowska K. and Paduch-Cichal E.
12	PDV-PE247	Peach cv. Kwiat Majowy	Poland*	AFU49023.1	Mroczkowska K., Paduch-Cichal E. and Sala-Rejczak K.
13	PDV-SWM1	Sweet cherry cv. Napoleona	Poland*	AFU49022.1	Mroczkowska K., Paduch-Cichal E. and Sala-Rejczak K.
14	PDV-PA63	Wild cherry F12/1 clone	Poland*	AFU49021.1	Mroczkowska K., Paduch-Cichal E. and Sala-Rejczak K.
15	PDV-SOF15P11	Sour cherry cv. Nomene	Poland*	AFU49020.1	Mroczkowska K., Paduch-Cichal E. and Sala-Rejczak K.
16	PDV-PA78	Wild cherry F12/1 clone	Poland*	AFU49019.1	Mroczkowska K., Paduch-Cichal E. and Sala-Rejczak K.
17	PDV-SOF17P17	Sour cherry cv. Big Core	Poland*	AFU49018.1	Mroczkowska K., Paduch-Cichal E. and Sala-Rejczak K.
18	PDV-SO40	Sour cherry cv. Korund	Poland*	AGD94945.1	Mroczkowska K., Paduch-Cichal E.
19	BMV-CZ	Agropyron repens	Czech Republic	ADW09016.1	Gadiou S. and Kundu J.K.
20	BMV-M2	Barley	USA	BAD83846.1	De Jong W. and Ahlquist P.
21	AMV 15/64	unknown	Netherlands	AAD04694.1	Thole V., Miglino R. and Bol J.F.
22	AMV-VRU	unknown	Netherlands	AAD04692.1	Thole V., Miglino R. and Bol J.F.
23	CMV-Legume	Cucumber	Japan	BAA03888.1	Karasawa A., Ito,A., Okada I., Hase S. and Ehara Y.
24	CMV-E5	Cucumber	Japan	BAA07676.1	Karasawa A., Ito A., Okada I., Hase S. and Ehara Y.
25	CCMV	Cawopea sp.	Netherlands	NP_613276.1	Allison R.F., Janda M. and Ahlquist P.

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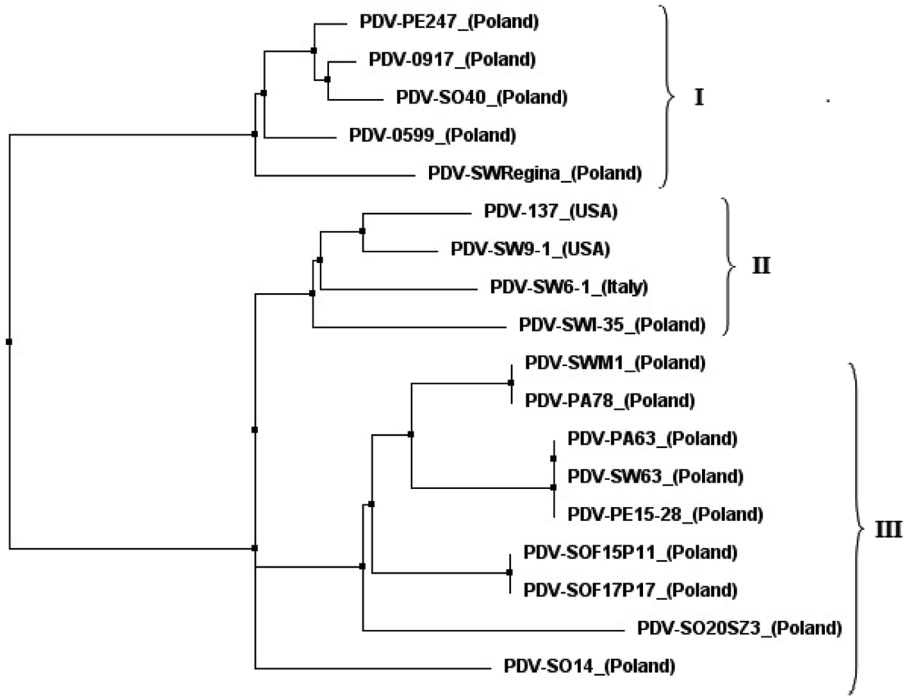

Fig. 1. Phylogenetic comparison of amino acid sequences of the MP for the studied PDV isolates: I-first group, II-second group, III-third group

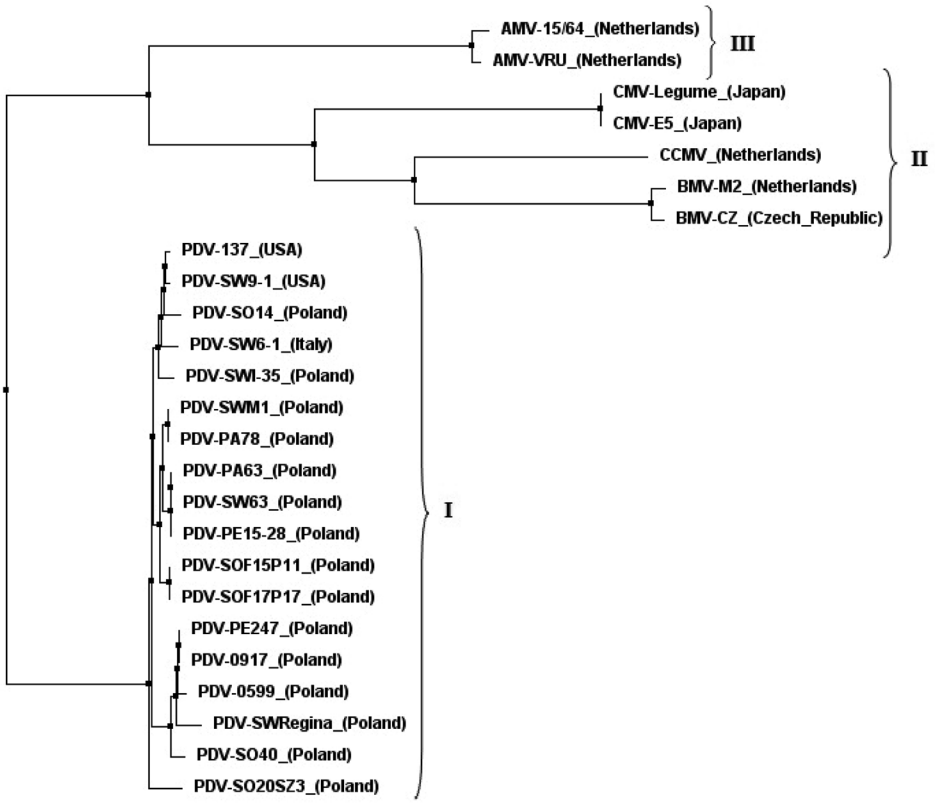

Fig. 2. Phylogenetic comparison of amino acid sequences of the MP for the studied groups of virus isolates: I – first group (PDV), II – second group (BMV, CCMV, CMV), III – third group (AMV)

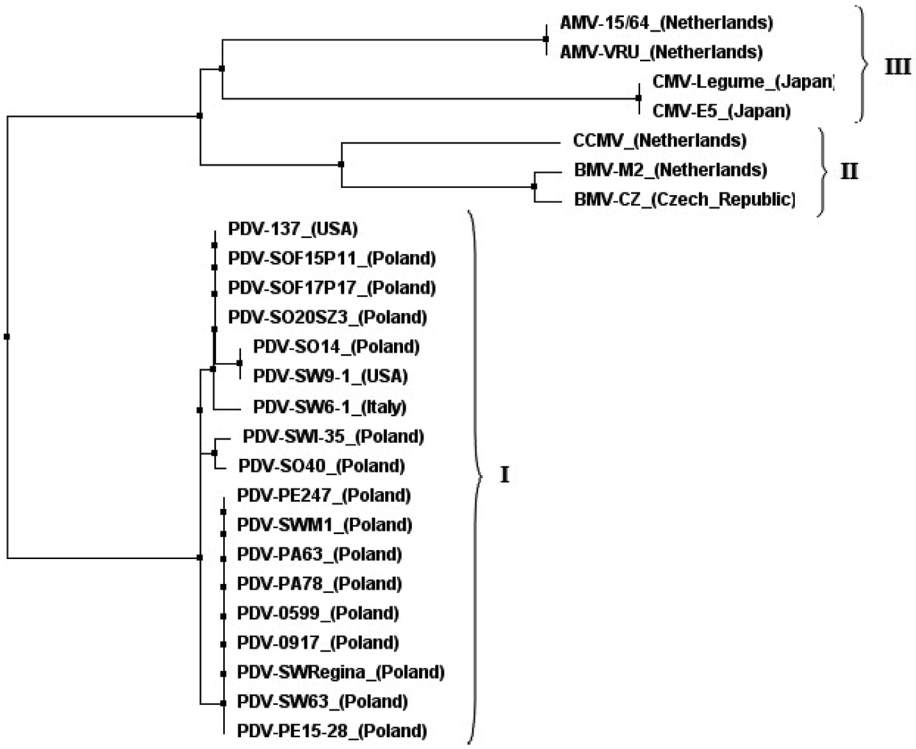

Fig. 3. Phylogenetic analysis of the RBD of the MP for the studied groups of virus isolates: I -first group (PDV), II – second group (BMV, CCMV, CMV), III – third group (AMV)

lates PDV-137 and PDV-SW9-1, the lowest (96.25%) for strains PDV-SWI-35 and PDV-SW6-1).

Group III contains nine isolates: PDV-SWM1, PDV-PA78, PDV-PA63, PDV-SW63, PDV-PE15-28, PDV-SOF15P11, PDV-SOF17P17, PDV-SO20SZ3, and PDV-SO14. The MP homology of MP between these isolates ranged from 94% to 100% (the highest 100% for example strains PDV-PA63 and PDV-PE15-28, the lowest 94.5%- for isolates PDV-SO20SZ3 and PDV-SO14).

COMPARATIVE ANALYSIS OF PDV, AMV, BMV, CCMV AND CMV ISOLATES REGARDING AMINO ACID SEQUENCES OF THE MOVEMENT PROTEIN

The length of the amino acid sequences of the movement protein of the viruses from the NCBI database ranged from 279 to 303 aa. The analysis made it possible to establish the homology of the sequence of MPs between PDV and AMV, BMV, CMV and CCMV strains; it ranged from 16% to 34% (the highest: 34% – for isolates PDV-137 and AMV-VRU, the lowest: 16.67% – for isolates PDV-SO20SZ3 and CMV-E5). All the sequences of the movement protein studied for PDV, AMV, BMV, CCMV, CMV differed by at least 197 aa but by no more than 240 aa. The phylogenetic analysis covering the MP sequences of the virus isolates made it possible to classify them into 3 groups (Fig. 2). The first group included 18 viral strains of PDV, with the 3 subgroups presented above. The second group contained strains of BMV, CMV and CCMV. The homology in this group ranged from 31% to 100% (the highest, 100% – for example strains CMV-Legume and CMV-E5, the lowest, 31% – for strains BMV-CZ and CMV-E5). The last group included only AMV strains: 15/64 and VRU with an amino acid sequence similarity level of 97%.

COMPARATIVE ANALYSIS OF THE RBD IN MOVEMENT PROTEIN PDV, AMV, BMV, CCMV AND CMV ISOLATES REGARDING AMINO ACID SEQUENCES

Comparative analyses of the *Bromoviridae* RBD between 56 and 85 aa (29 aa length) in movement protein amino acid sequences showed that homology ranged from 20% to 40%; so, the sequences differed by 17–23 aa. The highest homology was shown by isolates PDV-137, AMV-15/64 and AMV-VRU (40%), and the lowest by isolates PDV-137, CMV-Legume and also CMV-E5 (20%). The phylogenetic analysis covering the MP sequences of the virus isolates made it possible to classify them into 3 groups (Fig. 3). The first group contains PDV iso-

lates, and the second isolates of BMV and CCMV. In the third group there are the RBD sequences of AMV and CMV, which are the most similar to RBD PDV.

DISCUSSION

The results obtained in the present study concerning a comparison of the MP and RBD MP amino acid sequences among *Prune dwarf virus* demonstrate the existence of some level of differentiation. The MP amino acid sequences of MP differed by 4.55% on average, i.e. not more than 27 aa. These results are similar to the results presented by Paduch-Cichal (2000), who compared amino acid sequences of three PDV isolates differing in not more than in 20 positions, i.e. about 6.8%. The similarity of the results that we obtained demonstrates that MP amino acid sequences seem to be strongly conservative. Moreover amino acid sequence 56–85 residues (RBD) in MP have homology levels over 90% so this domain is also highly conservative in the studied sequences. In the identified groups, the third group was the most differentiated during the comparative analysis of MP amino acid sequences. The homology in MP amino acid sequences ranged from 96 to 99% (97% on average). The phylogenetic analysis of the amino acid carrier protein of PDV isolates conducted in this study, indicates a partial relationship between the geographical origin of PDV isolates and phylogenetic MP similarity. It appeared that in the first group (European) only isolates from the European continent are observed (Polish isolates: PDV-PE274, PDVSO40, PDV-0599, PDV-0917 and PDV-SWRegina). In the second group (mixed), four isolates from Europe (Italy: PDV-SW6-1, Poland: PDV-SWI-35) and North America (USA: PDV-137 and PDV-SW9-1) were noted. Probably, the first two European lines could have been separated, then one of the isolates from the second group, probably that from Italy (PDV-SW6-1 isolate) was transported with plants to the USA. This is confirmed by the evolutional distance between the PDV-SW6-1, and PDV-137 and PDV-SW9-1 isolates. Further changes within the sequence allowed for the formation of American isolates included in the mixed group together with European isolates. There is, however, a need for a larger number of MP sequences of PDV isolates to verify such a course of evolutional events. The studies by Scott et al. (1998), Vaskova et al. (2000), Hamond (2003) and Ulubas-Serce et al. (2009), which were based on comparative analysis of CP nucleotide sequences so far have excluded the existence of a relationship between isolate origin and phylogenetic similarity in the case of PDV. The conservativeness of MP and MP RBD amino acid sequences indicates the probably crucial role in PDV viral transport/infection. Many studies have shown that, in the infected plants, MPs are involved in the transport of viruses from cell to cell, either in the form of a virion or an RNP (ribonucleo protein) complex. According to intercellular transport forms, plant viruses could be classified into three groups. In the first one, the virus is transported in a non-virion (RNA-protein complex) form by the plasmodesmata of a plant cell (Tomenius et al., 1987; Lucas and Gilbertson, 1994). In this group we have two types of transport dependent on and independent of a capsid protein. The transport independent of the capsid protein is characteristic of *Tobacco mosaic virus* (TMV family *Virgaviridae*, genus *Tobamovirus*) and *Cowpea chlorotic mottle virus* (CCMV family *Bromoviridae* genus *Bromovirus*) (Herranz et al., 2005). The movement protein forms a complex with viral RNA (RNP complex-viral RNA and viral protein). MP causes an increase in the upper limit of plasmodesmata size (SEL) (Kawakami et al., 2004) and MP-RNA complexes are transported to the next cell protoplast (Wolf et al., 1989; Rao, 1997).

In the second group, both MP and CP are involved in viral transport (Kaplan and Palukatis, 1998). In this group we have two variants. The first is characteristic of *Cucumber mosaic virus* (CMV family *Bromoviridae*, genus *Cucumovirus*) (Schmitz and Rao, 1998). MP also causes an increase in SEL and therefore RNA-MP-CP viral complexes move from cell to cell. In the second variant, characteristic of *Cowpea mosaic virus* (CPMV, family *Secoviridae*, genus *Comovirus*) (Van Lent et al., 1990; Van Lent et al., 1991), *Alfalfa mosaic virus* (AMV, family *Bromoviridae*, genus *Alfamovirus*) (Flasiński et al., 1995; Rao and Grantham, 1995) and *Brome mosaic virus* (BMV, family *Bromoviridae*, genus *Bromovirus*) (Van der Vossen et al., 1994), the virus needs two proteins for intercellular transfer: MP and CP. The whole virion (Okinaka et al., 2001) is transported from cell to cell, CP proteins are used to bind it tubules connected to plasmodesmata. The movement protein is also localized in the tubules supporting virion transport inside the tubules. The third group includes *Potato virus* X (PVX, family *Alphaflexiviridae*, genus *Potexirus*), which requires CP for the transport; however, no integration with tubules has been noted (Cruz et al., 1998).

The results of the analysis conducted by Tzanedakis and Martin (2005) demonstrate that the *Prune dwarf virus* has the most similar MP sequence to *Fragaria chilonensis latent virus* (FCILV, family *Bromoviridae*, genus *Ilarvirus*), a virus also with an unknown viral transport mechanism. However, there was no homology of MP and

RNA binding domains together. Phylogenetic analyses of amino acid sequences of the PDV MP and MP RBD have shown that these sequences are more similar to AMV than to BMV, CMV and CCMV. Based on our results, the PDV mechanisms of cell-to-cell transport are probably similar to those of AMV. However, lack of studies concerning the precise mechanism of PDV transfer from cell to cell does not allow for an unequivocal classification. Moreover, van der Vossen et al. (1994) demonstrated that AMV with a mutant capsid protein (deletion of a considerable amount of C-terminal end of CP) does not form a virion structure, but maintains the ability to transfer to subsequent cell protoplasts. Flasiński et al. (1995) proved in turn that CP BMV mutants are able neither to encapsulate, nor to penetrate inside the cell through plasmodesmata. This may point to the existence of differences in the mechanism of virion transport within the *Bromoviridae* family (Dinant et al., 1993). Van der Vossen et al. (1994) and Rao and Grantham (1995) demonstrated that AMV and BMV in infected plant cells also exhibit the ability to induce tubules formation. Kasteel et al. (1997) claimed that the movement protein has a significant meaning in the stimulation of tubule formation, which seems to be confirmed in the research conducted in protoplasts by Jansen et al. (1998). Still, the mechanism of MP interaction with cell components requires further studies for a better understanding of the processes influencing pathological changes occurring in plant cells. It is possible that further studies will allow to discover new sources of resistance against PDV and more varieties, resistant to this virus will be cultivated.

CONCLUSION

Amino acid sequences of movement protein and MP-RNA binding domain of PDV are highly conservative – about 90% or higher, regardless of geographic origin of isolates. Comparative analyses of MP PDV sequences made it possible to classify isolates into three groups: two European and one mixed European-USA. The similarity between the amino acid sequences of the RBD/MP of AMV and PDV showed the important premise for the possible model of PDV transport in infected plants.

AUTHORS' CONTRIBIUTIONS

EK designed and did experiments, analyzed data and prepared the manuscript; KO analyzed data and prepared the manuscript; GG made suggestions to the manuscript. All authors declare no conflict of interest

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Program which creates phylogenetic trees – Jalview (http://www.ebi.ac.uk).