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QUARTERLY

Nucleotide sequence of the coat protein gene of the Skierniewice isolate of plum pox virus (PPV)*

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The coat protein (CP) gene of the Skierniewice isolate of plum pox virus (PPV-S) has been amplified using the reverse transcription — polymerase chain reaction (RT-PCR), cloned and sequenced. The nucleotide sequence of the gene and the deduced amino-acid sequence of PPV-S CP were compared with those of other PPV strains. The nucleotide sequence showed very high homology to most of the published sequences. The motif: Asp-Ala-Gly (DAG), important for the aphid transmissibility, was present in the amino-acid sequence. Our isolate did not react in ELISA with monoclonal antibodies MAb06 supposed to be specific for PPV-D.

The plum pox virus (PPV[1]) [1, 2], belonging to a large potyvirus group of plant viruses [3] (for review of potyvirus molecular biology see [4, 5]), is responsible for a severe disease (sharka) of plums, peaches and apricots. Its occurrence has been reported from many European countries as well as from Egypt [6]. In Poland it was first reported by Szczygieł [7] on plum. The presence of PPV in peaches and apricots, as well as the susceptibility of particular plum and peach cultivars to PPV were reported by Zawadzka [8] and Zawadzka & Malinowski [9]. Since PPV causes heavy losses in orchards, there is a great demand both for the methods of sensitive and reliable detection of infection and for obtaining virus resistant or tolerant cultivars producing high quality fruit. Unfortunately, conventional methods of breeding have so far failed to produce such cultivars. In the last years many reports on induction of the resistance to plant viruses in transgenic plants expressing viral coat proteins, have been published [10–12]. The initial step in construction of such plants is the isolation of the viral coat protein gene.

Particles of PPV contain plus-sense ssRNA of about 10 kb [13] coding for the polyprotein of M_r 354000. One of the cleavage products of this large protein is the coat protein (CP) gene of M_r 366000 [14]. The CP coding sequence is located at the 3′ end of its precursor gene.

The aim of our study was the construction of plums expressing the PPV CP gene, thus preparing a molecular probe for detection of PPV in plants, as well as a comparison of the Skierniewice isolate of PPV (PPV-S) with the PPV

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[1]Abbreviations: CP, coat protein; PPV, plum pox virus; RT-PCR, reverse transcriptase-polymerase chain reaction; PEG, polyethylene glycol.

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strains from other countries. In this paper we describe the isolation, cloning and sequencing of CP gene of PPV-S. We also discuss possible application of the restriction fragment length polymorphism (RFLP) for characterisation of the PPV strains.

MATERIALS AND METHODS

PPV isolation and purification. The plum pox virus (PPV-S) was isolated from the plum Dąbrowicka Prune grown near Skierniewice (central part of Poland). The culture of the virus was obtained by making repeated inoculations from single local lesions developed in *Chenopodium foetidum*. PPV was then propagated in pea. Virus particles were purified as follows. Infected pea leaves were homogenised in a mixture of 0.1 M Tris/thioglycolate buffer, pH 8.6, chloroform and carbon tetrachloride (100 g of leaves : 200 ml of buffer : 60 ml of $CHCl_3$: 60 ml CCl_4). The homogenate was centrifuged in glass tubes for 25 min at 3200 r.p.m. and the aqueous phase was collected and clarified (10 min at 10000 r.p.m.). Virus was precipitated by the addition of polyethylene glycol (PEG) 6000 and sodium chloride (4 g PEG 6000, 1.75 g NaCl/100 ml) followed by centrifugation (10 min at 10000 r.p.m.). Pellet was resuspended in 0.05 M borate buffer, pH 8.2, overnight. This mixture was clarified (10 min at 10000 r.p.m.) and virus was concentrated by centrifugation (60 min at 39000 r.p.m.). After the clarification in Eppendorf tubes (3 min at 12000 r.p.m.) the crude preparation was layered over 10%–40% sucrose density gradient and centrifuged in SW28 rotor at 27000 r.p.m. for 180 min. The fraction containing virus particles was diluted at least 3-fold with the borate buffer and centrifuged (45000 r.p.m. for 60 min). The final pellet was resuspended in borate buffer. Concentration of PPV particles in the solution was calculated from the UV absorption value at 260 nm, assuming that 0.1% solution of the virus gives $A_{260} = 3.0$ [15]. If the preparation was not used immediately, it was mixed with glycerol (1:1) and stored at –20°C.

Estimation of serotype. Serotyping of our isolate was made using the monoclonal antibodies MAb05 and MAb06 [16, 17] and positive controls PPV-W and PPV-A kindly given to us by Dr. Navratil. PPV-W and PPV-A represent serotype PPV-D and PPV-M [18], respectively. MAb05 reacts with PPV of both serotypes PPV-D and PPV-M. MAb06 reacts with PPV-D but does not recognise PPV-M. DAS-ELISA tests were carried out according to a standard procedure [19].

RNA extraction from virus particles. Purified virus particles (about 1 µg) were suspended in proteinase K solution (0.1 M Tris/Cl, pH 7.4, 50 mM NaCl, 10 mM EDTA, 0.2% (w/v) SDS, 200 µg/ml proteinase K), incubated for 2 h at 37°C, shaken for 10 min with the equal volume of phenol-chloroform mixture (500 g of phenol, 0.5 g 8-hydroxyquinolinone, 150 ml water mixed 1:1 with chloroform) and centrifuged for 10 min at 4°C. The aqueous phase was reextracted with the same mixture until no more protein appeared at the interphase, then twice with chloroform, and RNA was precipitated overnight with 2.5 vol. of ethanol at –20°C.

Primers for reverse transcription and PCR. The following oligodeoxyribonucleotides were designed as the primers on the basis of two published PPV CP gene sequences [20, 21]:

primer PPVCP1 : 5′ GAGATAT-
GGATCCcta<u>CTACACTCCCCTCACAC</u> 3′

primer PPVCP2 : 5′ GTGAATA-
GGATCCatg<u>GCTGACGAAAGAGAAGAC</u> 3′

The last 17 nucleotides of PPVCP1 (underlined) are complementary to the 3′ end of the fragment of viral RNA coding for PPV polyprotein. Upstream to this sequence PPVCP1 contains an extra termination codon (cta) and restriction site (bold type) for *Bam*HI followed by 7 nucleotides which make possible recognition of the restriction site by the enzyme. Primer PPVCP2 consists of a 18-nucleotide segment (underlined) corresponding to the 5′ end of the PPV RNA region coding for viral CP. Upstream to this segment PPVCP2 contains an initiation codon (atg), *Bam*HI restriction site (bold type) and extra 7 nucleotides.

Reverse transcription and PCR. RT-PCR was performed using an RNA PCR kit purchased from Perkin-Elmer Cetus. For reverse transcription about 0.1 µg of PPV-RNA was used. The reaction was run in a buffer containing 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 4 mM $MgCl_2$, 1 mM each of dNTP, 20 U RNase inhibitor, 0.75 µM PPVCP1 primer and 50 U M-MuLV reverse transcriptase. Final volume of the mixture was 20 µl. The reaction mixture was incu-

bated at 42°C for 45 min followed by 10 min at 95°C and 5 min at 5°C. During incubation each sample was covered with 50 µl of mineral oil. After reverse transcription the sample was extracted with chloroform and transferred to a new tube for PCR. To the mixture were added: 8 µl of 100 mM Tris/HCl buffer, pH 8.3, 4 µl of 25 mM $MgCl_2$, 1 µl of 15 µM PPVCP2 primer, 5 U of AmpliTaq DNA polymerase (Perkin-Elmer Cetus) and water up to 100 µl. The samples were then covered with mineral oil and incubated under the following conditions:

2 cycles	95°C	for 2	min
	50°C	for 1	min
	72°C	for 5	min
23 cycles	95°C	for 1.5	min
	63°C	for 1	min
	72°C	for 5	min
1 cycle	95°C	for 1.5	min
	63°C	for 1	min
	72°C	for 10	min

Cloning and sequencing of the PCR product. PCR product, extracted with chloroform, precipitated with ethanol and purified by Sea Plaque GTG agarose gel electrophoresis, was digested with *Bam*HI (Promega). The digested product was purified by gel electrophoresis and ligated to 50 ng of pBluescript II KS+ in the *Bam*HI site as described in [22]. The *E. coli* DH5αF′ cells were then transformed with the recombinant plasmid using the rubidium procedure [23]. Four clones were selected for sequencing of the PCR product and the recombinant plasmids isolated by the alkaline method [24] and purified by two-step CsCl gradient centrifugation [25]. The purified plasmids were used for sequencing either directly or after subcloning.

Sequencing of the recombinant plasmids (containing either the whole PCR product or its fragments) was performed by the dideoxy method [26] using [α-^{32}P]dATP, 3000 Ci/mmol (Amersham) and Taq DNA polymerase (Promega) under conditions recommended by the supplier of the enzyme.

RESULTS AND DISCUSSION

The results of ELISA (Fig. 1) clearly indicate that our PPV-S isolate reacted with the monoclonal antibody MAb05 but was not recognised by the MAb06 reacting with PPV-D. This means that PPV-S is different serologically from PPV-D. However, its similarity to PPV-M has not been checked with PPV-M specific antibodies.

Primers for the RT-PCR were constructed on the basis of the published sequences of the coat protein genes of PPV-Rankovic and PPV-D [20, 21]. If the sequence of the CP gene of PPV-S strain does not differ much from the previously known ones, the primers should enable us to amplify the region of 993 nucleotides of the PPV coding for 330 amino acids of the PPV CP. Gel electrophoresis of the PCR product (not shown) yielded only one band of the expected length. The product was cloned and its se-

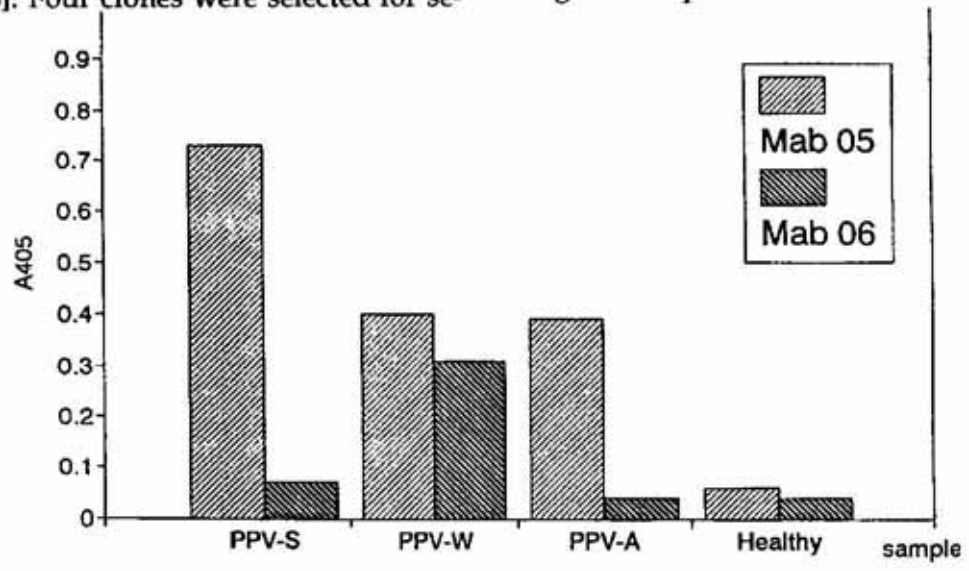

Fig. 1. The ELISA reaction of PPV-S with the monoclonal antibodies MAb05 and MAb06.

Table 1

Nucleotide sequence of the coat protein gene of PPV-S and its comparison with CP genes of other PPV strains.

Complete sequence is shown for the PPV-S isolate; for CP genes of other strains only differences are shown by letters, and identical nucleotides are marked with dots. Dashes denote deletions (PPV-NAT). Two 3-nucleotide long additions in the PPV-El Amar strain are indicated with arrow heads.

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PPV-strain                Nucleotide sequence of CP gene
                      10         20         30         40         50         60
A:(PPV-S)   GCTGACGAAA GAGAAGACGA GGAGGAAGTT GATGCAGGCA AGCCGATTGT AGTTACTGCA
B:(PPV-R)   .......... .......... .......... .......... ......G... ..........
C:(PPV-D)   .......... ....G..... .......... .......... .......... ..........
D:(PPV-NAT) .......... .......... .......... ......---- ---------- ----------
E:(PPV-PS)  .....T.... .G..G..... T..A...... ........A. GA..T.C... G..A......
F:(PPV-oS)  .....T.... AG..G..... T..A...... ........A. .A..C.C..C G..A......
G:(El Amar) .....T..G. AG.....^.. A........G ........G. G...TTTA...CACC...A..
                       add(GAT)^
                      70         80         90        100        110        120
A:(PPV-S)   CCGGCAGCAA CTAGCCCAAC ACTTCAACCA CCTCCAGTTA TACAGCCTGC ACCCCAGACT
B:(PPV-R)   .......... .......... .......... ........C. .......... .....G....
C:(PPV-D)   .......... .......... .......... ........C. .......... .....G....
D:(PPV-NAT) ---------- ---------- -......... ........C. .......... .....G....
E:(PPV-PS)  .......... ..GTGG.... GAC....... G.......G. ....A..... ......A..C
F:(PPV-oS)  .......... ..GTGG.... .A.C...... G.......G. ....A..... .ATT..A..C
G:(El Amar) .A.CAGC... T.GTTA.... .AC...G.A. A.....A.A. CGAGTA.AA. CTTA..AG..
                  add (ACC)^
                     130        140        150        160        170        180
A:(PPV-S)   ACGGCGCCAA TGTTCAACCC CATTTTCACG CCAGCAACAA CTCAACCAGC AACAAAACCA
B:(PPV-R)   ......T... ..C....... .......... .......... .......... ..........
C:(PPV-D)   .......... ..C....... .......... .......... .......... ..........
D:(PPV-NAT) .......... ..C....... .......... .......... .......... ..........
E:(PPV-PS)  ..A..A.... .......... .........T .......... ....G..T.. GGT..G....
F:(PPV-oS)  ...A.A.... .......... .........T .......... ....G..T.. GAT..G....
G:(El Amar) ...CA.G... ....T..T.. ...C.....T .....G..G. ..G.G..GA. C..T.GGA..
                     190        200        210        220        230        240
A:(PPV-S)   GTTTCACAGG TGTCAGGACC TCAACTGCAA ACTTTTGGAA CATATGGTAA TGAGGATGCC
B:(PPV-R)   .......... .......... .......... .......... .......... .........A
C:(PPV-D)   .......... ..C....... .......... .......... .......... .........A
D:(PPV-NAT) .......... .......... .......... .......... .....A..C. .........A
E:(PPV-PS)  ...C.T.CAA .T.....GA. CA...C..GG T........G TT.....A.. ...A..C..A
F:(PPV-oS)  .....T.TA. .T.....GG. ..C..C.... T........G TT.A...A.. .........A
G:(El Amar) ..GC.T..CA CAA..ACTA. .AC..CT.CT T........G TGATC..G.. .......A..
                     250        260        270        280        290        300
A:(PPV-S)   TCACCTAGCA ACTCAAACGC GCTAGTCAAC ACAAACAGAG ACAGGGACAT CGATGCAGGA
B:(PPV-R)   .......... .......... .......... .......... ........G. ..........
C:(PPV-D)   .......... .......... .......... .......... ........G. ..........
D:(PPV-NAT) .......... .......... .......... .......... .......TG. ..........
E:(PPV-PS)  .......... C.......A. TT.G..G..T ...GGA..G. .T......G. ..........
F:(PPV-oS)  .......... C.......A. TT.G..G... ...GGA..G. .T.....TG. ..........
G:(El Amar) G....C.ATG CT..C..T.. AG....TCGA ...GGT.... ....A..TG. T.....G..T
                     310        320        330        340        350        360
A:(PPV-S)   TCAATTGGAA CTTTCACAGT GCCACGTTTG AAGGCAATGA CTTCGAAACT ATCTTTGCCA
B:(PPV-R)   ...G...... ....T..... .......... .......... .......... ....C.....
C:(PPV-D)   .......... ....T..... .......... .......... .......... ....C.....
D:(PPV-NAT) ....C..... ....T..... .......... .......... .......... ....C.....
E:(PPV-PS)  ..G....... .....G.... .......C..A ..AA...... .A.....GT. ....C.A..G
F:(PPV-oS)  ..G....... .......... .......C..A ..AA...... .A.....GT. ....C.A..G
G:(El Amar) ..C.....G. .......... .......C... ..A....... .A..A..G.. C..AC.T...
                     370        380        390        400        410        420
A:(PPV-S)   AAGGTGAGGG GAAAGGCCAT TATGAACTTG AACCATTTGG CACATTATAG TCCTTCACAG
B:(PPV-R)   .......A.. .......T.. .......... .......... .......... ....G.....
C:(PPV-D)   .......A.. .......T.. .......... .......... .......... ....G.....
D:(PPV-NAT) .......A.. .......T.. .......... .......... .......... ....G.....
E:(PPV-PS)  .......ACC .......A.. .........A ..T....... .......C.. ....G....A
F:(PPV-oS)  .......AA. ....A..A.. .........A ..T....... .......C.. ....G....A
G:(El Amar) .......AA ..G..A..A.. ......T... ..T..CC... .TTTC..C.. ....G....A
                     430        440        450        460        470        480
A:(PPV-S)   GTTGATTTGT CAAACACGAG AGCTCCGCAG TCTTGTTTCC AAACTTGGTA TGAAGGAGTT
B:(PPV-R)   .....C.... .......... .......... .......... .......... ..........
C:(PPV-D)   .....C.... .......... .......... .......... .......... ..........
D:(PPV-NAT) .....C.... .......... .......... .......... .......... ..........
E:(PPV-PS)  .....C.... .......AC. ......A..A ..C..C.... .G........ ..........
F:(PPV-oS)  ........A. .......AC. ......A..A ..C..C.... .G........ ..........
G:(El Amar) ..G....... .G......C. ......A... ..C..C..T. ....A..... ..........
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                      490        500        510        520        530        540
A:(PPV-S)   AAGCGAGATT ATGATGTCAC GGACGATGAA ATGAGCATCA TTTTAAATGG TCTTATGGTT
B:(PPV-R)   .......... .......... .......... .......... .......... ..........
C:(PPV-D)   .......... .......... .......... .......... .......... ..........
D:(PPV-NAT) ........C. .......... .......... .......... .......... ..........
E:(PPV-PS)  .....T.... .......... A..T..CC.. .......... ....G..... CT.G......
F:(PPV-oS)  .....T.... .C........ A..T..G... .......... ....G..... C..G......
G:(El Amar) .GA..C..C. ....C..... T..T...... ........T. ....G..... G........C
                      550        560        570        580        590        600
A:(PPV-S)   TGGTGCATAG AGAATGGAAC ATCCCCGAAT ATCAATGGAA TGTGGGTGAT GATGGATGGG
B:(PPV-R)   .......... .......... .......... .......... .......... ..........
C:(PPV-D)   .......... .......... .......... .......... .......... ..........
D:(PPV-NAT) .......... .......... .......... .......... .......... ..........
E:(PPV-PS)  ........C. .A..C..... G..T.....C .......... .......... ..........
F:(PPV-oS)  ........C. .A..C..... G.....T..C .....C.... .......... ..........
G:(El Amar) ........T. .......... .......... ..T....... .......C.. ......C...
                      610        620        630        640        650        660
A:(PPV-S)   GAAACACAAG TGGAGTATCC AATAAAGGCA TTGTTGGATC ATGCGAAACC CACTTTTAGA
B:(PPV-R)   .......... .......... .......... .......... .......... ..........
C:(PPV-D)   .......... .....C.... .......... .......... .......... ..........
D:(PPV-NAT) .......... .......... .......... .......... .......... ..........
E:(PPV-PS)  ..G....... .......... .......C.. .......... .C........ ..........
F:(PPV-oS)  .......... .......... .......C.. .......... .C........ ..........
G:(El Amar) ..G....... .......... .......C.. .......... .......... ..........
                      670        680        690        700        710        720
A:(PPV-S)   CAAATTATGG CACATTTCAG TAACGTGGCT GAAGCGTATA TTGAAAAACG AAATTATGAA
B:(PPV-R)   .......... .......... .......... .......... .......... ..........
C:(PPV-D)   .G....G... ...G...... CG........ .......G.G .G........ ......C...
D:(PPV-NAT) .......... .......... .......... .......... .......... ..........
E:(PPV-PS)  .......... .......... .......... ........C. .......... .........G
F:(PPV-oS)  .......... .......... .......... .......... .......... G.....C..G
G:(El Amar) .......... .......... .......... .......... .......... G........G
                      730        740        750        760        770        780
A:(PPV-S)   AAAGCATACA TGCCAAGGTA TGGAATTCAG CGCAACCTGA CAGACTACAG CCTCGCCAGA
B:(PPV-R)   .......... .......... .......... .......... .......... ..........
C:(PPV-D)   .......... .......... .......... .......... .......... ..........
D:(PPV-NAT) .......... .......... .......... .......... .......... ..........
E:(PPV-PS)  .......... .......... .......... .......... ....T..... T..A......
F:(PPV-oS)  .......... .......... .......... .......... ....T..... ..........
G:(El Amar) .......... .......... ...T...... .......... .G..T..... ..........
                      790        800        810        820        830        840
A:(PPV-S)   TATGCCTTTG ATTTTTACGA AATGACTTCA ACGACACCCG TACGGGCACG TGAAGCTCAT
B:(PPV-R)   .......... .......... .......... ........A. .......... ..........
C:(PPV-D)   .......... .......... .......... .......... .......... ..........
D:(PPV-NAT) .......... .......... .......... .......... .......... ..........
E:(PPV-PS)  ..C....... .......... .......... ..A..G..T. .G..T..... ..........
F:(PPV-oS)  ..C......G .......... .......... ..A.....T. .G..T..... ..........
G:(El Amar) ..C....... .......... .......... ........T. .G..T..... ..........
                      850        860        870        880        890        900
A:(PPV-S)   ATCCAGATGA AGGCAGCAGC ATTGAGAAAT GCTCAAAATC GTTTATTTGG CTTGGATGGA
B:(PPV-R)   .......... .......... .......... .T........ .......... ..........
C:(PPV-D)   .......... .......... .......... .T........ .......... ..........
D:(PPV-NAT) .....A.... .......... .......... .T........ .......... ..........
E:(PPV-PS)  ..A....... .......... .......... .T........ .......... ..........
F:(PPV-oS)  ..A....... .......... .......... .T...G.... .......... ..........
G:(El Amar) ..A....... .......G.. .......... ..C..G.... .......... ..........
                      910        920        930        940        950        960
A:(PPV-S)   AACGTCGGAA CACAAGAAGA GGACACAGAG AGACACACCG CTGGTGATGT TAATCGCAAC
B:(PPV-R)   .......... .......... .......... .......... .......... ..........
C:(PPV-D)   .......... .......... .......... ........G. A......... ..........
D:(PPV-NAT) .......... .......... .......... .......... .......... ..........
E:(PPV-PS)  .......... .......... .......... ..G....... .......C.. ..........
F:(PPV-oS)  .......... .......... .......... ..G....... .......C.. G.........
G:(El Amar) ..T..T.... .......... .......... ..G....... .......... ..........
                      970        980        990
A:(PPV-S)   ATGCACAACC TCCTCGGTGT GAGGGGAGTG TAG
B:(PPV-R)   .......... .......... .......... ...
C:(PPV-D)   .......C.. .......... .......... ...
D:(PPV-NAT) .......... ........A. .......... ...
E:(PPV-PS)  .......... .......... .......... ...
F:(PPV-oS)  .......... .......... .......... ...
G:(El Amar) .....T.... ........A. .......... ...
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A:(PPV-S) - Skierniewice isolate of PPV, B:(PPV-R) - Rankovic strain of PPV [20], C:(PPV-D) - PPV-D strain [21], D:(PPV-NAT) - PPV-NAT strain [13], E:(PPV-PS) [32], F:(PPV-oS) [32], G:(El Amar) - PPV-El Amar strain [6].

quence was read in 4 independent experiments to avoid mistakes, possible when Taq DNA polymerase is used for DNA amplification. The nucleotide sequence of the PCR product and its comparison with the published sequences of CP genes of PPV is shown in Table 1. PCR amplification may occur even when there are some mismatches between the primer and the target sequence [27]. Therefore it should be stressed that the sequences of the first 18 and the last 17 nucleotides may actually originate from the sequences of primers. In preparing cDNA we have not used primers corresponding to the sequences outside the CP gene.

It is obvious from the data shown in Table 1 that PPV-S is different from the other PPV strains, the CP genes of which have been sequenced so far. The smallest differences are observed between PPV-S and PPV-Rankovic strain: there are only 16 substitutions of nucleotides (homology 98.4%) of which 10 lead to substitutions of amino acids (Table 2). PPV-D strain differs in the CP gene from PPV-S by 27 substitutions (homology 97.3%). In both cases the substitutions are distributed non-uniformly along the gene. There is an about 390 bp long stretch in the central part of the genes (positions 427–818) where the sequences of PPV-S and PPV-Rankovic are identical. PPV-NAT (not aphid transmissible) [28] is shorter than the aphid transmissible PPV-S, PPV-Rankovic and PPV-D by 45 nucleotides, and differs from PPV-S by 19 substitutions. Apart from this deletion, PPV-NAT shows 91.1% homology with PPV-S. The largest number of substitutions is observed in relation to the El Amar strain [6] (80.6% homology); in this case most of the changes are located in the 5′-end region of the CP gene (78% of all changes within 420 bp at the 5′-end), while the region corresponding to C-terminal part of the coat protein is much more conservative.

Wetzel *et al.* [27] showed that the restriction fragments length polymorphism of the 243 bp PCR product can be used as a tool for dividing PPV strains into two groups: strains with or without the *Rsa*I restriction site in the amplified fragment (GTAC at position 820–823 in Table 1). They also suggested that the presence or absence of the *Rsa*I restriction site is highly correlated with the particular PPV-D serotype group. The original definition of the PPV-D and PPV-M serotype was made [18] using polyclonal antibodies and the agar double diffusion technique. We have not done this kind of experiments with PPV-S. Therefore we do not know whether our isolate is an exception from this rule (it possesses the *Rsa*I site but its serological properties differ from those of PPV-D), or the classification based on the reaction with MAb05 and MAb06 does not fully correspond to the original distinction between the PPV-D and PPV-M type.

We suggest that the primers and method we have used for amplification of PPV-S CP gene can be applied to other PPV strains. It should thus be possible to distinguish either of each pair of PPV strains of known CP sequence by RFLP analysis of the PCR product. For example, PPV-S and PPV-El Amar, compared with PPV-Rankovic, PPV-D and PPV-NAT, lack *Hin*cII site at position 421 (due to substitutions $C_{426} \rightarrow T_{426}$ in PPV-S and $T_{423} \rightarrow G_{423}$ in PPV-El Amar) or *Hpa*II site at position 114 (substitution $G_{116} \rightarrow A_{116}$). This comparison might serve as a simplified version of the classification based upon sequence data [29], especially if the IC-RT-PCR method developed by Wetzel *et al.* [30] is used.

The amino-acid sequence of PPV-S coat protein (predicted from the sequence of the CP gene) differs less from the other PPV strains (Table 2) than does its nucleic acid sequence. Like other aphid-transmissible PPV-strains, PPV-S contains an Asp-Ala-Gly motif (positions 11–13) which was suggested to be important for aphid transmissibility [6], preceeded by a conserved stretch of 10 amino acids at the N-end of CP. Within this stretch in PPV-PS and PPV-oS [32] there are substitutions Glu-7 → Asp-7, and in PPV-oS and El Amar Arg-4 → Lys-4, but obviously these substitutions do not alter the nature of this stretch and are not incompatible with the suggestion of PPV strains being transmissible by aphids.

We have not checked the aphid transmissibility of PPV-S in a controlled experiment. However, PPV-S was isolated from a tree which had been infested with aphids. It has also been reported by Smolarz & Zawadzka [31] in 1978 that PPV occurring near Skierniewice is aphid transmissible.

Similarly as in the whole potyvirus group [4] the C-terminal part of PPV-S coat protein is, in general, much more conserved than its N-terminal part but, surprisingly, it differs in the

Table 2

Amino-acid sequence of the PPV-S coat protein predicted from the nucleotide sequence of the CP gene and its comparison with the CP proteins of other PPV strains.

Complete sequence is shown for PPV-Skierniewice only; identical amino acids in other strains are marked with dots. Deletion of 15 amino acids in PPV-NAT is marked with dashes. Addition of two amino acids in PPV-El Amar is indicated with arrow heads.

```
PPV strain          Amino acid sequence of CP
-------------------------------------------------------------------------------
                  10         20         30         40         50         60
A:(PPV-S)  ADERKDEEEV DAGKPIVVTA PAATSPTLQP PPVIQPAPQT TAPMPNPIPT PATTQPATKP
B:(PV-R)   .......... .....S.... ......I... ........R. ..S.L..... ..........
C:(PPV-D)  .......... .......... ......I... ........R. ....L..... ..........
D:(PPV-NAT).......... ..-------- -------... ........R. ....L..... ..........
E:(PPV-PS) ......D... ...R.T.... ....VA.T.. A......... ..T....... .......VR.
F:(PPV-oS) ...K..D... ...K.TA... ....VA.T.. A......I.. .T....... .......IR.
G:(El Amar)...K...... ...R.L.T.T QQPITT.T.. T.ITSTTL.A .QA....... PA..E.T.RT
           add D^                 add V^
                  70         80         90        100        110        120
A:(PPV-S)  VSQVSGPQLQ TFGTYGNEDA SPSNSNALVN TNRDRDIDAG SIGTFTVPRL KAMTSKLSLP
B:(PV-R)   .......... .......... .......... ......V... .V........ ..........
C:(PPV-D)  ....P..... .......... .......... ......V... .......... ..........
D:(PPV-NAT).......... .....SH... .......... ......V... .T........ ..........
E:(PPV-PS) .PPI..TKPR S..V...... ...T..T... .G....V... .....A.... .T.......
F:(PPV-oS) ..L...ATP. S..V...... ...T..T... .G....V... .......... .T........
G:(El Amar).PHTTTTTPP S..VI....T A.NA...V.R .G....V... .......... ..........
                  130        140        150        160        170        180
A:(PPV-S)  KVRGKAIMNL NHLAHYSPSQ VDLSNTRAPQ SCFQTWYEGV KRDYDVTDDE MSIILNGLMV
B:(PV-R)   ..K....... .........A. .......... .......... .......... ..........
C:(PPV-D)  ..K....... .........A. .......... .......... .......... ..........
D:(PPV-NAT)..K....... .........A. .......... .......... .......... ..........
E:(PPV-PS) ..K....... .........A. .......... .......... .........E. ..........
F:(PPV-oS) ..K....... .........A. .......... .......... .........E. ..........
G:(El Amar)..K....... ....F...A. .......... .......... R......... ..........
                  190        200        210        220        230        240
A:(PPV-S)  WCIENGTSPN INGMWVMMDG ETQVEYTIKP LLDHAKPTFR QIMAHFSNVA EAYIEKRNYE
B:(PV-R)   .......... .......... .......... .......... .......... ..........
C:(PPV-D)  .......... .......... .....H.... .......... R.V.R..D.. ..CV......
D:(PPV-NAT).......... .......... .......... .......... .......... ..........
E:(PPV-PS) .......... .......... ......P... .......... .......... ..........
F:(PPV-oS) .......... .......... ......P... .......... .......... ..........
G:(El Amar).......... .......... .......... .......... .......... ..........
                  250        260        270        280        290        300
A:(PPV-S)  KAYMPRYGIQ RNLTDYSLAR YAFDFYEMTS TTPVRAREAH IQMKAAALRN AQNRLFGLDG
B:(PV-R)   .......... .......... .......... .......... .......... V.........
C:(PPV-D)  .......... .......... .......... .......... .......... V.........
D:(PPV-NAT).......... .......... .......... .......... .......... V.........
E:(PPV-PS) .......... .......... .......... .......... .......... V.........
F:(PPV-oS) .......... .......... .......... .......... .......... V.........
G:(El Amar).......... .......... .......... .......... .......... ..........
                  310        320        330
A:(PPV-S)  NVGTQEEDTE RHTAGDVNRN MHNLLGVRGV
B:(PV-R)   .......... .......... ..........
C:(PPV-D)  .....KQ... ...D...... ..TF......
D:(PPV-NAT).......... .......... ......M...
E:(PPV-PS) .......... .......... ..........
F:(PPV-oS) .......... .......... ..........
G:(El Amar).......... .......... ......M...
```

For designations of PPV see Table 1.

C-terminal half from CP of PPV-D by 12 amino acids, while in the N-terminal half only by 7 amino acids. On the contrary, in CP of PPV-El Amar, the N-terminal half of which differs largely from that of PPV-S, the C-terminal part (positions 161–330) is identical except for one substitution (Val → Met, pos. 327).

As shown in Fig. 1, PPV-S CP reacts well with antibody MAb05 but is not recognized by antibody MAb06. This indicates that PPV-S is serologically different from PPV-D which reacts with both MAb05 and MAb06.

Assuming that antibodies can recognise the external part of CP in intact viral particles and

knowing from aphid transmissibility that the N-terminal region of CP is externally oriented, it can be suggested that the different reactivity of MAb06 with PPV-S and PPV-D resulted from differences in amino acids 27, 39 and/or 45 (Ile, Arg and Leu in PPV-D substituted by Thr, Glu and Phe in PPV-S). Since other amino acids can also contribute to the reactivity against antibodies, it would be interesting to check the reactivity towards MAb06 of CP of PPV-PS, PPV-oS and El Amar, where these residues are the same as in PPV-S (although there are numerous other substitutions in the respective CP region). Unfortunately, during the present investigation these strains were not available for direct comparison.

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