# PLUM POX VIRUS CAPSID PROTEIN MOBILITY IN SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS

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*Received January 12, 1999; accepted March 26, 1999*

**Summary.** – Slovak plum pox virus (PPV) isolates BOR-3 and KR-4 behaved atypically in polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) of their capsid proteins (CPs). Whereas other tested PPV isolates could be sorted by SDS-PAGE into groups corresponding to the M or D strains, as controlled by restriction fragment lenght polymorphism (RFLP) analysis, the two abovementioned isolates behaved as the M strain according to RFLP analysis but not SDS-PAGE. Slight mobility differences were observed also among the isolates belonging to the D strain. SDS-PAGE of the CP thus cannot clearly distinguish between these two main PPV strains. BOR-3 isolate has been shown atypical also in its biological properties, and it reacted very weakly with a monoclonal antibody (MAb) recognizing well both M and D strains in immunoblot analysis.

**Key words:** plum pox virus; capsid protein; immunoblot analysis; proteolysis

## Introduction

The plum pox (sharka) belongs to most important diseases of stone fruit trees in Europe. It is caused by a potyvirus, PPV, which has spread since the beginning of the 20th century from Balkan countries into nearly whole subcontinent. Several PPV strains have been described M, D, EA and C. The EA (El Amar) strain has been found in Egypt (Wetzel *et al.*, 1991). The C (Cherry) strain includes sour cherry (SoC) and sweet cherry (SwC) isolates described originally in Moldova and southern Italy, respectively (Nemchinov *et al.*, 1995; Crescenzi *et al.*, 1995). Although the PPV-C was already found also in middle Europe (Kölber *et al.*, 1998), most significant in our region remain the first two strains, M (Marcus) and D (Dideron). Originally, they were described as serotypes (Kerlan and Dunez, 1979). Later it was shown that both strains differ also in the mobility of their CPs (Adamolle, 1993) and in the presence (D) or absence (M) of an *Rsa*I restriction site in the cDNA corresponding to the RNA part coding for the CP C-terminus (Candresse *et al.*, 1994). Serotyping of PPV isolates by group-specific MAbs correlates very well though not completely with RFLP analysis (Candresse *et al.*, 1998).

Recently, a collection of Slovak PPV isolates was established, individual isolates were biologically characterized and grouped by RFLP analysis (Glasa *et al.*, 1998; Kúdela *et al.*, 1998). A MAb was prepared and used for immunological detection and characterization of PPV strains (Šubr and Matisová, 1999). In this paper we show and discuss differences in the SDS-PAGE mobility of CPs of Slovak PPV isolates.

## Materials and Methods

The germplasm bank of PPV isolates was kept in the form of chip-budded *Prunus persica* GF305 seedlings. PPV was multiplied in *Nicotiana benthamiana* infected by mechanical inoculation. Three-four weeks after infection the virus was purified according to Laín *et al.* (1988). Reference PPV isolates Marcus and Dideron were

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**Abbreviations:** CP = capsid ptotein; MAb = monoclonal antibody; PPV = plum pox virus; RFLP = restriction fragment length polymorphism; SDS = sodium dodecyl sulfate; SDS-PAGE = polyacrylamide gel electrophoresis in the presence of SDS

obtained from Dr. Quiot, INRA, Montpellier, France. Core CPs were prepared by mild trypsinolysis of purified PPV particles according to Shukla *et al.* (1988). SDS-PAGE (Laemmli, 1970) was done in 10% or 15% gels. In immunoblot analysis (Hirano and Watanabe, 1990) nitrocellulose membranes (Serva) were used and the staining was performed with a rabbit polyclonal IgG (kindly provided by Dr. Rabenstein, Federal Center for Breeding Research on Cultivated Plants, Aschersleben, Germany) or mouse MAb 2H8 (Šubr and Matisová, 1998), and a secondary alkaline phosphatase-labeled goat antibody (Dianova, Germany).

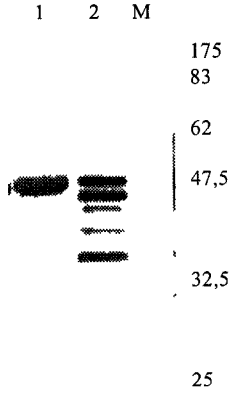


**Fig. 1**
**Immunoblot analysis of PPV VAR-2 isolate using MAb 2H8**
SDS-PAGE in 15% gel . The isolate was purified from *N. benthamiana* (lane 1) and *N. glutinosa* x *N. clevelandii* (lane 2). Size marker ($M_r$ in K values, lane M).

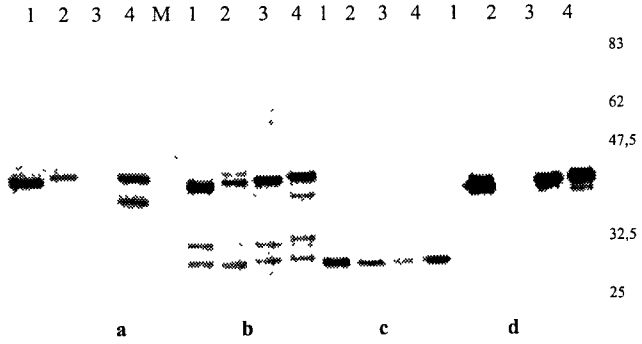


**Fig. 2**
**Immunoblot analysis of selected Slovak PPV isolates**
SDS-PAGE in 10% gel. Polyclonal IgG (a–c) and MAb 2H8 (d). Crude plant sap (a), purified virus (b and d), and purified virus after mild trypsinolysis (c). PPV isolates BOJ-3 (lanes 1), BOR-3 (lanes 2), VAR-2 (lanes 3), and KR-4 (lanes 4). Size marker ($M_r$ in K values, lane M).

## Results and Discussion

Although phenylmethylsulfonyl fluoride was added to the extraction buffer to final concentration of 1 mmol/l to prevent *in vitro* proteolysis, the quality of purified PPV CP depended on the host plant species used for virus multiplication. Whereas one major band was observed in immunoblots of PPV CP from *N. benthamiana* and *P. sativum*, a marked degradation of CP occurred after its purification from *N. clevelandii x N. glutinosa* hybrids (Fig. 1). When crude sap samples were analyzed, immunoblot patterns showed also progressive CP degradation in *N. clevelandii x N. glutinosa* in comparison to no degradation in samples from *N. benthamiana* (results not shown). Thus a higher content or other quality of plant proteases in the tobacco hybrid was obvious. Therefore we used *N. benthamiana* for the PPV multiplication in further experiments. On the other hand, no influence of the plant age or of material storage at -70°C on the SDS-PAGE pattern of CP was observed.

The SDS-PAGE mobility of nearly all the tested PPV isolates correlated with the respective PCR result regards strain afiliation, namely CPs of M isolates migrated more slowly than those of D isolates (Pasquini and Barba, 1997). Apparent $M_r$ values, based on determination of CP retention factors, were in our case lower than those described by Adamolle (1993): 35 K and 33.5 K compared to 38 K and 36 K for M and D isolates, respectively. Closer to our results are the values given by López-Moya *et al.* (1994), 36 K and 34 K, however, in this case no strain afiliation of differently migrating PPV isolates has been done.

Nevertheless, $M_r$ values estimated in this way can serve only for orientation and depend on used standards, gel systems and on the human factor. However, one may expect that relative CP mobilities can be reproducibly determined and included in grouping of PPV isolates.

In screening Slovak PPV-M isolates we found two exceptional cases of the CP mobility. Both isolates KR-4 and BOR-3 like other M isolates had no *Rsa*I restriction site in the cDNA region coding for the CP C-terminus. Nevertheless, the KR-4 CP had slightly slower mobility than expected and the BOR-3 CP migrated as a double band with mobilities lower and higher than those of typical M isolates (Fig. 2b). When analyzing the crude plant sap, the CP mobility of other M isolates was equal to that of KR-4 isolate (Fig. 2a). KR-4 CP is probably more stable in the purification process while CPs of other isolates are proteolytically shortened.

The BOR-3 isolate behaved equally in SDS-PAGE of CP from purified virus and crude plant sap. To exclude the possibility that BOR-3 is a naturally occurring mixture of different isolates we infected *Chenopodium foetidum* with the BOR-3 isolate and reinoculated single lesions to *N. benthamiana*. Immunoblot patterns of the crude sap from one-lesion-infected plants were identical with the original BOR-3 isolate double-band pattern (result not shown). We do not assume that both the longer and shorter CP molecules play an active role in the virus replication cycle. More probably, the original CP is shortened by host protease activity and the difference between

BOR-3 and other M isolates lies in the sensitivity level to this proteolysis and reflects differences in the CP amino acid sequences. Similar double-band patterns of PPV CP have been observed in other laboratories too (Navrátil *et al.*, 1998).

After mild trypsinolysis of PPV virions the core CP of all tested isolates migrated with the same velocity (Fig. 2c). This confirms that CP migration differences depend on the differences in protruding termini of CP molecules which are mostly exposed to intracellular proteolytic activity. It is known that core CP is more conserved and most differences between strains of respective potyviruses have been found in the surface-localized CP N-terminus (Shukla and Ward, 1988).

BOR-3 CP reacted very weakly with MAb 2H8, binding otherwise well all tested M and D isolates (Fig. 2d). The epitope reacting with MAb 2H8 is linear like probably most of PPV epitopes (Candresse *et al.*, 1998) and is located outside of the core CP (Šubr and Matisová, 1999). The amino acid sequence near to some of CP termini (most likely the N-terminus) is obviously different in the BOR-3 isolate. The real difference extent may be estimated only by the sequencing of BOR-3 CP gene. It cannot be excluded that some additional differences in the sequence of other genes will be found because the BOR-3 isolate shows also atypical biological properties (Glasa *et al.*, 1997).

Slight differences in the CP mobility were observed also among Slovak PPV-D isolates (Table 1). Similar results were presented by Dallot (1997) who found that CP of 10% of 200 tested French PPV isolates migrated more slowly than typical D isolates, although they behaved as PPV-D according to RFLP analysis. Natural or artificial proteolysis is a general problem connected with plant virus purification. As shown by us, even when processed in parallel by the same protocol, or even when an unprocessed plant sap is used, CPs of different isolates of the same PPV strains may display different SDS-PAGE mobilities. Therefore this method cannot be used without another (RFLP analysis, serotyping, or estimation of biological properties) for the grouping of PPV isolates. It is possible that in the future small differences in the CP mobility will be used for a more precise characterization of PPV isolates.

**Table 1. Comparison of SDS-PAGE mobilities of CPs of selected Slovak PPV isolates and type isolates**

| Isolate | Strain (RFLP) | Mobility |
|---|---|---|
| Dideron | D | f |
| BBR-1 | D | f |
| BOJ-3 | D | m |
| BIII/2 | D | m |
| Marcus | M | s |
| CAH-2 | M | s |
| VAR-2 | M | s |
| KR-4 | M | s |
| BOR-3 | M | s[d] |

f = fast; m = middle; s = slow.
[d]Double-band.

**Acknowledgements.** We thank Dr. Frank Rabenstein, Federal Center for Breeding Research on Cultivated Plants, Aschersleben, Germany, for the polyclonal PPV antiserum and Dr. Quiot, INRA, Montpellier, France, for the reference PPV isolates. This work was supported by grants Nos. 95/5305/012 and 2/4039/97 of the Grant Agency for Science.

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