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CURRENT SITUATION OF *PLUM POX VIRUS* VARIABILITY IN SLOVAKIA

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SUMMARY - Molecular variability of PPV isolates was studied using RT-PCR targeting 3´terminal part of CP gene and RFLP analysis of PCR products. The subgroup differentiation based on *Rsa*I polymorphism of 44 isolates from naturally infected plum, myrobalan, apricot, almond and peach trees revealed the presence of isolates belonging to PPV-M and PPV-D subgroups. PPV-M and PPV-D isolates were almost equally represented in tested samples. Recent studies have discovered the presence of recombinant isolates in Slovakia using a molecular tool based on PPV-M and D subgroup typing targeting P3-6K1, CI and CP regions of the PPV genome. Nearly all the PPV recombinants were recovered from plums and myrobalans; on the contrary, no recombinant was found on peach. The presence of atypical populations of recombinant PPV must be taken into account considering their viability in competition with conventional PPV-M and D isolates.

Key words: Slovakia, PPV, strain, recombinant, PCR, virus variability

RESUME *- La variabilité moléculaire des isolats du PPV a été évaluée par l'application de la RT-PCR, en utilisant comme cible l'extrémité terminale 3' du gène de la protéine capsidique. Successivement, les produits de la PCR ont été analysés à travers la RFLP. La différenciation des sous-groupes, basée sur le polymorphisme RsaI de 44 isolats provenant de pruniers, myrobolans, abricotiers, amandiers et pêchers infectés, a révélé la présence d'isolats appartenant aux sous-groupes PPV-M et PPV- D. Les isolats PPV-M et PPV-D étaient représentés presque dans la même mesure dans les échantillons testés. Des études récentes ont amené à la découverte d'isolats recombinants en Slovaquie, en effectuant le typage des sous-groupes PPV-M et PPV- D et en utilisant comme cibles les régions P3-6K1, CI et CP du génome du PPV. La quasi-totalité des recombinants du PPV ont été récupérés des pruniers et des myrobolans infectés, alors que, jusqu'à présent, aucun recombinant n'a été trouvé sur pêcher. La présence de populations atypiques de PPV recombinant devrait être prise en compte vu leur capacité de compétition vis-à-vis des isolats PPV-M et PPV-D conventionnels.*

Mots-clés: *Slovaquie, PPV, souche, recombinant, PCR, variabilité du virus.*

INTRODUCTION

Plum pox virus (PPV), the agent responsible for sharka disease, is the most important viral pathogen of stone fruit trees world-wide. Two main subgroups of PPV isolates have been determined, i.e. PPV-M and PPV-D, on the basis of serological or molecular tools focusing coat protein (CP) or CP gene. Two additional minor subgroups including cherry-infecting isolates and geographically limited El Amar isolates have also been confirmed (Lopez-Moya *et al*., 2000).

Based on symptomatology, the occurrence of sharka in Slovakia was reported for the first time around 1950 (Králiková, 1962). Later, the biological properties of several PPV isolates were characterized according to their reaction on selected herbaceous and woody test plants (Glasa *et al.*, 1997). Non-transmissibility of PPV by seed was confirmed (Glasa *et al*., 1999).

VARIABILITY OF PPV POPULATION

The first attempts to characterise the molecular variability of Slovak PPV isolates were carried out using RT-PCR targeting 3´terminal part of CP gene and subsequent RFLP analysis of PCR products (Wetzel *et al*., 1992). The subgroup differentiation based on *Rsa*I polymorphism of 44 isolates from naturally infected plum, myrobalan, apricot, almond and peach trees from commercial orchards and private gardens in 22 localities of western and middle Slovakia revealed the presence of isolates belonging to PPV-M and PPV-D subgroups (Fig. 1). PPV-M and PPV-D isolates were almost equally

represented in tested samples. Interestingly, the typing of PPV isolates from infected tolerant plum cultivars (Cacak´s cvs.) showed a large predominance of PPV-M isolates (Kúdela *et al*., 1998; Glasa *et al*., 1998). The PPV isolates were found to differ by the mobility of capsid proteins (CP) in the SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (Šubr and Glasa, 1999). To this date, no infection in sweet or sour cherry was identified in Slovakia.

RECOMBINANT ISOLATES OF PPV

Recently, a set of Slovak and French isolates was evaluated for the molecular variability in the P3-6K1 genomic region (Glasa *et al*., 2002a). Surprisingly, the Slovak BOR-3 isolate presented discordance in subgroup affiliation depending on different genomic regions and detailed sequence analyses confirmed that isolate is a natural recombinant between a D and an M PPV-type (Glasa *et al*., 2001). Biological assays demonstrated its capacity to be aphid- and graft- transmitted to various *Prunus* spp.

RNA recombination is thought to play an important role in the evolution, adaptation, repair and diversification of viral genomes. Therefore, further studies were undertaken to detect potential dissemination of recombinant isolates in Slovakia. A molecular tool based on PPV-M and D subgroup targeting P3-6K1, CI and CP regions of the PPV genome was used for recombinant identification. Closely related recombinant isolates were detected in various Slovak localities. Nearly all the PPV recombinants were recovered from plums and myrobalans. On the contrary, no recombinant was found on peach. Sequence analysis of the (Cter) NIb-(Nter) CP region of 10 recombinant isolates from Slovakia showed their high homology (over 98%) and an identical recombination breakpoint situated in the C terminus of the NIb gene (Glasa *et al*., 2002b). Moreover, the additional analyses of available Slovak PPV isolates identified as PPV-M typed in previous studies (Glasa *et al*., 1998) revealed that most of them have in fact recombinant characteristics.

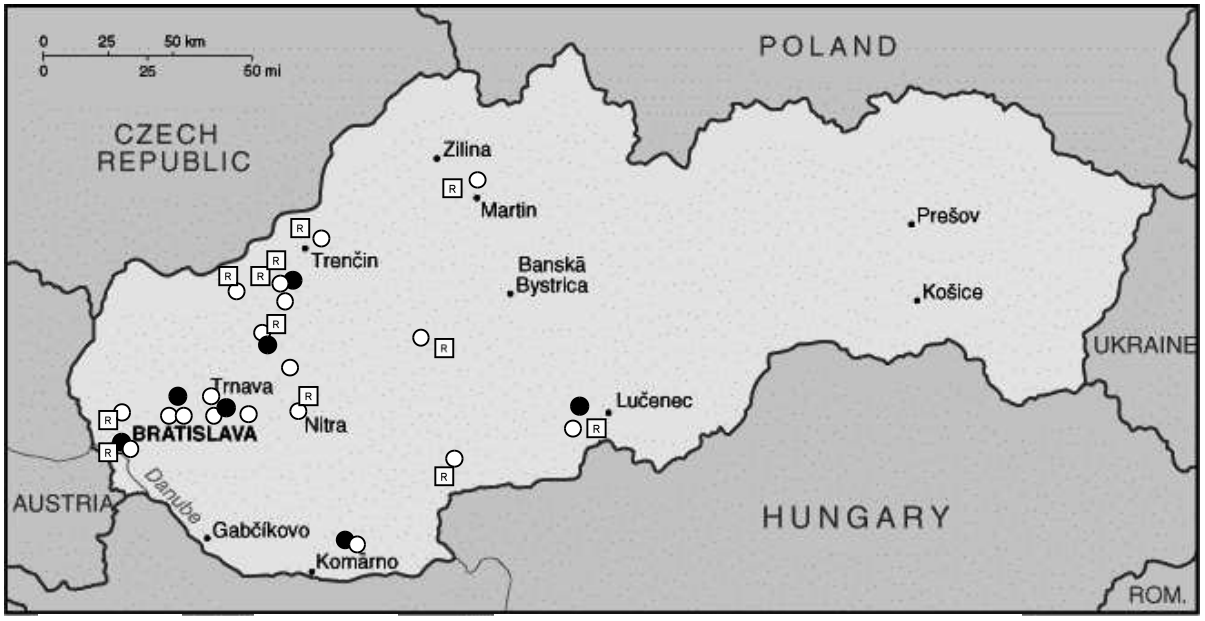

Fig. 1. A map marking the localities in western and central Slovakia where the PPV isolates were collected and analysed for recombinants

The exact point source of PPV recombinants could not be determined. However, some hypothesis could be put forward. Close molecular relationship of Slovak recombinant isolates supports the hypothesis that they arose from common ancestors. The linkage of recombinants to plum orchards of Bojnice and Krajné in western and central Slovakia (Fig. 1) planted in the early 1980's, as first noted with biological material originating from Yugoslavia, suggests a potential pathway. This hypothesis is also supported by the fact that the first reported PPV recombinant originated from former Yugoslavia (Cervera *et al*., 1993). In the late 1980's a new generation of tolerant plum cultivars selected in Cacak (Yugoslavia) dominated commercial plum plantings in Slovakia. Therefore, the coincidence of recombinants in plums,

could have been through unrestricted propagating material in the past, especially when health regulations were not enforced and in absence of available molecular detection techniques.

In summary, we revealed a high incidence of PPV in all expected localities in Slovakia. The presence of atypical populations of recombinant PPV must be taken into consideration as our studies demonstrated their apparent competitiveness with conventional PPV-M and D isolates.

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