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OCCURRENCE OF DIFFERENT *PLUM POX VIRUS* STRAINS IN SEVERAL STONE FRUIT SPECIES IN AUSTRIA

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SUMMARY - A survey was carried out in Austria to type PPV isolates in sone fruit orchards and hedges along their borders. A total of 181 isolates were typed by serological and molecular (IC-RT-PCR and PCR/RFLP in the CP and the Nib-genes) techniques. Selected isolates (54) were tested by DASI-ELISA using the following monoclonal antibodies (MAbs): 5B (universal), 4DG5 (PPV-D specific), AL (PPV-M specific), AC (PPV-C specific) and EA24 (PPV-El Amar specific). The vast majority of Austrian PPV isolates (159) belong to D strain and only a few isolates (16) could be assigned for sure to PPV-M. For 6 isolates, the serological and molecular data for strain identification were not in agreement, which could be explained by sequencing analyses.

Key words: Austria, stone fruits, PPV, virus strain, ELISA, PCR

RESUME *- Une enquête a été menée en Autriche afin de typer des isolats de PPV provenant de certains vergers fruitiers et des leurs haies de bordure. Au total, 181 isolats ont été typés en utilisant des techniques sérologiques et moléculaires (IC-RT-PCR et PCR-RFLP du gène de la protéine capsidique et du gène Nib). Des isolats sélectionnés (54) ont été testés en DASI-ELISA, en ayant recours aux anticorps monoclonaux (MAbs) suivants: le 5B (universel), le 4DG5 (spécifique pour le PPV-D), l'AL (spécifique pour le PPV-M), l'AC (spécifique pour le PPV-C) et l'EA24 (spécifique pour le PPV-El Amar). La grande part des isolats autrichiens de PPV (159) appartiennent à la souche D et seuls quelques isolats (16) pourraient être assignés sûrement au PPV-M. Pour 6 isolats, les données sérologiques et moléculaires concernant l'identification de la souche n'étaient pas concordantes, ce qui pourrait être expliqué par le séquençage.*

Mots-clés: *Autriche, espèces fruitières à noyau , PPV, souche virale, ELISA, PCR.*

INTRODUCTION

Dr. Vukovits (1961) reported the presence of PPV in Austrian plums in the Vienna area. To the question posed by Dr. Saric during the discussion about his thoughts on the origin of PPV in Austria, and how long the disease had been in the country, and how old the infected trees were, Dr Vukovits replied that, he suspected, diseased material had been probably imported during the war, and diseased plants were 10 20 years old. Although he announced for the future the intention in Austria to test stock material for viruses at least in the largest fruit tree nurseries (in the following years), little was done to raise the growers awareness on the importance of virus-free planting material. By 1988 in Styria, the major plum growing area, 21,000 trees had to be eradicated due to PPV and further 3,000 in 1989 (Keck, 1992). In 1986 at the IAM a project for the production of MAbs against PPV was initiated and by that time it was possible to identify infected material of *Prunus domestica, P. armeniaca* and *P. persica.*

MATERIALS AND METHODS

Over the last 4 years (1999-2002), 181 *Plum pox virus* (PPV) isolates were identified in different stone fruit growing areas in the eastern part of the country, i.e. Niederösterreich, Burgenland and Vienna (Fig. 1). Isolates were collected from plants in orchards, but also from hedges along the borders of the

orchards or along the roads in the close neighbourhood of the plots. These were in detail: *Prunus armeniaca* (27 isolates), *P. spinosa* (8), *P. cerasifera* (7), *P. persica* (11) and *P. domestica* (128).

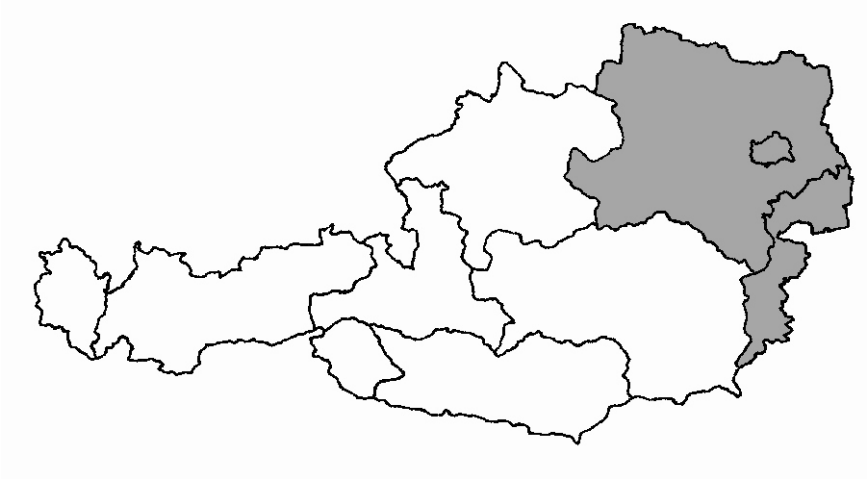

Fig. 1. PPV surveyed areas in Austria

Isolates were characterised by serological and molecular techniques (IC-RT-PCR, PCR/RFLP in the CP and the NIb-genes). Tissue cultures of *P. armeniaca* infected with selected isolates were established for a better availability of the pathogen the year round for the improvement of diagnostic and eradication procedures (Laimer, 2003), as well as for *in vitro* challenge infection experiments of transgenic plants (Laimer, 2002).

Some 54 selected isolates of wild and cultivated *Prunus* species (one from *P. armeniaca,* 2 from *P. cerasifera,* 2 from *P. persica* and 49 from *P. domestica*) were analysed by DASI-ELISA with the five internationally accepted monoclonal antibodies used for PPV detection and strain differentiation: 5B as universal antibody and 4DG5 as specific to PPV-D (Cambra *et al*., 1994), AL for PPV-M (Boscia *et al*., 1997), EA 24 for PPV-EA (Myrta *et al*., 1998a) and AC for PPV-C (Myrta *et al*., 2000).

Beyond that, seven PPV-specific MAbs, EA4, EA5, EA8, EA11, EA12 and EA18 (Myrta *et al*., 2001) were used to evaluate the intra-strain variability of Austrian isolates in comparison with other European isolates, in order to distinguish between different M isolates according to their geographic distribution: a Mediterranean and a Central - European subcluster. In our case this was of particular interest since we expected an answer on the geographic origin of M - strain isolates, should they appear.

RESULTS AND DISCUSSION

The vast majority of isolates encountered were of D - type, and only few isolates could be assigned with certainty to M - type (Table 1). The situation of PPV strains is similar to that reported for Slovakia (Glasa *et al*., 2003) and Czech republic (Polak *et al*., 2003) but in clear contrast to the results described from the Balkan countries (Varveri and Boutsika, 1998; Myrta *et al.,* 1998b; Dulic-Markovic, 2003; Kamenova *et al*., 2003; Stamo *et al*., 2003). According to the serological characterisation for intra-strain variability with the Mabs, they are M - isolates of the Mediterranean group (PPV-M_2), which could be correlated with the distribution pattern of planting material.

The differentiation by PCR/RFLP according to Wetzel *et al*. (1991) did not yield reliable results, since we found several isolates indicated as M - type strains with this method, which in the serological countercheck and by sequencing were found to be clearly D - type strains.

Table 1. Characterisation of Austrian PPV isolates

Number of isolates	Host plant	Strain typing	
		ELISA	PCR/RFLP
108	*Prunus domestica*	D	D
5*	" "	D	M
15	" "	M	M
26	*P. armeniaca*	D	D
1	" "	M	M
6	*P. cerasifera*	D	D
1*	" "	D	M
8	*P. spinosa*	D	D
11	*P. persica*	D	D

* Contradictory results

Sequencing of the amplified products confirmed a base pair exchange in the recognition site for the restriction enzyme (Fig. 2), which obviously leads to a false result (Mendonça, 2003). By comparing the sequences of selected isolates of PPV from Azores and Austria (Fig. 2), it was possible to verify that PPV-JA10 belongs to D type and PPV-33 to the M type, which is in agreement with serological and PCR-RFLP data. PPV-25, which was serologically recognised as D type, but was not digested in the PPV-D RsaI specific site (Wetzel *et al*., 1992), showed a sequence homology to PPV-M type in the putative RsaI restriction site, but the remaining sequence is typical for PPV-D strains (Mendonça, 2002). In fact, Olmos *et al*. (1997) developed their strain specific primers for PCR detection of different PPV strains exactly in this area, and one base pair mismatch versus four matches seem sufficient to overcome the problem.

Furthermore it might be advisable to use larger fragments, also of other sequence genomic regions, e.g. the NIb gene, for which Hammond *et al*. (1998) described an alternative protocol. However, these primers have also been improved (Hammond *et al*. in prep.).

CONCLUSIONS

Since Austria joined the EU in 1995 and adopted the respective legislation on the phytosanitary standards of stone fruit planting material (Directive 92/33/EEC), the increased activities of fruit consultants on the danger represented by PPV and the intensified inspection of nurseries by phytosanitary service have led to a general alert and therefore to a more rapid identification and subsequent removal of PPV-infected trees.

In this report, however, we could not include data on the situation concerning PPV in Styria, another major stone fruit producing area, but its presence has been confirmed and attempts to control it through the use of PPV-tolerant plum cultivars from Germany (Szith, pers. comm). In the other regions of Austria, stone fruits are grown mainly for domestic purposes. However, PPV has been detected by the Phytosanitary Control Agency also in plums from Tyrol (Riedle, pers. comm.).

In order to correctly estimate the incidence of this quarantine pest, we consider of upmost importance, besides strict inspections of nurseries, regular surveys of stone fruit orchards and neighbouring wild habitats. If populated by wild or native *Prunus* relatives, these could serve as intermediate hosts and allow unadvertised building-up of PPV inoculum.

Of major concern should be the list of species used for recommendation of plantation of wind belts or ornamental fences around villages, since they include sloe and elderberry, known hosts of PPV.

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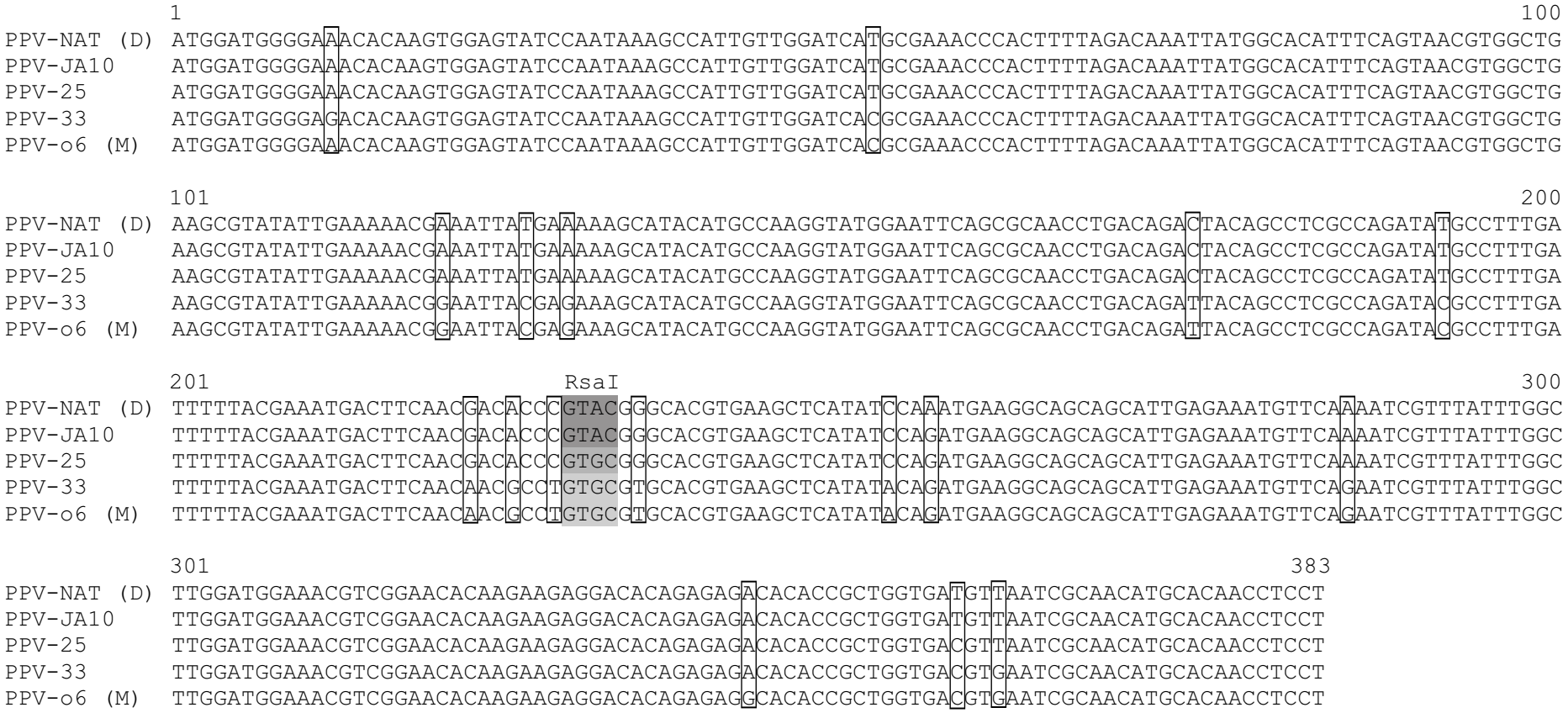
Fig. 2. Alignment of 3' terminal PPV-CP sequences from PPV isolates from Austria (PPV-25 and PPV-33) and Azores (PPV-JA10) with sequences obtained in the NCBI database from isolates of D type (PPV-NAT NC001445) and M type (PPV-o6 S57404).



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