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CHARACTERIZATION OF HUNGARIAN *PLUM POX VIRUS* ISOLATES

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SUMMARY - Characterization of 97 Hungarian PPV isolates was carried out by ELISA and PCR. PCR detected 59 PPV-M isolates, 25 PPV-D isolates and 15 D+M mixed infections. The serological analysis indicated 72 isolates belonging to PPV-M and 25 to PPV-D serotype. There was a reasonably good correlation between the results of ELISA and PCR. However, detection of PPV-D in mixed infections by PCR indicated the greater sensitivity of this method compared to ELISA. Contradictory results of serological and molecular characterization were obtained only in a single case.

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

RESUME *- Quatre-vingt-dix-sept isolats hongrois du PPV ont été caractérisés par ELISA et PCR- La PCR a détecté 59 isolats PPV-M, 25 isolats PPV-D et 15 infections mixtes M+D. L'analyse sérologique a fait ressortir 72 isolats appartenant au sérotype PPV-M et 25 au sérotype PPV-D. Quant au typage sérologique et moléculaire des souches PPV-D et M, une bonne corrélation a été mise évidence entre les résultats de l'ELISA et de la PCR. La détection des infections mixtes par la PCR a indiqué la sensibilité plus éleve de cette méthode par rapport à l'ELISA. Des résultats contradictoires de la caractérisation sérologique et moléculaire ont été obtenus dans un seul cas.*

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

INTRODUCTION

Four major types of P*lum pox virus* can be distinguished based on serological and molecular properties: PPV-Marcus (PPV-M) (Candresse *et al*., 1994), PPV-Dideron (PPV-D) (Kerlan and Dunez, 1979), PPV-Cherry (PPV-C) (Nemchinov and Hadidi, 1996) and PPV-El Amar (PPV-EA) (Wetzel *et al.,* 1991).

Reliable serotype-specific monoclonal antibodies (MAbs) are available for serological typing of PPV isolates: PPV-D (Lopez-Moya *et al.,* 1994; Cambra *et al.,* 1994; Navratil *et al.,* 1992), PPV-M (Boscia *et al.,* 1997), PPV-EA (Myrta *et al.,* 1998) and PPV-C (Myrta *et al.,* 2000).

First serological and molecular reports of the presence of D type isolates from plum and apricot in Hungary were provided by Lopez-Moya *et al.* (1997). Presence of M strain on plum and D strain on almond has been demonstrated using MAbs raised against different epitopes of PPV in DASI-ELISA (Pribék and Gáborjányi, 1997; Pribék *et al*., 1998). A coat protein fragment of PPV-D isolate from almond was sequenced (Pribék *et al.,* 2001). PPV-D and PPV-M isolates were detected during the screening of a high number of samples originating from plum, apricot, peach and almond trees by DASI-ELISA, nested PCR and RFLP (Szemes *et al.,* 2000; 2001).The difference of the SK 241 PPV-D peach isolate from the other D isolates was demonstrated by serotyping several PPV isolates from different countries (Myrta *et al.,* 2001). In this paper we report the latest typing results of the Hungarian PPV isolates.

MATERIALS AND METHODS

PPV isolates were collected during 1966-1996 from germplasm collections, orchards and nurseries from different parts of Hungary. The selected isolates originated from plum, apricot, peach, almond and ornamental *Prunus* trees and maintained on GF 305 (Table 1).

The collected isolates (97) were studied for their biological, serological and molecular characteristics. Indexing was done on four woody indicators (*Prunus domestica*, cv. Italian prune, *P. tomentosa*,

P. armeniaca seedling, *Prunus* hybrid GF 31) and three herbaceous species (*Chenopodium foetidum*, *Nicotiana clevelandii* and *N. benthamiana*).

PPV detection was confirmed by universal antibody MAb 5B (Cambra *et al.,* 1994). The serological typing of PPV isolates was carried out using the following MAbs: MAb 4DG11, (López-Moya *et al.,* 1994), MAb 03, MAb 06 (Navratil *et al.,* 1992) (D-specific); MAb AL (Boscia *et al.,* 1997) (M-specific); MAb EA (Myrta *et al.,* 1998) (EA-specific) and PPV-TUV (Myrta *et al.,* 2000) (C-specific).

D-, M-, El Amar- and C-specific primers were used for the molecular typing in a nested PCR system (Szemes *et al.,* 2000). RFLP of 243bp PCR products from CP was carried out with RsaI (Wetzel *et al.,* 1991).

Table 1. Characterization of Hungarian isolates of PPV

Year of isolation	Code of isolate	Source species	Origin (location)	ELISA	PCR
1966	SK-6	apricot	Budatétény	M	D,M
1966	SK-190	apricot	Budatétény	D	D
1968	SK-16	*P. glandulosa*	Vácrátót	M	M
1968	SK-43	apricot	Érd	M	M
1970	SK-18	*P. serotina*	Érd	M	D,M
1971	SK-41	apricot	Érd	M	M
1971	SK-44	apricot	Érd	M	M
1972	SK-22	peach	Balatonújhely	M	M
1972	SK-23	apricot	Siófok	M	M
1973	SK-12	plum	Bak	M	M
1973	SK-13	plum	Rákoskeresztúr	M	M
1973	SK-14	plum	Rákoskeresztúr	M	M
1973	SK-36	plum	Pesthidegkút	M	M
1973	SK-40	peach	Balatonújhely	M	M
1973	SK-82	plum	Pesthidegkút	M	M
1974	SK-2	plum	Siófok	M	M
1974	SK-3	peach	Érd	M	M
1974	SK-4	plum	Érd	M	M
1974	SK-48	greengage	Budapest	M	M
1974	SK-76	plum	Érd	M	M
1974	SK-77	plum	Érd	M	M
1975	SK-61	peach	Keszthely	M	M
1976	SK-54	peach	Siófok	M	D,M
1976	SK-55	peach	Sasad	M	M
1976	SK-56	peach	Sasad	M	M
1976	SK-58	peach	Sasad	M	M
1976	SK-59	peach	Sasad	M	M
1976	SK-69	plum	Érd	M	M
1978	SK-52	apricot	Cegléd	M	M
1978	SK-53	apricot	Cegléd	M	M
1978	SK-63	myrobalan	Cegléd	M	M
1978	SK-72	plum	Érd	M	M
1979	SK-64	apricot	Cegléd	M	D,M
1979	SK-74	plum	Érd	M	M
1979	SK-83	myrobalan	Cegléd	M	D,M
1979	SK-84	myrobalan	Cegléd	M	D,M
1982	SK-70	plum	Cegléd	M	M
1982	SK-91	apricot	Cegléd	M	D,M
1986	SK-30	plum	Pölöske	D	D
1986	SK-31	plum	Pölöske	M	D,M
1986	SK-33	plum	Pölöske	M	M
1986	SK-35	plum	Pölöske	M	M
1986	SK-37	plum	Pölöske	M	M
1986	SK-42	plum	Pölöske	M	M
1986	SK-45	plum	Pölöske	M	M

Year of isolation	Code of isolate	Source species	Origin (location)	ELISA	PCR
1986	SK-46	plum	Pölöske	M	M
1986	SK-47	plum	Pölöske	M	M
1986	SK-50	plum	Pölöske	M	M
1986	SK-60	plum	Pölöske	M	M
1986	SK-62	plum	Pölöske	M	M
1986	SK-65	plum	Pölöske	M	M
1986	SK-67	plum	Pölöske	M	D,M
1986	SK-68	plum	Pölöske	M	M
1986	SK-181	peach	Balatonboglár	M	D,M
1986	SK-185	peach	Balatonboglár	M	D
1986	SK-186	peach	Balatonboglár	M	M
1986	SK-188	peach	Balatonboglár	M	D,M
1991	SK-195	peach	Érd	D	D
1991	SK-196	peach	Érd	M	D,M
1991	SK-197	peach	Kecskemét-Borbás	D	D,M
1991	SK-198	peach	Kecskemét-Borbás	M	M
1991	SK-199	peach	Kecskemét-Borbás	M	M
1991	SK-201	peach	Kecskemét-Borbás	D	D
1991	SK-203	myrobalan	Cegléd	D	D
1991	SK-204	almond	Cegléd	M	M
1991	SK-205	wild apricot	Cegléd	M	M
1991	SK-206	peach	Cegléd	M	M
1991	SK-207	almond	Rákócifalva	M	M
1991	SK-208	apricot	Békéscsaba	M	M
1991	SK-209	peach	Békéscsaba	D	D,M
1991	SK-210	peach	Kondoros	D	D
1991	SK-211	peach	Érd	D	D
1991	SK-212	apricot	Velence	D	D
1991	SK-242	plum	Pölöske	D	D
1992	SK-233	peach	Kecskemét-Borbás	D	D
1992	SK-236	peach	Kecskemét-Borbás	D	D
1993	SK-192	apricot	Érd	M	M
1993	SK-193	peach	Soroksár	D	D
1993	SK-194	peach	Kecskemét-Borbás	M	D,M
1994	SK-237	peach	Siófok	M	M
1994	SK-238	peach	Csorna	D	D
1994	SK-239	peach	Csorna	D	D
1994	SK-240	peach	Csorna	D	D
1994	SK-241	peach	Csorna	D	D
1995	SK-243	peach	Siófok	D	D
1995	SK-244	peach	Siófok	M	M
1995	SK-245	plum	Debrecen	M	M
1995	SK-246	plum	Debrecen	M	M
1995	SK-247	apricot	Debrecen	D	D
1995	SK-248	plum	Csorna	D	D
1995	SK-249	wild almond	Ortilos	D	D
1996	SK-258	gage	Ádánd	D	D
1996	SK-261/A	plum	Kecskemét	M	M
1997	SK-251	plum	Alsótekeres	M	M
1997	SK-253	peach	Alsótekeres	D	D
1997	SK-256	apricot	Érd	D	D
1997	SK-257	myrobalan	Õrtilos	M	M

RESULTS AND DISCUSSION

It was demonstrated that the woody and herbaceous indicators responded with similar symptoms to PPV inoculation, therefore they were not suitable for the differentiation of strains.

PCR detected 58 PPV-M and 24 PPV-D and 15 D and M in mixed infection, whereas the serological analysis indicated 72 isolates belonging to PPV-M and 25 to PPV-D serotypes. PCR and ELISA results were contradictory in a single case. Isolate SK 185 was characterized by PCR as a PPV-D strain, while ELISA indicated PPV-M type. The RFLP results supported PCR typing. Some 32 of 58 M isolates detected by PCR originated from plum (56%), 14 from peach (24%), 9 from apricot (15%), 2 from almond (3%) and 1 from ornamental *Prunus* (2%). On the other hand, 14 of 24 D isolates were from peach (58%); 5, plum (20%); 4, apricot (16%) and one almond (4%) (Table 2).

Table 2. Distribution of PPV strains detected by PCR on different *Prunus* species in Hungary

Species	No of plants	M strain	D strain	D+M
		Nr.	Nr.	Nr.
plum	41	32	5	4
apricot	16	9	4	3
peach	35	14	14	7
almond	3	2	1	-
ornamental *Prunus*	2	1	-	1
Total	97	58	24	15

CONCLUSIONS

Based on our results, the following conclusions can be drawn:

in Hungary, the incidence of M strains is higher (about double) than D strains;

increased incidence of D strain had been observed since the middle of the '80s. It may be explained by the social and economic changes that took place in Hungary at that time, i.e. more liberal importation of new varieties was allowed, which potentially pses a higher risk to introduce materials infected with PPV-D.

Some remarkable observations were made concerning the presence of the particular PPV strains on certain fruit tree species:

M strain is most frequently on plum, and D strain on peach;

incidence of strains D and M is similar on apricot;

in Hungary, both strains are reported on almond.

Regarding serological and molecular typing of the PPV-D and M strains, there was a reasonably good correlation between the results of ELISA and PCR. Detection of mixed infection by PCR indicates the higher sensitivity of this method over the ELISA. Contradictory results of serological and molecular characterization were obtained only in a single case. Further studies on these topics are in progress.

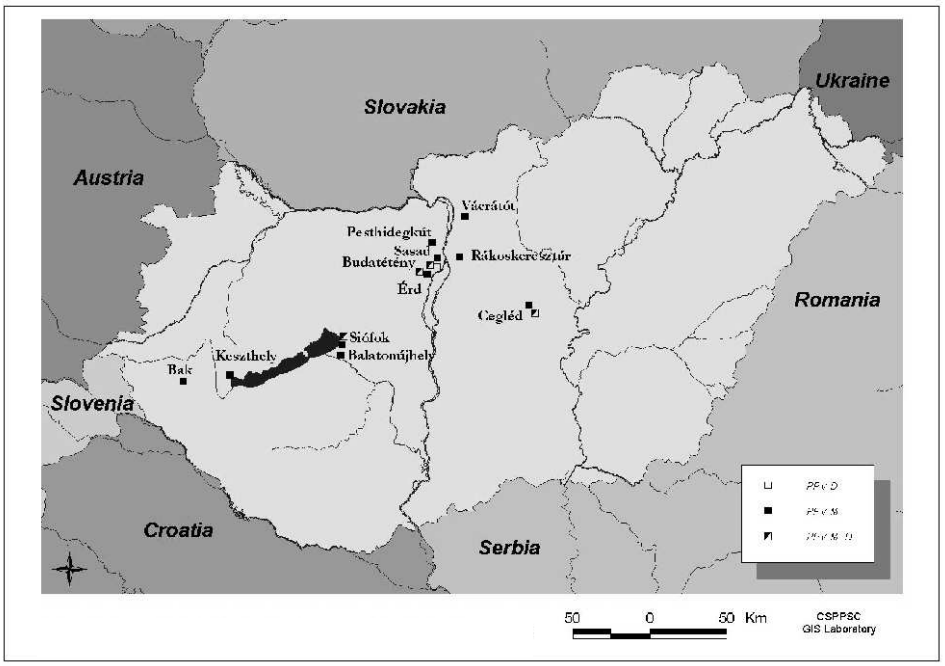

Fig.1. Map of PPV strains until 1985 in Hungary

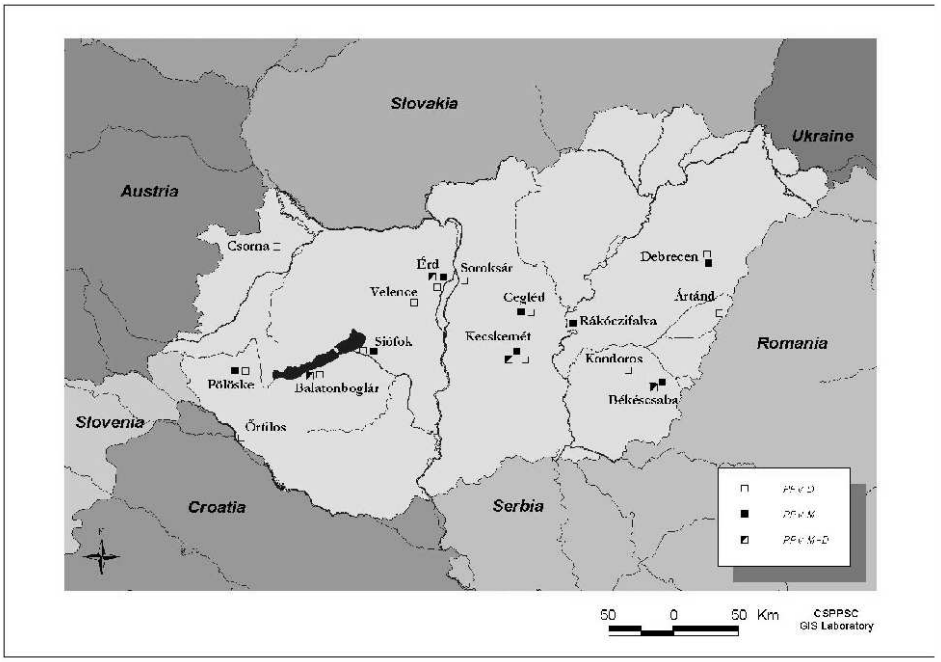

Fig.2. Map of PPV strains after 1985 in Hungary

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