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*in*

Myrta A. (ed.), Di Terlizzi B. (ed.), Savino V. (ed.).  
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Bari : CIHEAM

Options Méditerranéennes : Série B. Etudes et Recherches; n. 45

2003

pages 61-63

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=3001771>

To cite this article / Pour citer cet article

Dulic-Markovic I. **Plum Pox Virus strains in Yugoslavia**. In : Myrta A. (ed.), Di Terlizzi B. (ed.), Savino V. (ed.). *Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region*. Bari : CIHEAM, 2003. p. 61-63 (Options Méditerranéennes : Série B. Etudes et Recherches; n. 45)



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# PLUM POX VIRUS STRAINS IN YUGOSLAVIA

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**SUMMARY** - Plum and peach samples were collected from different regions in Yugoslavia and strain identification was done by using: (i) PCR-RFLP; (ii) RT PCR with strain specific primers; (iii) DAS-ELISA with PPV-D and PPV-M specific Mabs; and (iv) EBIA. Results showed the existence of three groups of isolates belonging to M and D serotypes. In the first two groups, PPV-M predominated (120 of 143 isolates studied), while the remaining 23 isolates belonged to PPV-D and were scattered sporadically through the area sampled. A third group of 34 isolates that were collected in plum and apricot from central Serbia were serologically PPV-M (coat protein migration) and negative against D-specific panel of MABs. However, all of them were found to have an *Rsa*I restriction site on the 243 bp amplified fragment, which is typical of D isolates. Grafting experiments supported previous findings at Cacak Centre that PPV isolates from central Serbia do not infect peach. Considering the differences in epidemic potential and pathogenicity, PPV typing based on serotypes may not accurately reflect strains based on pathogenicity.

**Key words:** Yugoslavia, stone fruits, PPV, virus strain, ELISA, PCR

**RESUME** - Des échantillons de prunier et pêcher ont été prélevés dans différentes régions de la Yougoslavie et l'identification des souches a été réalisée en ayant recours aux techniques suivantes : la PCR-RFLP, la RT-PCR avec des amorces spécifiques pour les souches, la DAS-ELISA avec des Mabs spécifiques pour le PPV-D et le PPV-M et l'EBIA. Les résultats ont montré la présence de trois groupes d'isolats appartenant aux sérotypes M et D. Dans les deux premiers groupes, le PPV-M était prédominant (120 isolats sur 143 étudiés), alors que les 23 isolats restants appartenaient au PPV-D et étaient répandus sporadiquement dans la région à l'étude. Un troisième groupe de 34 isolats, qui avaient été collectés sur des pruniers et abricotiers en Serbie centrale, étaient sérologiquement des PPV-M (migration de la protéine capsidique et réaction négative contre les Mabs spécifiques pour le D). Cependant, tous ces isolats affichaient un site de restriction *Rsa*I sur le fragment amplifié de 243 paires de bases, qui est typique des isolats D. Des essais de greffage ont confirmé les données précédemment obtenues au Centre de Cacak, d'après lesquelles les isolats du PPV de la Serbie centrale n'infectent pas le pêcher. Compte tenu des différences du potentiel épidémique et de la pathogénicité, le typage du PPV basé sur les sérotypes ne reflète pas toujours correctement la pathogénicité des souches.

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

## INTRODUCTION

Sharka, caused by *Plum pox virus* (PPV), is an economically important disease of plum, apricot and peach in Yugoslavia. First recognized there in 1935, it is now widespread over Europe. Sharka control is based on the use of tolerant and resistant varieties. This approach demands PPV strain identification, because isolates vary in their rate of spread by aphid, host range and pathogenicity.

## MATERIALS AND METHODS

Plum and peach samples were collected from different regions in Yugoslavia and strain identification was done using 4 methods: PCR-RFLP (Wetzel *et al.*, 1991), RT-PCR with strain-specific primers (Candresse *et al.*, 1995), DAS-ELISA with D and M specific monoclonal antibodies and by EBIA (Quiot-Douine *et al.*, 1990). Testing methods used Czech (Navratil *et al.*, 1998) and Italian MABs (Boscia *et al.*, 1997), limiting serological studies to the detection of M and D serotypes.

## RESULTS AND DISCUSSION

Survey in Yugoslavia showed the existence of three groups of PPV isolates belonging to serotypes M and D (Table 1). In the first two groups, PPV-M predominates (120 of 143 isolates), while the remaining 23 isolates were the D serotype and were scattered sporadically through the area sampled. All Yugoslavian peach samples were of the M type, except for one aberrant D isolate. Typical M isolates were identified from peach and plum in northern (Vojvodina) and southern regions of Serbia. A separate collection of 12 plum PPV isolates made in the early 1970s near Novi Pazar and maintained on *P.tomentosa* at the Cacak Fruit and Viticulture Research Centre, were also of the M serotype.

A third group of 34 isolates, representing plum and apricot collections from central Serbia (near Cacak and Valjevo) (Fig. 1) gave different serological and PCR results from the samples above. All 34 samples were serologically of the M serotype, showed coat protein migration characteristic of the M serotype; and tested negative against panel of D Mabs. However, all possessed an *RsaI* restriction site on the 243 bp amplified fragment, typical of D isolates. Grafting experiments supported previous findings of Cacak Centre that PPV isolates from central Serbia do not infect peach.

Table 1. Differentiation of PPV isolates on the basis of their reaction with D and M specific monoclonal antibodies, coat protein mobility, *RsaI* polymorphism of 243 bp fragment and their pathogenicity to peach

Strain	Nr. of isol.	MAbs				CP	RFLP *		PCR **		Peach
		NO3 (D)	NO6 (D)	NO8 (D)	AL (M)		<i>RsaI</i>	<i>AluI</i>	PD	PM	
M (PS)	120	-	-	-	+	38 K	-	+	-	+	+
M <i>RsaI</i>	34	-	-	-	+	38 K	+	+	-	+	-
Valj						40 K					
D	14	+high e.	+ high e.	+high e.	-	36 K	+	+	+	-	+
D peach (Dora)	1	+low e.	+low e.	+low e.	-	36 K	+	+	+	-	+
D08(98/OH)	8	-	-	+	-	37 K	+	+	+	-	0

\* RT PCR-RFLP (Wetzel *et al.*, 1991)

\*\* RT PCR RT PCR with strain specific primers PD i PM (Candresse *et al.*, 1995)

+ positive reaction; - negative reaction; 0 no results

Results of grafting experiment on the pathogenicity of different isolates on different varieties and hybrids showed that tolerance, resistance and immunity were greatly dependent on the virus isolate (Table 2).

Table 2. Reaction of different varieties on different strains

Isolate	Variety*	Cacanska Rodna		Cacanska Najbolja		Opal		G12	
		May 1	May 14	May 1	May 14	May 1	May 14	May 1	May 14
M (PS)		++	+++	+	+++	++	++	-	+
D08 (98/OH)		++	++	+	+++	-	+	++	++
D (Dora)		-	+++	+	++	++	+++	-	-
M <i>RsaI</i> (Valj)		++	+++	-	-	++	++	-	-
D (NAT)		+	+++	+	+++	+++	+++	+++	+++

\* Artificial inoculation by chip budding on March 16 in greenhouse. Visual assessment, - proved by ELISA and PCR

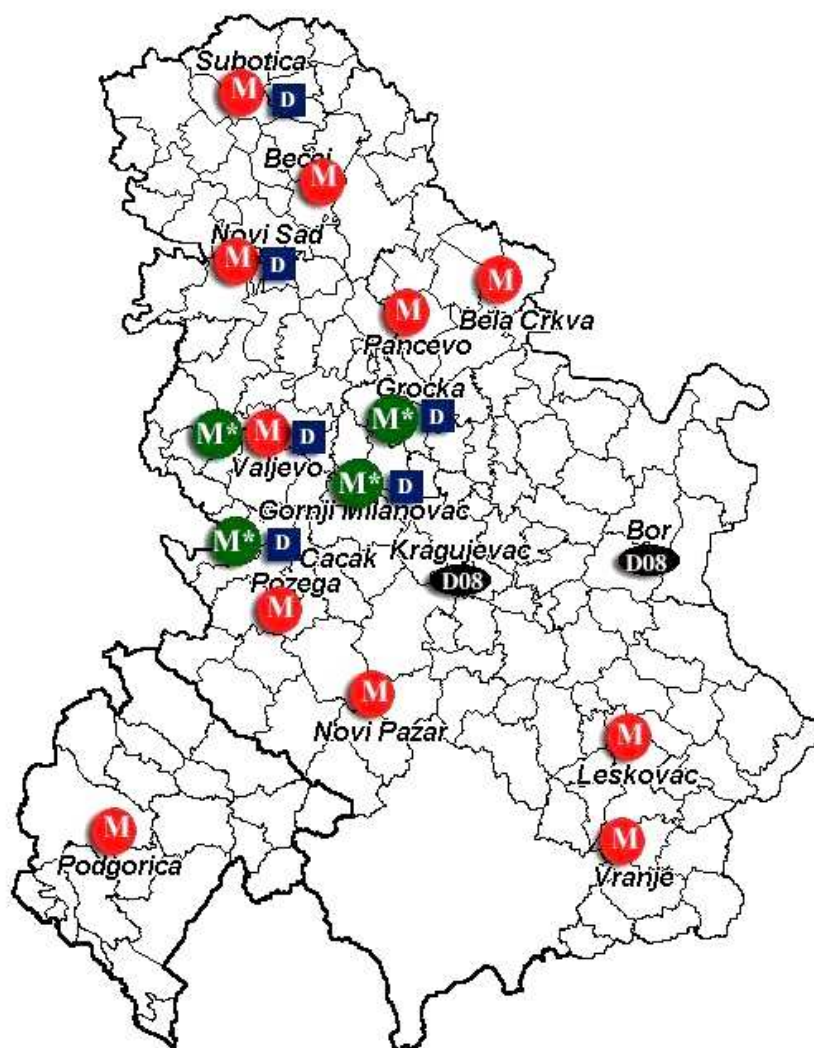


Fig. 1. Distribution of PPV strains in Yugoslavia

The results of this survey on typing Yugoslavian PPV isolates provide a possible explanation for inconsistencies found in some previous PPV studies with regard to the reactions of stone fruit cultivars and hybrids against PPV infection, the rapidity of PPV spread, and the ability of PPV to infect the peach. All this may ask for reflections on PPV strain differences in epidemic potential and pathogenicity. PPV strains based on serotypes may not accurately reflect virus strains based on pathogenicity.

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