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SEROLOGICAL CHARACTERIZATION OF *PRUNE DWARF VIRUS* ISOLATES

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Abstract: *Prune dwarf virus* (PDV), a worldwide pathogen of stone fruit trees, has many isolates with different biological, serological and molecular properties. Monoclonal antibodies (MAbs) to the *Prunus mahaleb* isolate of PDV were used to investigate the serological variability of virus isolates, by TAS-ELISA (triple antibody sandwich enzyme-linked immunosorbent assay). The twenty-two PDV isolates from Germany (1), Italy (7), Poland (13) and the USA (1) were characterised against eight single MAbs. The virus showed high serological variability. Analysis of the MAbs reaction allowed for the identification of 13 serogroups.

Key words: *Prune dwarf virus*, MAbs, serogroups, TAS-ELISA

INTRODUCTION

Prune dwarf virus (PDV) is a member of the *Bromoviridae* family, genus *Ilarvirus*. PDV possesses a tripartite genome and isometric to bacilliform particles (Fauquet *et al*. 2005). *Prune dwarf virus* is widespread in different stone fruit species (Fulton 1970). Several isolates with different biological, serological and molecular characteristics have been described (Waterworth and Fulton 1964; Paduch-Cichal 2000; Vascova *et al*. 2000; Fonseca *et al*. 2005; Ulubas *et al*. 2009).

Monoclonal antibodies (MAbs) have been produced and used for differentiating PDV isolates. Nine out of 77 MAbs, detected virus isolates from California, Washington, Italy, Bulgaria, Germany, Hungary or France and identified *Prunus cerasus* L. and *Prunus persica* Borkh.) serotypes (Jordan *et al*. 1991). Boari *et al*. (1997) used eight monoclonal antibodies in the serological characterization of eight PDV isolates by TAS-ELISA (triple antibody sandwich enzyme-linked immunosorbent assay). The results demonstrated that serological differentiation among these virus isolates was possible. The results showed that PDV-B, one of the serotypes identified, seems preferentially associated with almond.

The purpose of our study was to determine the serological variability among 22 PDV isolates from different *Prunus* species. Classification of these new virus isolates used eight monoclonal antibodies.

MATERIALS AND METHODS

Twenty-two PDV isolates were collected from virus – infected breaking buds of *Prunus amygdalus* Batsh., *P. avium* L., *P. cerasus* L., *P. domestica* L. and *P. persica* Borkh. trees. The isolates were from Germany (1), Italy (7), Poland (13) and USA (1) (Table 1).

Eight MAbs (PD-3, PD-6, PD-7, PD-8, PD-9, PD-10, PD-11 and PD-12) were used in this study. Boari *et al*. (1997) used PDV isolate (PDV IAM11) from *Prunus mahaleb* as antigen to obtain these specific antibodies. Then, eight hybridoma lines were selected and cloned. Monoclonal antibodies were received from Dipartimento di Protezione delle Piante e Microbiologia Applicata (Università degli Studi di Bari and CNR, Istituto di Virologia Vegetale,Unit of Bari, Italy).

PDV was detected in different stone fruit species by DAS-ELISA (double antibody sandwich enzyme-linked immunosorbent assay) (Clark and Adams 1977) using a commercial kit of rabbit polyclonal antibodies (PAbs) (LOEWE Biochemica GmbH). After the first screening, 22 representative virus isolates were selected based on geographical origin, host species and symptoms. PDV-positive samples were then tested using the TAS-ELISA technique (Spiegel *et al*. 1996) with a panel of 8 MAbs.

The results were assessed by measuring absorbance at 405 nm. A_{405} values above 0.200 were considered positive for PDV. The control values were below this threshold.

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Table 1. Twenty two *Prune dwarf virus* isolates collected from virus – infected breaking buds of almond, peach, plum, sour cherry and sweet cherry trees, from Germany (1), Italy (7), Poland (13) and the USA (1)

Isolate	Original host	Origin of isolate
PDV-Almond2	almond cv. unknown	Italy
PDV-PE15-28	peach cv. unknown	Germany
PDV-PE247	peach cv. Kwiat Majowy	Poland
PDV-Plum5	plum cv. unknow	Italy
PDV-PL13	plum cv. Armed	Poland
PDV-PL1-19	plum cv. unknown	Italy
PDV-SO14	sour cherry Kormed	Poland
PDV-SO20SZ1	sour cherry cv. Royal Burgundy	Poland
PDV-SO20SZ3	sour cherry cv. Royal Burgundy	Poland
PDV-SO40	sour cherry cv. Korund	Poland
PDV-SOF15P1	sour cherry cv. Big Lory	Italy
PDV-SOF15P11	sour cherry cv. Nomene	Italy
PDV-SOF17P17	sour cherry cv. Big Core	Italy
PDV-SW6-1	sweet cherry cv. unknown	Italy
PDV-SW7	sweet cherry cv. Poznańska	Poland
PDV-SW9-1	sweet cherry cv. unknown	USA
PDV-SW63	*Prunus avium* clone F12/1	Poland
PDV-SW78	*Prunus avium* clone F12/1	Poland
PDV-SW145W	sweet cherry cv. Büttnera Czerwona	Poland
PDV-SWI-35	sweet cherry cv. unknown	Poland
PDV-SWM1	sweet cherry cv. Napoleona	Poland
PDV-SW-Regina	sweet cherry cv. Regina	Poland

RESULTS AND DISCUSSION

The virus-specific PAbs (polyclonal antibodies) used in this study detected PDV, regardless of the original host or geographic origin. The virus-specific PAbs did not differentiate among the twenty-two PDV isolates (not shown).

Since each MAb recognised a different panel of isolates, it was assumed that the 8 MAbs targeted a different epitope. MAbs 8, 11, 7, 9 and 6 reacted with 18, 17, 16, 14 and 13 isolates, respectively. MAbs 10, 12 and 3 reacted with 8, 3 and 1 isolates, respectively. Analysis of the MAbs reaction allowed the identification of 13 serogroups, including the 'PDV-B' of Boari *et al*. (1997) corresponding to serogroup 12 of this study (Boscia *et al*. unpublished). None of the eight tested MAbs reacted positively with our PDV-PL13 and PDV-SOF15P1 isolates (Table 2). The variability of isolates from the same host was noted (Table 3). Nine sweet-cherry isolates were assigned to five serogroups. Seven sour-cherry PDV isolates were divided into six different serogroups. Three plum and two peach isolates were grouped into three and two separate serogroups, respectively. One PDV isolate from almond represented subgroup number 4 (positive with MAb PD-8, -9, -11), different from serogroup; PDV-B; described by Boari *et al*. (1997), positive with MAb PD-6, -7, -8, -9, -10, -11 (Boscia *et al*., unpublished).

Virus variability as related to country of origin is shown in table 4. There were nine serogroups representing the isolates that occurred in a single country: five in Poland (represented by PDV-SO20SZ1, PDV-SO-20SZ3, PDV-SW78, PDV-SO14, PDV-SW145W), three in Italy (represented by PDV-Almond2, PDV-Plum5, PDV-SO15P11), and one in Germany (represented by PDV-PE15-28). The remaining isolate population (13 isolates) contained isolates from more than one country.

Jordan *et al*. (1991) and Boari *et al*. (1997) used monoclonal antibodies for the serological differentiation of PDV isolates. The results of these investigations have been confirmed in the study presented in this article. This is the first case of such serological characterization of PDV isolates tested in Poland. In our experiment, the serological characterization of virus isolates was performed by TAS-ELISA. Differentiation among 22 PDV isolates was obtained using eight of MAbs including 3, 6, 7, 8, 9, 10, 11 and 12 in TAS-ELISA. Analysis of the MAbs reaction allowed us to identify 13 serogroups. The serological variability could not be correlated with host species or country of origin. Boari *et al*. (1997) obtained three different serotype groups, one of which (PDV-B) was only comprised of the two *Prunus amygdalus* Batsh. isolates. Their examination of 120 samples collected from field-grown PDV-infected *Prunus* trees (40 of which were almonds), confirmed the natural existence of a wide range of serological PDV variants, which yielded 36 different types of reactants. The PDV-B serotype was detected in 38% of the almond trees tested, but only in three of the other 86 PDV-infected *Prunus* plants. The possible prevalence of serogroup PDV-B in almond isolates was not confirmed in this study.

The serological analysis showed that PDV isolates can be differentiated by a panel of 8 MAbs, as a possible consequence of the occurrence of a large number of serotype specific amino acid substitutions.

Table 2. Serological reaction of twenty-two *Prune dwarf virus* isolates obtained from infected breaking buds of almond (PDV-Almond2), peach (PDV-PE15-28, PDV-PE247), plum (PDV-Plum5, PDV-PL13, PDV-PL1-19), sour cherry (PDV-SO14, PDV-SO20SZ1, PDV-SO20SZ3, PDV-SO40, PDV-SOF15P1, PDV-SOF15P11, PDV-SOF17P17) and sweet cherry (PDV-SW6-1, PDV-SW7, PDV-SW9-1, PDV-SW63, PDV-SW78, PDV-SW145W, PDV-SWI-35, PDV-SWM1, PDV-SW-Regina) trees with eight MAbs: PD-3, PD-6, PD-7, PD-8, PD-9, PD-10, PD-11, PD-12 and MAbs mixture (PD-3, PD-6, PD-7, PD-8, PD-9, PD-10, PD-11, PD-12)

Isolate	MAbs									Serogroup
	3	6	7	8	9	10	11	12	M*	
PDV-PL13	–	–	–	–	–	–	–	–	–	1
PDV-SOF15P1	–	–	–	–	–	–	–	–	–	1
PDV-SO20SZ1	–	–	+	–	–	–	–	–	–	2
PDV-SO20SZ3	–	–	+	–	–	–	–	–	+	2
PDV-SW78	–	–	–	+	–	–	–	+	+	3
PDV-Almond2	–	–	–	+	+	–	+	–	+	4
PDV-PE15-28	–	–	+	+	+	–	+	–	+	5
PDV-Plum5	–	+	–	+	+	–	+	–	+	6
PDV-SO14	–	–	–	+	+	+	+	–	+	7
PDV-PE247	–	+	+	+	+	–	+	–	+	8
PDV-PL1-19	–	+	+	+	+	–	+	–	+	8
PDV-SO40	–	+	+	+	+	–	+	–	+	8
PDV-SW6-1	–	+	+	+	+	–	+	–	+	8
PDV-SW63	–	+	+	+	+	–	+	–	+	8
PDV-SW9-1	–	+	+	+	–	+	+	–	+	9
PDV-SWI-35	–	+	+	+	–	+	+	–	+	9
PDV-SWM1	–	–	+	+	+	+	+	–	+	10
PDV-SOF15P11	+	+	+	+	+	+	+	–	+	11
PDV-SW7	–	+	+	+	+	+	+	–	+	12
PDV-SW-Regina	–	+	+	+	+	+	+	–	+	12
PDV-SOF17P17	–	+	+	+	+	+	+	–	+	12
PDV-SW145W	–	+	+	+	+	+	+	+	+	13

+ a positive reaction; – a negative reaction; M* – MAbs (monoclonal antibodies) mixture: PD-3, PD-6, PD-7, PD-8, PD-9, PD-10, PD-11 and PD-12; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 - number of serogroup

Table 3. Distribution of serogroups within host species. Number of *Prune dwarf virus* isolates and number of serogroups on the basis of the reaction of each of eight MAbs with twenty-two *Prune dwarf virus* isolates originating from different *Prunus* species (almond, peach, plum, sweet- and sour-cherry)

Host species	Tested isolates [No.]	Identified serogroups [No.]
Sweet-cherry	9	5
Sour-cherry	7	6
Plum	3	3
Peach	2	2
Almond	1	1

Table 4. Distribution of PDV serogroups by country of origin. Number of *Prune dwarf virus* isolates and number of specific serogroups on the basis of the reaction of each of eight MAbs with twenty-two *Prune dwarf virus* isolates originating from different countries: Poland, Germany, Italy and the USA

Country	Isolates [No.]	Hosts [No.]	Identified serogroups [No.]	Country specific serogroups [No.]	Isolates in country specific serogroups [No.]
Poland	13	4	8	5	5
Italy	7	4	6	3	3
Germany	1	1	1	1	1
USA	1	1	1	none	none

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