

SEROLOGICAL CHARACTERISATION OF MEDITERRANEAN *PRUNUS NECROTIC RINGSPOT VIRUS* ISOLATES

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SUMMARY

Prunus necrotic ringspot virus (PNRSV), a worldwide pathogen of stone fruits, has many isolates with different biological, serological, and molecular properties. Monoclonal antibodies (MAbs) to a peach isolate of PNRSV were used to investigate the serological variability of PNRSV isolates by DASI-ELISA. Isolates from different stone fruit species and different Mediterranean countries were first identified with polyclonal antisera (PAbs), then 81 isolates were selected from Albania (3), Cyprus (10), Greece (5), Italy (31), Lebanon (6), Malta (5), Tunisia (7), Turkey (7) and Spain (1) and characterised against 10 single MAbs. Six U.S. isolates were included as controls. The virus showed high serological variability as shown by the identification of 34 serogroups, many of which were host-specific (64%) and country-specific (67%).

Key words: PNRSV, MAbs, serological characterisation, serogroups, DASI-ELISA.

INTRODUCTION

Prunus necrotic ringspot virus (PNRSV), a member of the genus *Illarvirus*, possesses a tripartite genome and isometric to bacilliform particles. The virus is widespread in different stone fruit species (Barbara, 1988; Mink, 1992; Diekmann and Putter, 1996). Many isolates with different biological, serological, and molecular characteristics have been described, some being

sufficiently distinct so as to be identified as strains (Barbara *et al.*, 1978; Crosslin and Mink, 1992).

The serological variability of PNRSV is well known. Mink *et al.* (1987) grouped cherry isolates of PNRSV in three serotypes (CH3, CH9 and CH30) using polyclonal antisera. The serological diversity of isolates from rose (Casper, 1973) and hops (Smith *et al.*, 1986) has also been documented.

Monoclonal antibodies (MAbs) have been produced and used for differentiating PNRSV isolates. For example (i) Halk *et al.* (1984) identified 3 serotypes from 9 isolates tested; (ii) production at the University of Bari (UBA) of 10 MAbs and their use against 38 isolates primarily from Italy, allowed identification of 17 serological variants (Boari *et al.*, 1998); (iii) Spiegel *et al.* (1999), using eight of the UBA MAbs against 16 virus isolates recognised four serological subgroups, three clear-cut and one tentative.

The purpose of this study was to determine the serological variability in a large PNRSV population from different stone fruit species in the Mediterranean.

MATERIALS AND METHODS

Source of isolates. Eighty-one PNRSV isolates were collected from nine different Mediterranean countries and the USA. Virus-infected leaf samples were from almond, apricot, peach, plum and cherry trees. The isolates were from: Albania (3), Cyprus (10), Greece (5), Italy (31), Lebanon (6), Malta (5), Tunisia (7), Turkey (7) and Spain (1). Six US isolates were also included as controls.

Monoclonal antibodies (MAbs). These were obtained from an Italian peach isolate of PNRSV by Boari

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Table 1. PNRSV serogroups established by means of 10 Mabs.

Serogroup	MAbs											Isol. no.	F (%)
	116	460	294	141	348	399	563	41	503	236	M*		
1	+	+	+	+	+	+	+	+	+	+	+	12	14.9
2	+	+	+	+	+	+	+	+		+	+	1	1.2
3	+	+		+	+	+	+	+	+	+	+	11	13.7
4	+	+	+	+	+	+	+	+	+		+	2	2.5
5	+	+		+	+	+	+	+	+		+	6	7.6
6	+	+	+	+	+	+	+	+			+	2	2.5
7	+	+	+	+	+	+		+	+		+	4	4.9
8	+	+		+	+	+	+	+			+	4	4.9
9	+	+		+	+	+		+	+		+	1	1.2
10	+	+	+	+		+		+	+		+	1	1.2
11	+	+	+	+	+	+		+			+	1	1.2
12	+	+	+	+		+	+	+			+	1	1.2
13	+	+		+		+	+	+			+	1	1.2
14	+	+	+	+	+				+		+	1	1.2
15	+	+	+	+	+	+					+	1	1.2
16	+	+		+		+		+			+	1	1.2
17	+	+	+	+	+						+	2	2.5
18	+	+		+	+				+		+	1	1.2
19	+	+	+	+					+		+	1	1.2
20	+	+		+	+					+	+	2	2.5
21	+			+	+		+	+			+	1	1.2
22	+	+		+		+	+				+	1	1.2
23	+	+	+	+							+	2	2.5
24	+		+	+	+						+	1	1.2
25	+	+	+		+						+	2	2.5
26	+	+		+							+	3	3.7
27	+		+	+							+	1	1.2
28	+	+			+						+	1	1.2
29	+	+	+								+	1	1.2
30	+			+							+	1	1.2
31	+	+									+	7	8.8
32		+	+								+	1	1.2
33	+										+	1	1.2
34		+									+	2	2.5
no.	32	29	18	27	19	16	11	15	10	4	34	81	
F (%)	94.1	85.2	52.9	79.4	55.8	47.0	32.3	44.1	29.4	11.7	100.0		100.0

* MAb mixture; F: frequency.

et al. (1998), and were numbered 41, 116, 141, 236, 294, 348, 399, 460, 503 and 563.

Virus detection and isolate characterisation.

PNRSV was detected in different stone fruit species was by DAS-ELISA (Clark and Adams, 1977) using commercial (Loewe, Sauerlach, Germany) or locally produced kits. After a first screening, a number of representative isolates were selected based on geographical origin, host species and symptoms. PNRSV-positive samples were then tested by DASI-ELISA (Cambra *et al.*, 1994) using the panel of 10 MABs.

RESULTS

Twelve of 81 isolates reacted with all the MABs, whereas three isolates were recognised by only one MAB. Mabs 116, 460 and 141 reacted with 79, 76 and 73 isolates respectively, whereas Mab 236 reacted only with 26 isolates. Since each MAB recognised a different panel of isolates (Table 1), it was assumed that the 10 MABs targeted to a different epitopes. Analysis of the MAB reactions allowed identification of 34 serogroups (Table 1).

As to the variability of isolates from the same host, apricot isolates showed the highest rate of diversity (91%) as 10 of 11 isolates fell in different serogroups. Isolates from peach, plum, cherry and almond showed diversity ranging from 57 to 64% (Table 2). There were 22 host-specific serogroups (64%) representing nearly 30% of the virus isolates.

Virus variability as related to country of origin is shown in Table 3. About 67% of the serogroups, representing 32% of the isolates, occurred only in a single country, whereas nearly 68% of the isolate population

was composed by isolates coming from more than one country.

DISCUSSION

To our knowledge, our investigation is the largest-scale serological characterisation of PNRSV isolates performed by DASI-ELISA using monoclonal antibodies. This new set of anti-PNRSV MABs has provided wider serological information than currently available, as predicted by Halk *et al.* (1984).

Although coat proteins of PNRSV isolates from different host species and geographic origins are extensively conserved (Scott *et al.*, 1998; Aparicio *et al.*, 1999), the virus showed very high serological variability, consistent with previous reports. The present findings show good correlation with the results of molecular analysis (PCR-RLFP) and can be relied upon for typing of PNRSV isolates. Grouping of PNRSV isolates as proposed by Spiegel *et al.* (1999) was confirmed by us when only the first eight MABs reported in Table 1 were taken into account. When two extra MABs (530 and 236) were included in the tests, a much more complicated serotyping resulted.

Based on molecular variability, Aparicio *et al.* (1999) clustered a large number of Mediterranean PNRSV isolates into three main phylogenetic groups. This grouping was essentially maintained when sixteen new isolates from the Czech Republic were characterized (Vaskova *et al.*, 2000). Comparison between the groupings obtained by molecular and serological analysis showed that members belonging to a defined phylogenetic group can be differentiated by our panel of 10 MABs as a possible consequence of the occurrence of a large number of serotype-specific amino acid substitutions.

Table 2. Distribution of serogroups within host species.

Host species	Tested isolates no.	Origin countries no.	Identified serogroups no.	Host-specific serogroups no.	Isolates in host-specific serogroups no.	Variability within the host (%)	Relation of serogroups to host (%)	Relation of isolates to host-specific serogroups (%)
	[a]		[b]	[c]	[d]	[b/a]	[c/b]	[d/a]
Almond	26	5	15	6	7	57	40	27
Peach	25	8	16	7	8	64	50	32
Plum	12	5	7	4	5	58	57	42
Apricot	11	4	10	5	5	91	50	45
Cherry	7	2	4	0	0	57	0	0
Total	81	10	34	22	25		64	30

Table 3. Distribution of PNRSV serogroups by country of origin.

Country	Isolates no.	Hosts no.	Identified serogroups no.	Country-specific serogroups no.	Isolates in country-specific serogroups no.	Isolate variability within the country (%)	Relation of serogroups to the country (%)	Relation of isolates to country-specific serogroups (%)
	[a]		[b]	[c]	[d]	[b/a]	[c/b]	[d/a]
Italy	31	5	18	11	12	58	66	39
Cyprus	10	4	6	1	1	60	16	10
Tunisia	7	1	6	2	2	85	33	29
Turkey	7	2	3	1	1	42	33	14
Lebanon	6	2	6	2	2	100	33	33
USA	6	3	3	1	1	50	33	17
Greece	5	3	4	2	3	80	50	60
Malta	5	2	3	1	1	60	33	20
Albania	3	1	2	2	3	66	100	100
Spain	1	1	1	0	0	100	0	0
Total	81	5	34	23	26		67	32

ACKNOWLEDGMENTS

This study was carried out in the framework of the Mediterranean Network for Virus Disease Assessment and Sanitation of Stone Fruit Trees (MNFT), promoted by CIHEAM/IAM-Bari. We are grateful to Prof. G.P. Martelli for helpful discussion and critical reading of the manuscript.

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Received 5 September 2000
Accepted 27 November 2000