European Journal of Plant Pathology **107:** 845–848, 2001.

Short communication

Existence of two serological subclusters of *Plum pox virus*, strain M

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Accepted 3 July 2001

Key words: PPV, strain M, serological subcluster, MAbs, ELISA

Abstract

A large-scale serological characterisation of *Plum pox virus* (PPV) isolates was carried out with 19 monoclonal antibodies (MAbs), including the universal MAb5B and the following strain-specific MAbs: AL (specific to PPV-M), 4DG5 (specific to PPV-D), TUV and AC (specific to PPV-C), and EA24 (specific to PPV-EA). The study involved 108 PPV isolates of different geographical origin (Albania, Bulgaria, Cyprus, Czech Republic, Egypt, France, Germany, Greece, Italy, Hungary, Moldova, Romania, Slovakia, Spain, Turkey and Yugoslavia) and hosts (almond, apricot, peach, plum and cherry). The inter- and intra-strain serological relationships of PPV isolates were evaluated by DASI-ELISA. High serological variability was detected, not only between strains, but also among isolates of the same strain. Computer-assisted analysis of serological data support the hypothesis of the existence of two distinct subclusters, denoted PPV-M_1 and PPV-M_2, which seem to prevail in Mediterranean and Eastern–Central European countries, respectively.

Plum pox virus (PPV) isolates are grouped into four strains which can be differentiated by biological, serological, molecular and epidemiological characteristics: Marcus (M), Dideron (D), El Amar (EA) and Cherry (C) (Pasquini and Barba, 1997; Candresse et al., 1998). As monoclonal antibodies (MAbs) were shown to be extremely useful for the discrimination and identification of potyviruses, several PPV-specific MAbs were raised and used in the characterisation of PPV isolates (Navrátil et al., 1992; Cambra et al., 1994; López-Moya et al., 1994; Asensio et al., 1995; Pasquini et al., 1995; Boscia et al., 1997; Myrta et al., 1998, 2000). To date, serotype-specific MAbs are available also (Cambra et al., 1994; Boscia et al., 1997; Myrta et al., 1998, 2000; Navrátil et al., 1998). This work reports the results of investigations on the serological variability of PPV isolates belonging to the four known PPV strains using MAbs.

One hundred and eight isolates of PPV, originating from different geographical areas and host species, were collected from Albania (5), Bulgaria (1), Cyprus (2), Czech Republic (6), Egypt (9), France (5), Germany (3), Greece (5), Italy (12), Hungary (34), Moldova (2), Romania (1), Slovakia (8), Spain (5), Turkey (7) and Yugoslavia (3). MAbs used were those newly obtained from the El-Amar isolate (Myrta et al., 1998) and MAbAL specific to PPV-M (Boscia et al., 1997), MAbEA24 specific to PPV-EA (Myrta et al., 1998), MAbAC and TUV specific to PPV-C (Myrta et al., 2000), MAb4DG5 specific to PPV-D and the universal MAb5B (Cambra et al., 1994). DASI-ELISA (Cambra et al., 1994) was carried out for testing PPV isolates.

The presence or absence of each putative epitope was recorded, in a binary matrix (Blanco-Urgoiti et al., 1996) for every isolate. Epitope strings were aligned with CLUSTAL W (Thompson et al., 1994). Tentative clusters were constructed with the NEIGHBOR, SEQBOOT, PROTDIST, and CONSENSE programmes of the PHYLIP package (Felsenstein, 1989) and

Table 1. PPV serogroups based on the reaction of 19 MAbs against 108 virus isolates

Sero-groups	Serogical clustering	No. isolates	Origin of isolates	MAbs identifying different epitopes											
				EA18	EA12	EA11	EA9	EA 8	EA5	EA4	EA24	AC	4DG5	AL	5B
I	EA	9	9 (EG)	+	+	+	+	+	+	+	+				+
II	M_1	3	2 (GR), 1 (SK)	+	+	+	+	+	+	+				+	+
III	M_1	5	5 (TY)	+	+	+	+	+	+	+			+	+	+
IV	M_1	9	2 (GR), 2 (AL), 1 (FR), 1 (IT), 1 (CYP), 1 (GER), 1 (CZ)	+	+	+	+	+	+					+	+
V	D	1	1 (TY)*	+	+	+	+	+	+				+		+
VI	M_1	1	1 (TY)	+		+	+	+	+	+			+	+	+
VII	M_1	3	2 (IT), 1 (GR)	+		+	+	+	+					+	+
VIII	M_1	7	5 (IT), 1 (ROM), 1 (YU)	+		+	+		+					+	+
IX	D	10	4 (HU), 3 (CZ), 2 (SP), 1 (IT)		+		+	+	+	+			+		+
X	M_2	3	2 (SK), 1 (HU)		+		+	+	+	+				+	+
XI	D	1	1 (HU)*		+	+	+	+	+	+			+		+
XII	M_2	22	17 (HU), 2 (FR), 1 (YU), 1 (GER), 1 (CYP)		+		+	+	+					+	+
XIII	D	6	2 (HU), 2 (CZ), 1 (SP), 1 (YU)		+		+	+	+				+		+
XIV	D	3	3 (HU)		+		+		+	+			+		+
XV	D	2	1 (SK), 1 (SP)		+			+	+	+			+		+
XVI	M_2	1	1 (AL)				+	+	+				+	+	+
XVII	M_2	3	2 (HU), 1 (SK)				+	+	+					+	+
XVIII	C	2	1 (IT), 1 (MO)		+			+	+			+			+
XIX	M_2	1	1 (HU)		+		+	+						+	+
XX	D	3	2 (HU), 1 (BUL)		+		+	+					+		+
XXI	D	1	1 (SK)					+	+	+			+		+
XXII	M_2	2	1 (HU), 1 (FR)		+		+		+					+	+
XXIII	M_2	2	2 (AL)				+		+				+	+	+
XXIV	M_2	2	2 (SK)				+		+					+	+
XXV	D	1	1 (GER)					+		+			+		+
XXVI	D	2	2 (IT)							+			+		+
XXVII	D	2	1 (MO), 1 (lab)		+			+					+		+
XXVIII	M_2	1	1 (FR)		+				+					+	+
Total		108		8	18	9	21	21	23	12	1	1	15	15	28

*isolates not included in any cluster; shadowed areas indicate the results of universal (5B) and strain-specific MAbs. Identical results: EA18 or (EA2, EA7, EA13, EA14, EA15, EA16); AC or TUV.

Al-Albania; BUL-Bulgaria; CZ-Czech Republic, CYP-Cyprus, EG-Egypt, FR-France, GER-Germany, GR-Greece, IT-Italy, HU-Hungary, MO-Moldova, ROM-Romania, SP-Spain, SK-Slovakia, TY-Turkey, YU-Yugoslavia.

visualised with TREEV 32 program. The outgroup used in the analysis was *Apple mosaic virus* (ApMV).

MAb5B recognised all the 108 isolates, confirming its nature of universal reagent (Asensio et al., 1995; Candresse et al., 1998), whereas no isolate was recognised by all of the 19 MAbs. As shown in Table 1, the 108 virus isolates gave 28 distinct serological reaction patterns (serogroups) confirming the alleged CP variability among PPV isolates (Cambra et al., 1994; Asensio et al., 1995). From the reaction of each of the 19 MAbs with the tested isolates, it appears that some MAbs can be grouped according to their capacity for identifying the same antigens (epitopes), for example, MAb EA2, EA7, EA13, EA14, EA15, EA16 and EA18; MAb AC and TUV. Assuming that each of these two groups represents a single epitope, and that other MAbs recognise distinct single epitopes, it can be concluded that the 19 MAbs utilised identify 12 different PPV epitopes. Thus, the analysis of 28 different epitope strings (each of 12 number positions) expressed as binary matrix (i.e., serogroup I: 111111110001), resulted in a tree with clear-cut serological clusters (Figure 1).

The only exceptions were two isolates V and XI, respectively Turquie (TYR) and SK241 (HU), which were not included in any of the major clusters. In the case of isolate Turquie, the serological analysis (PPV-D) is in agreement with previous molecular studies, that identified this isolate as an outside member of the PPV-M strain (Candresse et al., 1998). SK241 was typed as PPV-D also by PCR followed by enzymatic digestion (RsaI polymorphism) (Szemes et al., 2001) and its distance from other PPV-D isolates could be due to differences in other ELISA-target epitopes. Six Turkish and three Albanian isolates reacted against both M- and D-specific MAbs. The Albanian isolate PPV-AL (serogroup XXIII) was already identified with serotype-specific primers as being a natural mixture of the two strains (M + D) (Candresse et al., 1998). The other isolate was from a neighbouring tree and, therefore, likely to host a mixed infection. The third Albanian isolate, Plum-KO (serogroup XVI), was collected in a varietal collection where both strains (M and D) were present as single or mixed infections (Myrta, unpublished). The six Turkish isolates

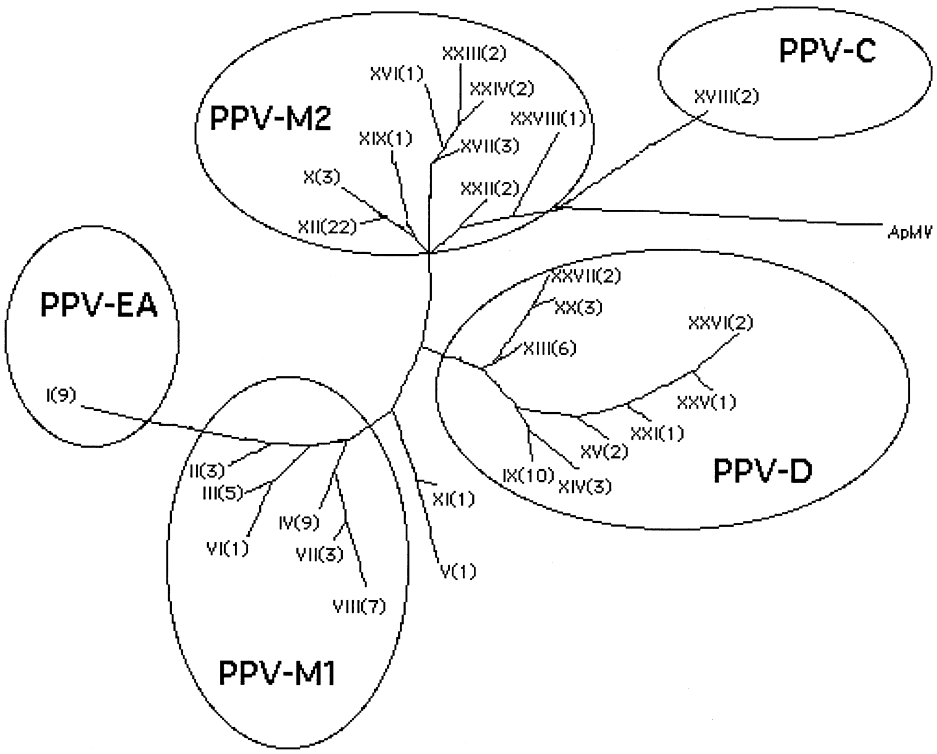

Figure 1. Serological clustering of 28 PPV serogroups. For detailed information on isolate origin refer to Table 1.

(serogroups III and VI) seemed to be PPV-M isolates also containing the D-specific epitope. The existence of two PPV-M subgroups is also indicated by the analysis of Asensio et al. (1995) carried out with different PPV-M isolates and elaboration system.

Interestingly, serotype M isolates clustered in two subgroups apparently correlated with their geographical origin. The first subgroup is composed mainly (28 out of 37, nearly 75%) with isolates from Central and Eastern Europe (Hungary, Slovakia, Germany), whereas from the rest of 9 isolates, 8 are originated from adjacent countries. The second subgroup, represented from 28 isolates, is constituted mostly (24, nearly 86%) of isolates originated from Mediterranean countries (Greece, Italy, Turkey, Albania, Cyprus, France and Yugoslavia). MAbEA18 (or EA2, 7, 11, 13, 14, 15 and 16) can specifically identify the two new PPV-M subgroups. Intriguingly, PPV-EA seemed to derive from the Mediterranean cluster of PPV-M showing much lower affinity with any of the other subgroups.

The present results demonstrate a clear-cut clustering of PPV isolates based on their serological properties, which may also be of phylogenetic significance. The use of other MAbs, in addition to those known to be serotype-specific, can perhaps help in determining the status of isolates with ambiguous serotype assignment. Possibly due to evolutionary differentiation, PPV serotype M separates into two subgroups that seem to prevail in two geographically defined areas (i.e., the Mediterranean and Central–Eastern Europe) which can be tentatively identified as PPV-M_1 and PPV-M_2, respectively. However, whether this serological classification finds support at the molecular level and whether it is linked with a differential epidemiological behaviour remains to be determined.

Acknowledgements

Grateful thanks are expressed to Prof. G.P. Martelli for helpful discussion and critical reading of the manuscript. Many thanks to Drs. V. Pallás, S. Sabanadzovic and F. Grieco for their helpful comments during the elaboration of the serological data; K. Çaglayan, I. Gavriel, W. Hartmann, G. Ghanem, M. Glasa, M. Navrátil and N. Minoiu for supplying PPV isolates.

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