Evaluation of different peach genotypes for resistance to *Plum pox virus* strain M: preliminary results

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Abstract

Different responses to the experimental inoculation with a PPV-M isolate were observed in peach germplasm derived from crosses between *Prunus persica* and peach related species showing resistance to several pathogens. The response evaluation was performed by correlating the phenotypic analysis with serological and molecular tests.

Twenty-one individuals, asymptomatic and with low concentration of the virus inside the leaf tissues, have been selected. This germplasm can be considered 'highly tolerant' or 'resistant' and must be submitted to further investigations.

Keywords: PPV, Prunus persica, P. davidiana, P. ferganensis, tolerance.

Introduction

Plum pox virus (PPV) is the most detrimental stone fruit pathogen, generating significantly high management costs, estimated at over 10.000 million euros during the past 30 years (Cambra et al., 2006b).

The availability of genotypes which are resistant to PPV would represent a starting point to respond to economic, health and environmental problems caused by this pathogen. Some commercial apricot and plum varieties turned out to be resistant or immune to the less severe PPV-D strain. Even some commercial peach varieties resulted to be highly tolerant to the same strain. In peach germplasm, no genes conferring resistance to PPV or tolerance to PPV-M were found. Therefore, we looked for the sources of such resistance and evaluated them within the peach related species. In 1998, the wild *Amigdalus* species *Prunus davidiana*, clone P1908, which originates from China, turned out to be resistant to several pathogens of *Prunus persica*, including PPV (Pascal et al., 1998). His resistance, introduced into the *P. persica* genome using a backcross strategy, was studied on the basis of phenotypic evaluation and mapping of related candidate genes, and displayed a complex pattern of quantitative inheritance (Deqroocq et al., 2005). In this work, the preliminary results of the resistance/tolerance evaluation of different selections derived from crosses between *P. persica* and different parental genomes showing resistance to PPV or to other pathogens are reported.

The evaluation was performed by correlating the phenotypic analysis with the results obtained from different diagnostic tests. The data correlation was done using the classification scale reported in Faggioli et al., (1999), appropriately modified by introducing the real time RT-PCR (rt RT-PCR) analysis (Tab. 1).

Tab. 1 Classification of the plant response by correlation of symptoms expression on rootstock and selections and the different sensitivity of the diagnostic tests.

Phenotypic analysis		Diagnostic test			
Rootstock	**Selection**	**ELISA**	**RT-PCR**	**rt RT-PCR**	**Reaction type**
+	+	+	+	+	sensitive
+	-	+	+	+	tolerant
+	-	-	+	+	highly tolerant
+	-	-	-	+	resistant
-	-	-	-	+	highly resistant
-	-	-	-	-	immune

Materials and methods

Several selections (122), provided by the C.R.A. – Fruit Tree Research Centre of Rome - have been evaluated. All individuals derived from crosses between *P. persica* and different parental genomes showed resistance to either PPV only, or to several pathogens. The sources of resistance were represented by *P. davidiana, P. ferganensis* and some PPV-D tolerant commercial peach varieties crosses with 'Nettarina pendula' (Tab. 2).

Tab. 2 Peach selections assayed to evaluate the resistance/tolerance to PPV-M infection.

Group	Number of individuals	Parental genome
1	92	F1 hybrids: cv 'Maria Aurelia' x (*P. persica* x *P. davidiana)* (SD45).
2	6	F1 hybrids: *Prunus ferganensis*, IF7310828, F1P72 (ibrido *P. ferganensis* x IF7310828), BC1 19, BC1 25, BC1 61
3	24	*P. persica* (Glohaven, Nectaross, Flavorcrest, O'Henry) x 'Nettarina pendula' clone S2678

All selections were double-grafted in autumn on the 'GF 305' indicator-rootstock and experimentally inoculated the following spring by chip budding. The source of inoculum was a PPV-M isolate (PPV-0019-G), provided by DPPMA-UBA, University of Bari (Italy). The inoculation was performed on 3 repetitions and a non-inoculated plant was kept as the negative control. All the plants were kept in a greenhouse for two years, and both the selection and indicator shoots were left to grow. In order to exclude possible interferences in the resistance/tolerance to PPV-M due to cross-infections by other common pathogens present in peach germplasm, all the selections were assayed in advance with DAS-ELISA and RT-PCR to check for the absence of other viruses (*Apple mosaic virus*, *Prune dwarf virus*, *Prunus necrotic ringspot virus*, *Apple chlorotic leafspot virus*) and one viroid (*Peach latent mosaic viroid*), respectively. All results were negative: none of the tested pathogens was present (data not shown).

The evaluation of the phenotypic expression of PPV-specific symptoms was performed the following spring, both on the selection and indicator shoots, on the basis of an appropriate scale, based on symptom intensity across the whole plant (Tab. 3).

Tab. 3 Phenotypic scale applied for the evaluation of PPV-specific symptoms on whole plants.

Scale	Symptoms
0	No symptoms
1	Symptoms on 1-3 leaves
2	Symptoms up to 50% of leaves
3	Symptoms on more than 50% of leaves

Serological analyses were performed by TAS-ELISA with the universal MAb 5B (Cambra et al., 2006a). Molecular analyses were done by RT-PCR (Wetzel et al., 1991) and TaqMan real time (rt) RT-PCR (Olmos *et al*., 2005) using, as template, total RNA extracted from leaves by a commercial kit (RNeasy Plant Mini kit, Qiagen - Inc.,Valencia, CA). The symptoms' evaluation and the diagnostic tests were performed every fifteen days during the spring period, and the correlation between phenotypic analysis and the results obtained by the different diagnostic tests was used to classify the PPV-M infection response of the tested germplasm using the scheme reported in Table 1.

Results

Two years after the experimental PPV-M inoculation, the symptom evaluation, performed on the basis of the established scale, revealed that 17.2% (21/122) of the tested selections did not show any symptoms: 13.1% (16/122) of these asymptomatic individuals did not show any symptoms either on the selection or the GF 305 indicator shoots, whereas the remaining ones (5/122) showed symptoms only on the GF 305 indicator rootstock (Tab. 4 and 5).

Tab. 4 Percentages of symptom distribution referred to the established scale, evaluated for each groups of selections.

Symptoms scale	Group 1	Group 2	Group 3	Tot
0	13%	0%	37.5%	17.2%
1	23.9%	83.3%	8.3%	24.6%
2	49%	16.7%	37.5%	44.3%
3	14.1%	0%	16.7%	13.9%
Total of selections	92	6	24	122

Tab. 5 Percentage of symptom expression on the selection and on GF 305 indicator, evaluated for each group.

Symptoms	Group 1	Group 2	Group 3	Tot
On selection and GF 305	82.6%	100,0%	54.2%	77.9%
Only on GF 305	5.4%	0%	0%	4.1%
Only on selection	4.4%	0%	8.3%	4.9%
Asymtomatic	7.6%	0%	37.5%	13.1%
Total of selections	92	6	24	122

Starting in April, all theses were subjected to an ELISA test and all individuals that showed symptoms on the selection shoots were positive and evaluated susceptible (in accordance with the scale reported in Table 1), while all the 21 asymptomatic individuals gave negative results. Nevertheless, the asymptomatic selections showed positive results when assayed using the TaqMan rt RT-PCR (Tab. 6).

Tab. 6 Results of different diagnostics tests, periodically performed to evaluate the temporary evolution of host/virus interaction.

N°	Selection	Symptoms	rt RT-PCR 5/04/09	ELISA 21/04/09	RT-PCR 21/04/09	ELISA 04/05/09	ELISA 26/05/09
1	Dofi 06-08-005	no	15.97	1.7	+	n.t.	n.t.
2	Dofi 06-08-012	only on GF 305	29.18	0.13	-	0.12	0.11
3	Dofi 06-08-023	no	16.22	0.3	+	n.t.	n.t.
4	Dofi 06-08-024	only on GF 305	27.58	0.12	-	0.19	1.58
5	Dofi 06-08-028	only on GF 305	22.55	0.5	+	n.t.	n.t.
6	Dofi 06-08-035	no	30.05	0.13	-	0.10	0.13
7	Dofi 06-08-045	no	29.08	0.13	-	0.12	0.10
8	Dofi 06-08-052	only on GF 305	28.34	0.12	-	0.11	0.10
9	Dofi 06-08-061	no	29.33	0.12	-	0.11	0.12
10	Dofi 06-08-063	no	16.23	2	+	n.t.	n.t.
11	Dofi 06-08-076	only on GF 305	14.65	1.5	+	n.t.	n.t.
12	Dofi 06-08-088	no	26.74	0.14	-	1.09	n.t.
13	394Q-XXXVII 55	no	26.69	0.11	-	0.10	1.68
14	193R-XLIII 127	no	26.99	0.12	-	0.12	0.11
15	394Q-XXXVII 52	no	27.92	0.14	-	0.12	0.14
16	194R-XXXVII 55	no	24.26	0.12	-	1.45	n.t.
17	393Q-XIV 55	no	29.29	0.11	-	0.10	0.14
18	195R-XLIII 123	no	29.6	0.12	-	1.89	n.t.
19	194 Q-XXXIX 100	no	28.18	0.16	-	1.56	n.t.
20	394 Q-XXXVII 54	no	15.42	0.32	+	n.t.	n.t.
21	194 Q-XXXIX 118	no	29.43	0.14	-	0.12	0.14
22	Healthy peach	-	undet	0.11	-	0.12	0.11

ELISA results are reported as absorbance values at 405 nm. Absorbance values two times higher then healthy control were considered positive; RT-PCR results are reported positive when a band of the expected size was observed in 1.2% agarose gel; rt RT-PCR results are reported as Ct values; n.t. = not tested.

ELISA and RT-PCR analyses, used to monitor the evolution of host/virus interaction through time, showed the same sensitivity; therefore, only the ELISA test was periodically performed on the asymptomatic individuals. Some individuals showed positive signals in ELISA, indicating an “in-progress” evolution of the PPV infection and, at the end of May, only nine of theses were confirmed to be negative in serological analysis (Tab.7).

Tab. 7 Ct values obtained in rt RT-PCR performed on GF 305 leaves collected from ELISA negative theses

Selections	Symptoms	Ct values
Dofi 06-08-012	only on GF 305	18.10
Dofi 06-08-035	no	27.09
Dofi 06-08-045	no	32.28
Dofi 06-08-052	only on GF 305	21.02
Dofi 06-08-061	no	undet
394Q-XXXVII 55	no	undet
193R-XLIII 127	no	33.78
394Q-XXXVII 52	no	32.02
194R-XXXVII 55	no	34.14
Healthy	no	undet

In order to verify the translocation of the virus in the indicator rootstocks, an rt RT-PCR was performed on GF 305 leaves from the final nine ELISA-negative individuals, and only two of them resulted negative for the presence of the virus (Table 8).

Tab. 8 Classification of evaluated germplasm response to PPV infection

N°	Group	Selection	Type of reaction
1	1	Dofi 06-08-005	tolerant
2	1	Dofi 06-08-012	resistant
3	1	Dofi 06-08-023	tolerant
4	1	Dofi 06-08-024	tolerant
5	1	Dofi 06-08-028	tolerant
6	1	Dofi 06-08-035	higlhy resistent
7	1	Dofi 06-08-045	higlhy resistent
8	1	Dofi 06-08-052	resistant
9	1	Dofi 06-08-061	higlhy resistent
10	1	Dofi 06-08-063	tolerant
11	1	Dofi 06-08-076	tolerant
12	1	Dofi 06-08-088	tolerant
13	3	394 Q-XXXVII 55	higlhy resistent
14	3	195R -XLIII 127	higlhy resistent
15	3	394Q -XXXVII 52	higlhy resistent
16	3	194R XXXIX 65	tolerant
17	3	393Q XIV 55	higlhy resistent
18	3	195R XLIII 123	tolerant
19	3	194Q XXXIX 100	tolerant
20	3	394Q XXXVII 54	tolerant
21	3	194 Q XXXIX 118	tolerant

Discussion

A high percentage of asymptomatic selections (17.2%) were obtained after two years from the experimental inoculation with the PPV-M strain, indicating an interesting level of resistance/tolerance of the evaluated germplasm. Only the selections derived from the *P. ferganensis* parental genome resulted to be sensitive, as they showed specific symptoms both on the selection and indicator shoots, making it pointless to identify genes conferring resistance or tolerance to PPV-M from this specific germplasm.

At the end of the evaluation tests, nine asymptomatic selections resulted positive only in RT-PCR, indicating the presence of very low concentration of the virus inside the plant tissues. According to the new classification scheme now in use, these selections may be classified as highly resistant germplasm, which renders these results particularly interesting. Moreover, in two individuals, the asymptomatic GF 305 indicator resulted negative in rt RT-PCR analysis for the presence of PPV, confirming that the virus had failed to relocate itself to the lower part of the plant, a result already found in a previous paper (Decrooq et al., 2005). The PPV-M infection response of the remaining asymptomatic individuals ranged from tolerant to resistant (Tab. 8). All these promising individuals have been derived both from 'Maria Aurelia' x SD45 F_1 hybrid (*P. persica* x *P. davidiana*) and from some commercial peach cultivars crossed with weeping peaches (S2678).

Contemporarily to this investigation, a framework molecular map of the evaluated progenies was obtained by genetists (Micali et al., 2009) and further investigation is taking place to implement the results and to correlate the genome mapping with the plants response.

The 21 asymptomatic selections will be kept for one more year in the screenhouse so as to verify the temporal stability of their response to the experimental PPV-M infection. If the resistance/tolerance response will be confirmed, field trials will be established in areas where the disease is endemic, so as to also verify the validity of the evaluated response under the natural inoculum pressure by aphid vectors.

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