Preliminary results on resistance to PPV-M in *Prunus persica* (L.) Batsch

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Abstract

Preliminary results of trials evaluating peach cultivar resistance to *Plum pox virus* (PPV) and aphid colonization were obtained. Data after one vegetative cycle since an inoculation assay showed that most of the peach cultivars analyzed were susceptible to PPV-M isolate, used as inoculum source. Also, in our experiments two cultivars, Ambra and Cappucci 18, were found to be tolerant while cultivars Fei Cheng, Harrow Blood, Jing Yu and Rosa Dardi were resistant.

Moreover, to evaluate the possible epidemiological impact of the peach cultivars NJ WEEPING and S6699 resistant to aphid colonization, experimental transmissions were carried out. This was done by using a clonal culture of *Myzus persicae* (Sulzer) as vector and PPV-M isolate as inoculum source under controlled acquisition access period. The results showed that in our conditions aphids were not able to transmitted PPV-M isolate to healthy plants of NJ WEEPING and S6699. Under the same conditions PPV-M was transmitted by aphids from infected to healthy GF305 plants.

Keywords: sharka, peach, ELISA, aphid transmission, virus tolerance

Introduction

Plum pox virus (PPV) is the causal agent of sharka disease, one of the most dangerous diseases of stone fruits and in particular peach. PPV is present in several areas of the world including North America and Asia. In Europe, where sharka was first reported and where it is still spreading, PPV is infecting both fruit trees and wild plant species. In these cases, the application of quarantine measures is time consuming, expensive and not effective, not even when virus-free material is employed for the plantation of new orchards.

A fast spread of the strain PPV-M recently occurred in several areas in Europe and in particular in Italy, where the disease is causing severe losses to the peach crop, and threatens the nursery industry as well. Where sharka is endemic the only sustainable strategy is the employment of resistant cultivars. In the present study, we report preliminary results on the identification of peach cultivars naturally resistant to infections by PPV, or to the colonization by PPV aphid vectors.

Material and methods

Molecular Characterization of PPV Isolates: Total RNA was extracted from symptomatic leaves of 9 naturally infected peach trees using a silica method (MacKenzie et al., 1997). RT-PCR reactions were performed to amplify three different genes (HC-Pro, P3 and CP) for all the PPV isolates investigated.

Amplified fragments were purified and cloned into pCRII vector using the TA cloning kit (Invitrogen). The clones obtained were sequenced and nucleotide sequences were aligned using Bioedit software version 7.0.5.3. CP, HC-Pro and P3 gene sequences were used to construct different phylogenetic trees. Minimum evolution analysis was carried out using the neighbourjoining method and bootstrap replicated 1000 times with the software Treecon version 1.3b. PPV-PS (AJ243957) isolate was used as the outgroup.

Evaluation of Resistance to PPV of Peach Cultivars: Fifteen peach cultivars (Table 1) were tested for PPV resistance by green-grafting on inoculated *P. cerasifera* plants, with two different PPV-M isolates (Fig. 1), in two independent assays. Plants were maintained in a screenhouse during the winter season and transferred to a greenhouse at 22-24°C for PPV symptom observation. At the end of May, leaf samples were collected from analyzed peach cultivars and from *P. cerasifera* for detection of PPV-M by ELISA (Cambra et al., 1994) and RT-PCR (Wetzel et al., 1991) assays.

Tab. 1 Results of resistance trials obtained inoculating fifteen different peach cultivars with PPV-M isolates.

Peach cultivars	*P. cerasifera*		*P. persica*		
	Symptoms	**ELISA**	**Symptoms**	**ELISA**	
AMBRA	+	+	-	+	Tolerant
CAPPUCCI 18	+	+	-	+	Tolerant
Chimarrita	+	+	+	+	Susceptible
Contender (Nct 544)	+	+	+	+	Susceptible
Fei Cheng	+	+	-	-	Resistant
Harrow Blood	+	+	-	-	Resistant
Hardy Red	+	+	+	+	Susceptible
Jing Yu	+	+	-	-	Resistant
Kamarat	+	+	+	+	Susceptible
Maycrest	+	+	+	+	Susceptible
May Fire	+	+	+	+	Susceptible
Nj 307	+	+	+	+	Susceptible
Rosa Dardi	+	+	-	-	Resistant
S 5898:128	+	+	+	+	Susceptible
T 16	+	+	+	+	Susceptible

Fig. 1 Chip budding trials

Peach cultivars were considered to be susceptible when they showed symptoms of the disease, tolerant when symptoms were absent on the scion but ELISA was positive, and resistant when there were neither symptoms nor a reaction in ELISA (Bazzoni et al., 2004).

Aphid Transmission: Aphid transmission experiments were conducted under controlled conditions using a clonal culture of *Myzus persicae* (Sulzer) as a vector and plants of *P. persica* cv. GF305, inoculated with PPV-M, as an inoculum source. The aphids, raised on healthy pea plants, were placed, with the aid of a small brush, onto the leaves of infected *P. persica* GF305 (acquisition period). Later, these aphids were placed onto healthy peach GF305 used as positive control, and onto plants of the two cultivars NJ WEEPING and S6699. Four leaves for every healthy peach tree were used for the inoculation period; in this the branch of leaves was isolated from the remaining part of the plant (Fig. 2).

Fig. 2 Aphid transmission trials with *M. persicae* and two peach cultivars NJ WEEPING and S6699

The plants, after insecticide treatment, were put in a greenhouse under controlled conditions. The occurrence of PPV transmission was diagnosed by symptoms observation, ELISA and RT-PCR assays.

Results and discussion

Molecular Characterization of PPV Isolates: The sequence analyses of 9 PPV isolates confirmed their classification within PPV-M strain and in particular they clustered with PPV-M isolate SK68 (M92280), a sharka strain isolated in Hungary (Fig.3). All isolates have high level of identity with PPV-M SK68 and maintain the DAG and PTK motifs in the CP and HC-Pro genes indicating their aphid transmissibility. These data confirmed previous results reported in Italy (Bianco et al., 2004; Bianco et al., 2005).

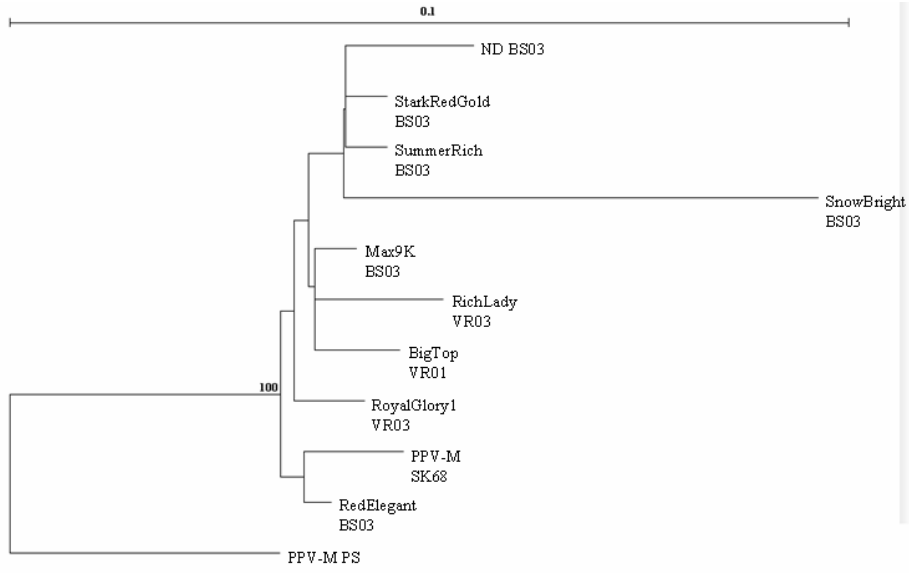

Fig. 3 Phylogenetic tree obtained with Treecon program analyzing the nucleotide sequence of nine PPV-M isolates collected from naturally infected peach.

Two of these viruses, from Snow Bright and Big top cultivars, were used in the assays to evaluate resistance in peach cultivars. Only one PPV-M isolate, from Big Top, was employed for the aphid transmission trials.

Evaluation of Resistance to PPV of Peach Cultivars: The results obtained in the evaluation of sharka resistance of the different peach cultivars are shown in Table 1. Fei Cheng, Harrow Blood, Jing Yu and Rosa Dardi proved to be resistant (no evident symptoms and ELISA negative assays) to two PPV-M isolates after the first cycle of experiments. Harrow Blood was reported as resistant also by Palmisano and colleagues (2008). Ambra and Cappucci 18 were shown to be tolerant (no evident symptoms but with ELISA positive assays) as already reported by Palmisano and colleagues (2008). The remaining cultivars were found to be susceptible to the disease on the basis of their symptom expression and ELISA positive assay.

Aphid Transmission: The transmission experiments confirmed that PPV isolate from Big top cultivar was aphid transmitted from infected to healthy *P. persica* GF305 plants as verified by ELISA and RT-PCR assays. On the contrary, inoculated peach trees of cultivars NJ WEEPING and S6699 were asymptomatic. Moreover, ELISA and RT-PCR, conducted on leaves collected from inoculated plants, were negative indicating that aphids were not able to transmit the virus. Also, samples collected from GF305, used as the rootstock for NJ WEEPING and S6699, were negative in the same analysis and no symptoms were observed. These results would indicate that these two cultivars may be resistant to aphid colonization. In fact other results suggest that these peach cultivars are PPV susceptible. Further experiments will be conducted in order to validate these data with the aim of supplying suitable cultivars for those areas where sharka disease is endemic and no efficient containment measures are available.

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