Scientific Papers, Series B, Horticulture, Vol. LVI, 2012
ISSN Online 2286-1580, ISSN-L 2285-5653

SEROLOGICAL AND MOLECULAR RESPONS OF SEVERAL APRICOT ROMANIAN VARIETIES TO THE ARTIFICIAL INFECTION OF PPV (PLUM POX VIRUS)

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Abstract

Sharka disease, caused by this virus (PPV) is one of the most serious viral diseases of stone-fruit crops, including peach (Prunus persica L.), apricot (P. armeniaca L.), plums (P. domestica L. and P. salicina Lindl.) as well as sweet and sour cherries (P. avium L. and P. cerasus L.) that may be systemically infected by a few unique PPV strains. The goal of this work is to evaluate a large number of local apricot varieties concerninhg the resitance to PPV, and using them on the valuable breeding programs, is an interesting perspective in limiting the spread of this virus. In support of this idea we studied a large number of genotypes grafted on the mirobolan rootstocks and GF305 (considered indicator to PPV), that were previously artificial infected with PPV by chip budding. The rootstocks and the apricot varieties were tested by Elisa and RT-PCR.

Keywords: *plum pox virus, resistance genotypes, strains*

INTRODUCTION

Apricot is the third most important species among the stone fruit crops with a world production of approximately 2.69 million tons (FAO 2004). In Europe, PPV is the most important virus affecting Prunus fruit crops and the most limiting factor for the apricot cultivation in terms of economics [9]

The implementation of an aggressive eradication program to control its spread is an extremely costly way of controlling PPV. Ultimately, the introduction of resistant cultivars of stone fruits into the orchards is the best long-term solution in order to control the virus. [10]

Several PPV resistance programs aimed to obtain resistant or partially resistant apricot cultivars are carried out in Europe [3], [6], [5], [11], [12]. The majority of existing apricot cultivars show different level of susceptibility to PPV. Breeding for resistance is one of the effective measures for a protection against the Sharka disease.

Resistant apricot cultivars, along with the apricot's small genome size of 294 Mb [1] and haploid number of n=8, facilitate the study of the genetics of PPV resistance. Recently, three genetic maps for apricot have been published [4], [8], [13].

The goals of the work presented in this communication are the identification of a natural source of resistance to PPV, introduce this resistance into commercial cultivars well adapted in our country, and the implementation of marker-assisted selection (MAS), based on markers tightly associated with resistance, as a measure to substantially streamline the breeding process.

MATERIAL AND METHOD

The several Roumanian apricot varieties :" Traian", "Auras", "Ceres", "Sirena', "Olimp", "Ovidiu", "SEO", "Euxin", "Harcot", "Tudor","Augustin", "Amiral", "Danubiu", "Histria", were tested in the artificial infected conditions, in the greenhouse.

The young apricot sticks were grafted onto inoculated GF305 (used like susceptible rootstock) ready for testing to PPV resistance.

They were inoculated with a chip-bud collected from three experimental field plots containing conventional varieties planted at Fruit Research Station, Bistrita, Romania**.**

Phenotyping methods

For phenotyping this Romanian progenies, plants without sharka symptoms on shoots growing from the inoculum bud and with

negative enzyme-linked immunosorbent assay (ELISA) reaction were re-inoculated. PPV infection was evaluated over three consecutive growth periods through visual symptoms and ELISA [11]

For Elisa method the mashed leaves (samples) in extraction buffer (AFT 0,2 % + Dieca 2% + PVP – 10) were placed in holes in a plate previously tapisated with polyclonal immunoglobulins conjugated (anti-PPV) and incubated at 4 ^{0}C for 16h. After 3 washes (with AFT- Tween) were added 200 µI specific monoclonal antibodies for PPV and incubated at 37 ^{0}C for 2 h. The last step was the implementation of immunoglobulins conjugated with alkaline phosphatase 1:1000 (200µI) and incubated for 2h at 37 ^{0}C. The reading was made at 405 nm considering the positive values exceeding twice the value of negative test reading (T-x 2). (Figures 1 and 3)

Pruning was performed at the beginning of each growth period to induce vigorous new shoots for symptom scoring. The plants, in which PPV was not detected by ELISA, were tested by reverse transcription polymerase chain reaction (RT-PCR) using the PPV specific primers P1 and P2, [15] that amplifies a 243 bp fragment located at the C-terminus of the PPV CP gene. PPV was trapped with PPV-polyclonal antibodies adsorbed on an Eppendorf micro tube. Enhanced Avian kit provided by Sigma was used for RT-PCR. The thermal cycling scheme used was the following: RT- 30 min at *50°C,* denaturation / RT inactivation - 2 min at 94°C followed by *35* cycles: template denaturation - 30 s at 94°C, primer annealing - 45 s at 61°C and DNA elongation- 60 s at 72°C. Following to the last cycle, amplified DNA was elongated for 10 min at 72°C. An aliquot of the amplified products (10 µI) was fractionated onto *1.5%* agarose gel electrophoresis in 1x TBE buffer. Bands were visualized by ethidium-bromide staining under UV light. [7] ,[15] .(Fig 2)

Plants were classified as resistant if they did not show symptoms and positive ELISA or RT-PCR reaction in the last three growth periods that were evaluated.

RESULTS AND DISCUSSIONS

In artificial infection conditions in the greenhouse the results presented in Figure 1 (in the top of the Elisa plate) shows that the samples belonging to susceptible GF 305 rootstock were found to be positive compared with most samples of apricot genotypes,(in the bottom of the Elisa plate) even if they were collected on the same plant.

Under these conditions the virus is able to infect susceptible peach rootstock but not the majority of the apricot genotypes like Traian', 'Auras', 'Ceres', 'Euxin', 'Tudor', 'Augustin' [2].

These potential resistants individuals were tested in terms of molecular techniques to confirm the nature of resistance to sharka.

Results concerning the molecular detection performed by RT-PCR) using a primer pair (Pl/P2) that amplifies a 243 bp fragment located at the C-terminus of the PPV CP gene, proved, that some apricot varieties that were found to be negative after Elisa test, were revealed to be positive after molecular testing like 'Ovidiu' (Fig 1 and Table 1). This, it show us that it supports the sensitivity of molecular testing [15].

Plants were classified as resistant if they did not show symptoms and/or positive ELISA or RT-PCR reaction in the last three growth periods that were evaluated. Resistant individuals were coded as heterozygous for the trait and those susceptible were coded as homozygous recessives (consistent with [14].

Table1. Results concerning scoring to the Romanian apricot varieties after 2 years evaluation to the PPV infection.

Genotypes	2011 Das Elisa	2011 RT - PCR	2012 DAS Elisa	2012 RT-PCR
Traian	-	-	-	-
Auras	-	-	-	-
Ceres	-	-	-	-
Sirena	+	+	+	+
Olimp	+	+	+	+
Ovidiu	-	+	-	+
SEO	-	-	-	-
Harcot	-	-	-	-
Euxin	-	-	-	-
Tudor	-	-	-	-
Augustin	-	-	-	-
Amiral	+	+	+	+
Danubiu	+	+	+	+
Histria	+	+	+	+

The apricot genotypes 'Traian', 'Auras', 'Ceres', 'Euxin', 'Tudor', 'Augustin' and the recognized varieties 'SEO and Harcot' were found resistant to PPV as demonstrated by both serological and molecular tests (RT-PCR).

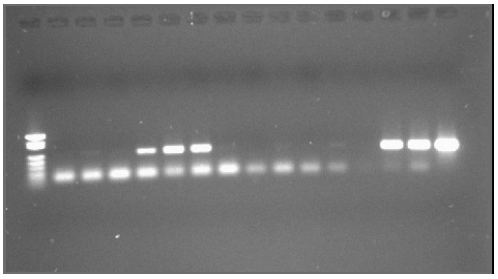

Fig. 1 The RT- PCR test concerning the PPV resistance on the Romanian apricot varieties.

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14

CONCLUSIONS

The identifying of a natural source of resistance to PPV, using this resistant source into new crosses with Romanian commercial cultivars well adapted in our country, and the implement of marker-assisted selection (MAS), based on markers tightly associated with resistance, as a measure to substantially streamline the breeding process, may be a promising strategy to obtain apricot varieties with natural genetic resistance to PPV.

ACKNOWLEDGEMENTS

This study was financed by project SharCO "Sharka containment"- FP7/2007 - Grant Agreement no 204429 funded by European Union.

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