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STUDIES CONCERNING THE SEROLOGICAL DETECTION ANALYSIS FOR THE PPV (PLUM POX VIRUS) ON SOME PEACH VARIETIES

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Abstract

Plum pox is a serious worldwide problem with a severe impact on the productivity and quality of Prunus fruits (PPV), a virus against which no chemical or biological curative treatment is available. Infections of susceptible plants cause a hormonal disruption in which antagonist hormones are induced simultaneously, while in resistant varieties, the accumulation of antagonist hormones shows a sequential pattern. Identifying key factors underlying the spread of disease is an essential but challenging condition for delivering management strategies. Identification of new genotypes of natural genetic resistance to Sharka; the study of the genetic mechanisms behind the expression of these characters and alternative methods of limiting the propagation of this quarantine virus are objectives of interest in finding long-term solutions to limit propagation of this virus. The Romanian peach varieties 'Alexia', 'Triumf', 'Alex' and 'Flacăra' following the Elisa serological tests proved negative at PPV in conditions of natural infection in the field.

Key words*: peach, varieties, Plum pox virus, Elisa test.*

INTRODUCTION

Plum pox potyvirus (PPV) is a devastating disease of wild and cultivated *Prunus* species. 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. In areas where plum pox is endemic, such as eastern Europe, the disease can be severe with a possibility of 80-100% yield losses (Kolber et al., 2001). Symptoms vary with virus strain, host species, and cultivar. In susceptible plum cultivars, typical symptoms of leaf chlorosis and necrosis lead to tattered leaves and fruit showing chlorotic rings. Infected apricot cultivars produce misshapen and necrotic fruit. In peach, symptoms are less obvious for many cultivars but chronic infection leads to premature yield reductions and death of trees. Because high concentrations of the virus are not produced and the virus is unevenly distributed in trees, it is difficult to detect and verify in disease surveys. In addition, diseased trees often remain symptom less for several years after infection and function as reservoirs for PPV survival and spread to neighboring trees and orchards.

The virus is transmitted by vegetative propagation and is spread by at least 20 different aphid species in a no persistent manner, allowing epidemics to develop rapidly (Damsteegt, 2001). Among the four identified groups of PPV, the PPV-D and PPV-M strains are the most prevalent and differ in disease severity among *Prunus* species, especially in their ability to infect peach trees via aphid transmission. Fast spreading outbreaks are mostly associated with the PPV-M strain (Zagrai, 2009). The disease manifests as yellow rings and blotches on leaves or fruit. Although not a danger to consumers, PPV can ruin the fruit's marketability by increasing acidity and causing deformities.

In Europe, 100 million trees are infected. PPV eradication efforts hinge on continual surveying of aphid and weed populations. The development and cultivation of new, resistant cultivars could be the definitive solution to this problem (Martinez-Gomez et al., 2000).

In order to plan an efficient breeding program to obtain cultivars resistant to Sharka, it is important to know the genetic control of this resistance.

The objectives for this work are to obtain the serological detection of PPV on local peach varieties ('Flacăra', 'Alexia', 'Alex', 'Triumf'). For study the limitation of PPV infection we are using the Romanian rootstock of interest concerning the resistance to PPV. These genotypes were tested in artificial infected conditions (in greenhouse). After the tests the apricot progenies have been grafted onto a inoculated (PPV-D, provenance of SCDP Voinesti) GF305, susceptible peach. During the vegetation period 2019 was tested by Elisa method.

MATERIALS AND METHODS

Plant material

'Flacăra'. Peach variety 'Flacăra' was created at SCDP Băneasa. The habit of tree is upright to spreading, with a medium anthocyanic pigmentation of flowering shoot, on which floral buds are seated two or more. The type of flower is campanulate with five narrow elliptic petals. The leaf blade is of medium length and width. The length of petiole is medium with reniform shape of nectarines. The size of fruit is large, ovate shaped (in ventral view), the shape of pistil end (without mucrone tip) pointed, the depth of stalk cavity deep and width broad and preeminence of suture medium. The ground color of skin to the fruit is yellow and the hue of over color of skin dark red; the relative area of over color of skin is medium. The color of flesh is orange-yellow with anthocyanin coloration of flesh in central part and around the stone week. The shape of stone is obovate and the intensity of brown color dark. Fruits reach maturity in the first two decades of September, have 240-260 g, the firmness of flesh is medium, without fibers (Figure 1).

Figure 1. 'Flacăra' variety

'Alexia'. Peach variety 'Alexia' was created at SCDP Băneasa. The habit of tree is upright to spreading, with a medium anthocyanic pigmentation of flowering shoot, on which floral buds are seated two or more. The type of flower is rosette with five circular petals. The leaf blade is of medium length and width. The length of petiole is short with reniform shape of nectaries. The size of fruit is medium, circular shape (in ventral view), the shape of pistil end (without mucrone tip) pointed, the depth of stalk cavity deep and width medium and preeminence of suture medium. The ground color of skin to the fruit is cream white and the hue of over color of skin medium red; the relative area of over color of skin is large. The color of flesh is cream without anthocyanin coloration of flesh in central part and around the stone. The shape of stone is obovate and the intensity of brown color medium. Fruits reach maturity in the last decade of June, have 100-110 g, the firmness of flesh is medium, with fibers.

'Alex'. Peach variety 'Alex' was created at SCDP Băneasa. The habit of tree is upright to spreading, with a weak anthocyanic pigmentation of flowering shoot, on which floral buds are seated two or more. The type of flower is rosette with five broad elliptic petals. The leaf blade is of medium length and width. The length of petiole is medium with reniform shape of nectarines. The size of fruit is medium, broad oblate shape (in ventral view), the shape of pistil end (without mucrone tip) weakly pointed, the depth of stalk cavity and width medium and prominence of suture medium. The ground color of skin to the fruit is greenish white and the hue of over color of skin light red; the relative area of over color of skin is medium. The color of flesh is white without anthocyanin coloration of flesh in central part and around the stone. The shape of stone is oblate and the intensity of brown color light. Fruits reach maturity in the first decade of August, have 125-150 g, the firmness of flesh is strong, without fibers.

'Triumf'. Peach variety 'Triumf' was created at SCDP Băneasa. The habit of tree is upright to spreading, with a medium anthocyanic pigmentation of flowering shoot, on which floral buds are seated two or more. The type of flower is campanulate with five narrow elliptic

petals. The leaf blade is of medium length and width. The length of petiole is medium with reniform shape of nectarines. The size of fruit is large, circular shape (in ventral view), the shape of pistil end (without mucrone tip) weakly pointed, the depth of stalk cavity deep and width medium and preeminence of suture medium. The ground color of skin to the fruit is yellow and the hue of over color of skin medium red; the relative area of over color of skin is large. The color of flesh is white without anthocyanin coloration of flesh in central part and around the stone week. The shape of stone is obovate and the intensity of brown color light. Fruits reach maturity in the second decade of July, have 245-260 g, the firmness of flesh is medium, without fibers (Figure 2).

Figure 2. 'Triumf' variety

Methods

A. Evaluation of the characteristic symptom of direct viruses on plants

To evaluate the symptoms characteristic of the studied viruses and establish their incidence, in May-July, the following were determined: frequency, intensity and degree of attack on leaves (number of organs analyzed 200) and on fruit (number of organs analyzed 100).

a) The frequency of the attack (F%) is the relative value of the number (n) of plants or organs of the plant attacked by a phytopathogenic agent relative to the total number (N) of observed plants or organs. The obtained results were calculated using the formula: F% = n/N x 100.

b) Intensity of attack (I%) is the value by which the degree of coverage or spread of the attack is given, reporting the surface attacked against the total observed area. The relative expression of attack intensity was calculated using the formula: I% = ∑(i x f)/b, where:

- i = the attacked area (%);
- f = the number of attacked cases per percent;
- n = total number of cases with attack.

c) Degree of attack (DA%) is the expression of the extent of the attack of the crop on the total number of plants we make the observations. The degree of attack was calculated using the formula: DA% = (F% x I%)/ 100.

Interpretation of plum pox genotype behavior in leaf virus attack was performed according to the scale:

* DA% = 1-5% ↔ very weak attack indicating genotypes with high tolerance;
* DA% = 6-10% ↔ weak attack indicating medium-tolerance genotypes;
* DA% = 11-20% ↔ medium attack indicating slightly susceptible genotypes;
* DA% = 21-30% ↔ strong attack indicating genotypes with moderate susceptibility;
* DA% > 30% ↔ very strong attack indicating genotypes with very high susceptibility.

d) Severity of attack and the virus virulence. Interpretation of the response of plum genotypes to Plum pox infection was determined using the interpretation scale of 1-9, the number of samples analyzed being 100. The plum genotypes were framed in:

* Degree of attack 9 ↔ it does not show symptoms on the fruit showing tolerable genotypes;
* Degree of attack 8 ↔ it show slight discoloration of fruit and sporadic premature fall of fruit indicating slightly susceptible genotypes;
* Degree of attack 5-7 ↔ more than 50% of the samples show symptoms and a premature fall of the fruits indicating genotypes with moderate susceptibility;
* Degree of attack 1-4 ↔ more than 75% of the samples show symptoms and a premature fall of fruits indicating very susceptible genotypes.

The influence of the virus on the production of some plum genotypes was determined; the effects of viral infection were expressed in the affected (t/ha) production.

Changes in the physiological characteristics due to the Plum pox virus were determined in 4 peach genotypes: 'Alexia', 'Triumf', 'Alex' and 'Flacăra'.

B. Detection of viral infection

Detection of viral infection was performed by biological methods and serological methods:

- The biological method was performed by bio-testing on wood indicators, respectively on peach GF305, by chip-budding grafting of a variable number of genotypes. Observations on the material were made at 3 months, 6 months and 1 year.
- The serological method comprised 2 variants:

* DAS-ELISA (Double Antibody Sandwitch Enzyme Linked Immunosorbent Assay) for Plum pox viruses (PPV), Prunus necrotic ring spot (PNRSV), Prune dwarf (PDV) and Apple chlorotic leaf spot (ACLSV) (Clark & Adams, 1977);
* TAS-ELISA (Triple Antibody Sandwitch Enzyme Linked Immunosorbent Assay) for identification of strains PPV-M and PPV-D to the virus Plum pox (Cambra et al., 1994).

RESULTS AND DISCUSSIONS

Understanding virus movement in woody plants is complicated by the fact that fruit tree viruses follow the sieve movement. Evidence of infection is based on typical symptoms. The symptoms of infection by a single virus are relatively easy to recognize (Zagrai I. et al., 2005).

The incidence of symptom development and the use of serological and molecular tests revealed the pathogenic effects of this virus. Local Romanian varieties of peach were tested concerning the resistance to the PPV.

In Table 1 the peach varieties were tested by serological tools the Romanian varieties and rootstocks, and among them, the variety 'Alexia' and 'Alex' are resistant to artificial infection with PPV-D.

To highlight the influence of rootstock resistant to PPV, we are grafted the peach resultants progenies on to the peach GF305 used like susceptible to PPV (Figures 3 and 4). Grafted plants and also peach varieties were monitored by visual inspection and ELISA, completed by RT-PCR for the PPV symptoms (Table 1). Results showed that infection not spread after inoculation on the different resistant rootstocks. Results confirm that, PPV infection was translocated from the inoculums buds to varieties but the virus remained close to the inoculation site for the varieties 'Alexa' and 'Alex', indicating a possible inhibition of virus replication. Some Romanian local varieties could be an important promise to limit the infection of the virus on peach orchards.

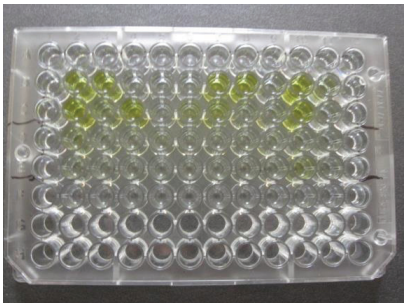

Figure 3. DAS-ELISA for Plum pox viruses (PPV)

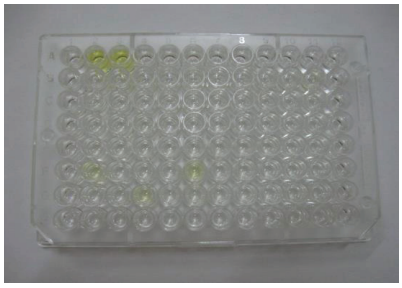

Figure 4. DAS-ELISA for Plum pox viruses (PPV)

Figure 5. Symptoms of Plum pox viruses (PPV)

Figure 6. Symptoms of Plum pox viruses (PPV)

Table 1 Observation of peach response to PPV-D strains infection and PPV detection in different parts of the graft-inoculated four peach genotypes, under artificial conditions

Nr. crt	Genotype	Analyzed part of plant	2011 (1rst scoring)			2011 (2nd scoring)	
			DASI-ELISA (DO = 405nm)	IC-RT-PCR	PPV symptoms intensity	DASI-ELISA	PPV symptoms intensity
1	'Falcăra'	bottom half	++	+	+	++	+
		top half	+	+	+	+	+
2	'Alexia'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
3	'Alex'	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
4	'Triumf'	bottom half	+++	+	++	+	+
		top half	+	+	+	+	+
C-	Negative Control	bottom half	-	-	-	-	-
		top half	-	-	-	-	-
C+	'Tuleu dulce' (control)	bottom half	++++	+	+++++	+++++	+++++
		top half	++++	+	+++	++++	+++

CONCLUSIONS

The peach GF305 rootstocks are a good indicator for susceptibility to PPV. The subsequent grafting protocol was optimized, and a Romanian PPV-D isolate was identified and used as inoculum's source. Analyzing the Alexa's and Alex's response to PPV infection remained close to the inoculation site, and revealed an interaction between the host genotype and the virus.

The PPV infection was translocated from the inoculums buds to varieties but the virus remained close to the inoculation site indicating a possible inhibition of virus replication.

Therefore, this genotype can be characterized at most as resistant to PPV.

Considering the results of peach 'Alexa' and 'Alex' were manifested a resistance to the artificial infection with PPV, the detection of the virus was performed by serological and molecular tools.

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