Serological determinations of romanian apricot varieties in PPV infection (*Plum px virus*)

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Abstract In Europe, PPV (*Plum px virus*) is the most important virus affecting *Prunus* fruit crops and the most limiting factor for the apricot cultivation in terms of economics. In order to find more permanent solutions to the problem of Sharka disease, cultivation of resistant genotypes is a very intrest way. The varieties *‘Stark Early Orange’* and *‘Traian’*, are two main sources of resistance, used in the Romanian breeding program. The goals of the work presented in this communication are the identification of a natural source of resistance to PPV (*Plum px virus*), introduce this resistance into commercial cultivars well adapted in our country. Disease resistance phenotypes are often simple and oligogenic in nature, yet the difficulties in establishing reliable inoculation and scoring methods can challenge even the best plant pathologist or breeder.

*Variola* (Sharka) caused by *Plum px virus* is transmitted both mechanically by grafting and naturally via aphids (1, 2). Vectors (aphids) reduction by chemical control is inefficient and causes the occurrence of aphids clones resistant to insecticides, PPV exists in some forms or variances called strains.

Serological and molecular characterisations of the isolated Plum pox virus (PPV) performed by Western European researchers have made possible to establish their belonging to different viral strains and also to identify recombed forms (3). Seven PPV strains have been identified and characterised serologically and molecularly until now: D (Dideron) isolated for the first time on apricot in South-Eastern France; M (Marcus) identified on peach in Northern Greece (10, 9); E. A. (El Amar) described in Egypt on apricot (15); SoC (sour cherry) detected in Republic of Moldova (12); Swc (sweet cherry) identified in Italia (4); PPV-Rec resulted from the recombination of the two major strains (M and D), being discovered in Albania, Bulgaria, Czechia, Germany and Slovakia (6). Suşa PPV-W (Winona) was identified in Canada (11). Recombination plays an important role in the evolution of riboviruses, having determined the emergence of new strains with unpredictable effects on pathogenity (5).

Natural recombination between PPV-D and PPV-M strains formed the emergence of a new viral strain, called PPV-Rec, reported for the first time (7). Recent studies performed on 16 isolates from Turkey led to the identification of new viral strains. Their sequencing has led to the observation of a recombination point in the HC-Pro viral region, at item 1566 of the viral genome. Therefore, the Turkish researchers proposed that this new recombed strain to be called PPV-T (Turkey), the geographical distribution in Turkey and possibly in other countries to be established by the following studies (9). These strains may be identified by lab tests. One of the most commons strains identified in Europe are PPV-D and PPV-M, which differ from the virulence and spreading point of view. The most efficient spreading vectors of the virus are the aphids (e.g. *Myzus persicae*). The aphids virus transmission mechanism is called non-persistent transmission, meaning that once the aphids have acquired the virus from an infected orchard, it shall remain contagious and may be only transmitted for a short period (minutes).

In some European countries, PPV occurrence in various geographical regions was associated with introduction of infected plants stocks from other countries.

One of the less known aspects pertaining to the PPV spreading is the potential role of native plants or weeds, in PPV survival and spreading. To this extent, the European lab tests have identified several common plants and weeds which may be inoculated mechanically with PPV (13).

**Matherial and Methods**

**Plant material**

The Plant material is formed of 8 native apricot varieties grafted on PPV-sensitive GF305 rootstock. For a concrete assessment of the genetic resistance to PPV, artificial infections have been made in controlled conditions (greenhouse to limit the PPV spreading).
Viral Material

Plum pox virus (PPV) is the responsible virus for the Sharka disease, the main viral disease of trees fruit Prunus, which includes several varieties important economically such as the apricot, peach or cherry trees. This disease affects the stone fruits in the Mediterranean basin and most of European countries. The situation continues to be critical in East European countries where the disease is endemic with a contamination rate between 15-70%. In general, fighting against viral plants disease is difficult due to the lack of curative means. At present, the pathogen viruses may only be controlled by preventive methods. Consequently, the fight against Sharka is based, mainly, on certified plantations and on campaigns of systematic eradication of attacked trees. This paper studies the interaction of Prunus genotypes with the PPV virus, the host plant being, as such, a woody plant. Fundamentally – the Prunus/PPV System forms an interesting sample for the research of plant-potyvirus interactions, considering the perennial plants specifications, grafted varieties, vegetative multiplications and a long selection cycle. On the other hand, this study represents an applied objective: a better understanding of the genetic resistance bases with Sharka shall allow the development of genetic resistance markers and commissioning of some techniques, such as markers assisted selection (SAM) of PPV-resistant varieties (cultivars). The viral material was formed of local PPV strains „Marcus and Dideron” from SCDP Bistriţa Romania.

Work methods

For phenotype, these studied progenies and which failed to reveal symptoms of Sharka disease on growing shoots were visually monitored serologically using the enzyme-linked immunosorbent assay (ELISA) and in addition PCR tests were performed on genotypes that were negative (without symptoms) on visual and serological inspections. (Figures 1 and 2). Thus, PPV infection was evaluated over three consecutive growth periods by visual determinations and Elisa.

The cut was carried out at the beginning of each growing period in order to induce strength to new shoots and to foster symptoms occurrence. Genotypes having neither developed symptoms in PPV visually nor by ELISA test, have been tested by the reverse transcription of polymerase chain reaction (RT-PCR) using specific primers for PPV: P1 and P2 (Wetzel et al. 1991), amplifying a segment of 243 bp on the C-terminal of the PPV CP gene. PPV was caught with PPV-polyclonal antibodies absorbed on an Eppendorf micro-tube. Kit procured from Sigma and used for RT-PCR. Thermal cycles schedule: RT-30 min at 50 °C, degeneracy / RT inactivation - 2 min t 94 °C, followed by 35 degeneracy cycles if the matrix: - 30 s to 94 °C, primer lining - 45 s at 61 °C and DNA-elongation 60 s at 72 °C. Further to the last cycle, the amplified DNA was elongated for 10 mins at 72°C.
An aliquot party from the amplified products (10 μl) were split into an agarose gel of 1.5% with 1x TBE electrophoresis buffer. Fragments were viewed by ethidium co-bromide colouring under UV light. (Kegler et al. 1998, Wetzel et al. 1991).

The plants were classified as resistant, if they did not show ELISA or RT-PCR positive reactions and symptoms during the last three growing periods that were assessed during three vegetational cycles.

**Results and Discussions**

Given that many of the Romanian apricot cultivars are not well enough supported and promoted on the Romanian market, we intend herein, among others, to assess some Romanian apricot cultivars already acknowledged in the cultivation as regards their PPV resistance. To this extend, certain Romanian apricot cultivars such as: "Iulia", "Traian", "Andrei", "Monica", "Callatis", "Bihoreanca", "Silvana", "Ioana", were grafted and infected on the GF305 rootstock (used as PPV indicator).

<table>
<thead>
<tr>
<th>Nr.crt.</th>
<th>Apricot cultivars</th>
<th>Variants</th>
<th>GF 305 - portaltoil</th>
<th>IC-RT-PCR</th>
<th>Soiul altoit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>‘Traian’</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>‘Andrei’</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>‘Monica’</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>‘Callatis’</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>‘Silvana’</td>
<td>6</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>‘Iulia’</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>‘Bihoreanca’</td>
<td>6</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>‘Ioana’</td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Photo. 3 and 4. - Aspects of response apricot varieties grafted on GF 305 after artificial infection with PPV

Legend Photo. 2.:  
1Kb - marker  
1. “Traian” – PPV resistant  
2. “Andrei” - sensitive  
3. “Monica” – sensitive  
4. “Callatis” – sensitive  
5. “Silvana” – sensitive  
6. “Iulia” – resistant  
7. “Bihoreana” – resistant  
8. “Ioana” - resist

The results have shown that the apricot resistant cultivars (“Traian”, “Iulia”, “Bihoreana”, “Ioana”) were able to limit the PPV virus infection, according to the previous results noted by other authors in the apricot research of Spain and France (Dosba et al, 1992; Dicenta and Audergon, 1998; Dicenta et al, 2000).

The “Monica” cultivar does not present serological and visual symptoms, but was RT-PCR positive (Table 1). The DAS-ELISA test was the most frequently used to diagnose PPV. However, molecular techniques which may detect even the smallest concentrations of a virus are more adequate to detect the virus in explants. RT-PCR, that combines the serological and molecular determination, provides more efficient results than ELISA (Martinez-Gomez et al. 2000). The genetic control hypotheses for the PPV resistance with apricot mentioned by two different authors (Ranković M et al. 1994), Ko LBER M (2001), (Audergon JM, et al. 1994) deem that the resistance allele is dominant. The infection process was different for each separate individual, in some plants the PPV presence was detected after the first repause period from the three vegetative cycles while certain plants failed to get infected.

Under artificial infections in the greenhouse, the results show that samples of GF 305 sensitive peach used as rootstocks were found to be positive as compared to most samples of apricot genotypes (bottom of the ELISA plate), collected from the same plant. Under these conditions, the virus is able to infect the peach rootstocks sensitive to Sharka but not certain apricot genotypes as “Traian”, “Bihoreanca”, “Iulia”, results correlating with those of other researchers such as Polák J (1994). These potential resistant individuals were tested using the molecular techniques in order to confirm the Sharka resistance nature.

The results on the molecular detection made by RT-PCR using a pair of primers (P1/P2) which amplifies a fraction of 243 bp located at the C-terminal end of PPV CP gene have proved that certain apricot genotypes, which appeared to be negative after the ELISA test, have been deemed to be positive after the molecular testing as the Monica genotype. This comes in support of the molecular testing sensitiveness (Wetzel. et al. 1991).
Conclusions

Because more of the Romanian apricot cultivars are not well enough supported and promoted on the Romanian market, we intend in this paper, among others, to also assess some Romanian apricot cultivars already acknowledged in the cultivation as regards their PPV resistance. Further to their indexing on GF305 and the serological and molecular tests, some cultivars such as “Traian”, “Bihoreanca”, “Iulia”, “Ioana” proved to be PPV resistant.

Identification of new natural sources of PPV resistance, use of these genotypes in the melioration programs where Romanian commercial cultivars well attuned to our country and, moreover, the implementation of the Molecular Markers Assisted Selection (MAS) based on the close association thereof with PPV resistance are measures to simplify significantly the melioration process which may be a promising strategy for the obtaining of PPV natural genetic resistance apricot cultivars.

References