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(RESEARCH ARTICLE)

Research into the serological response to *Plum pox virus* infection in certain indigenous peach genotypes

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Abstract

Sharka disease, caused by the *Plum pox virus* (PPV), is considered the most significant viral disease affecting stone fruit species, having a major impact on fruit yield and quality. Due to the severity of symptoms in susceptible varieties and the high efficiency of virus transmission via aphids, this disease represents one of the greatest threats to global fruit growing. The presence of PPV in a region significantly affects the production, propagation and marketing of planting material, as the virus is subject to national and international phytosanitary regulations concerning quarantine pests. Consequently, the occurrence of the disease necessitates the implementation of strict measures for monitoring, certification, containing the spread and, in some cases, the eradication of infected plants. The economic impact of Sharka disease is determined by both direct and indirect losses. Direct losses are caused by reduced production, a decline in fruit quality, the removal of infected trees, the implementation of quarantine measures, and the payment of compensation for destroyed plant material. Added to these are the indirect costs generated by plant health surveillance programmes, official inspections, diagnostic methods, the certification of planting material, and restrictions imposed on domestic and international trade in plants and horticultural products. Globally, the economic impact of the disease is considerable; estimates suggest that, since the 1970s, the costs associated with managing and controlling Sharka have exceeded 10 billion euros, which highlights the importance of developing effective strategies for prevention, diagnosis and genetic improvement to breed PPV-resistant *Prunus* varieties. It is therefore important to identify new genotypes within the genus *Prunus* that exhibit some degree of resistance to PPV. The present study aimed to investigate native peach genotypes that did not exhibit symptoms of PPV under field conditions. It was thus found that the varieties Armonie, Ștef, Dundi and *Prunus davidiana* exhibited symptoms of PPV following serological and molecular assessments.

Keywords: *Plum Pox Virus*; Resistance; *Prunus*; Simptoms; Pathogen.

1. Introduction

Plum pox virus (PPV), belonging to the species *Plum pox virus*, the genus Potyvirus and the family Potyviridae, is the aetiological agent of Sharka disease, considered the most serious viral disease affecting stone fruit species [1]. The virus is transmitted non-persistently by over 20 species of aphids and can also spread through vegetative propagation. To date, PPV infection has been reported in almost all species belonging to the genus *Prunus* [2]. The symptoms of the disease are associated with numerous adverse effects on production, including reduced fruit quality, fruit deformation, a decline in commercial value and premature fruit drop, resulting in significant economic losses for the fruit-growing sector. Due to its major economic impact and high potential for spread, PPV is classified in many countries as a phytosanitary quarantine organism [3]. In this context, the identification and development of sources of genetic

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resistance to PPV is one of the main objectives of breeding programmes for species of the genus *Prunus*. Recent research has shown that grafting certain peach genotypes onto the GF305 rootstock, which is recognized as being highly sensitive to the PPV-D strain, can lead to a gradual reduction in disease symptoms and a decrease in viral accumulation within the plant's tissues. This response appears to be specific to the interaction between peach and almond and is associated with the activation of complex defense mechanisms [4]. Graft-induced resistance is linked to changes in the plant's hormonal metabolism, particularly through the activation of the salicylic acid (SA)-mediated signalling pathway, accompanied by increased expression of genes involved in the defence response. At the same time, an increase in the concentrations of gibberellic acid and abscisic acid has been observed, alongside a reduction in jasmonate levels; these changes appear to help limit the replication and spread of the virus in infected plants [5]. Recent studies have shown that small RNAs (sRNAs) derived from three different PPV viruses, present in the almond scion, can migrate to the peach rootstocks, suggesting their involvement in defence mechanisms against viral infection. However, the protective effect observed is unlikely to be the direct result of gene silencing of the PPV genome, as there are significant differences between the nucleotide sequences of these viruses and those of PPV. [6] It is hypothesised that mobile sRNAs contribute to the activation or 'priming' of the host plant's defence mechanisms, thereby enhancing its ability to respond to viral infection. Furthermore, it has been demonstrated that mobile regulatory RNAs, together with other factors involved in intercellular signalling, can induce stable epigenetic modifications that are transmitted via both mitotic and meiotic division. These changes may influence the expression of genes involved in the plant's defence response and suggest that the protective effect exerted by the almond on the grafted peach may be associated, at least in part, with epigenomic regulatory mechanisms.[7] *Plum pox virus* (PPV) can cause symptoms in several plant organs. Characteristic symptoms can be observed on leaves, shoots, bark, petals, fruit and even the stones, with their severity varying depending on the variety, viral strain and environmental conditions (Figures 1 and 2).

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Figure 1 and Figure 2 Plum pox virus symptoms

The symptoms caused by *Plum pox virus* (PPV) are generally evident at the start of the growing season and are observed primarily on the leaves. These manifest as light green discoloration, chlorotic spots, chlorotic bands or rings, discoloration of the veins, as well as yellowing and deformation of the leaf blade. In some varieties, floral symptoms may also appear, consisting of discoloration of the petals. The fruit of infected plants frequently displays chlorotic spots and rings or light yellow linear patterns. As the infection progresses, the fruit may become misshapen or take on an irregular form, and brown or necrotic tissue may develop in the discolored areas, significantly affecting the quality and commercial value of the crop [8]. In European plums and apricots, PPV infection can cause premature fruit drop before ripening, whilst in Japanese plums and peaches, spots and rings on the fruit surface are characteristic. In the case of apricots, symptoms may also be observed on the stones, where light-coloured rings or spots appear, considered characteristic of the disease. In sweet cherries and sour cherries, the infection can cause fruit deformation and premature fruit drop [9]. The almond tree is considered one of the species in which symptoms are minimal; infected plants are often asymptomatic or show only occasional, subtle symptoms on the leaves. Furthermore, the severity of symptoms varies depending on the ripening period of the varieties, with symptoms generally being more pronounced in early-ripening varieties than in late-ripening ones[10]. In addition to fruit tree species of the genus *Prunus*, under

experimental conditions, PPV can also infect numerous herbaceous species frequently used as host plants in studies concerning the virus's biology and the relationships between the pathogen and the host plant [10].

2. Material and methods

2.1. Plant material

A one-step real-time PCR technique was used for the rapid detection of PPV infection in six local peach genotypes from private gardens. A total of six different peach genotypes from the national collection ("Crevedia", "Floarea", "Armonie", 'Nuca', 'Ştef', 'Dundi', etc.), including the controls and the positive control, were tested for their susceptibility to PPV infection under natural field conditions.

2.2. Viral material

Typical of potyviruses, the PPV genome is a positive-sense single-stranded RNA (ssRNA) with a protein bound to its 5′ end and a 3′-terminal poly-A tail. It is encapsidated by a single type of capsid protein (CP) in rod-like, flexuous particles and is translated into a large polyprotein, which is proteolytically processed into at least 10 final products: P1, HCPro, P3, 6K1, CI, 6K2, VPg, NIapro, NIb and CP. Furthermore, it is thought that P3N-PIPO is produced via a translational frame shift.

2.3. Methodology

The ELISA test was developed in 1976 by Clark and Adams and was initially used to detect *Plum pox virus* (PPV) in stone fruit species. Over time, this method has been referred to by various names, the best-known being DAS-ELISA (Double Antibody Sandwich ELISA).

The ELISA method allows viruses to be identified even when they are present in very low concentrations. Furthermore, in certain situations, viruses can be detected directly from vector organisms, making this technique extremely useful in phytopathological diagnosis.

The principle of the method is based on the ability of specific antibodies to bind to the viral antigen and to be conjugated with an enzyme. To identify a virus in a plant sample, antibodies specific to the pathogen of interest are added. The test result can be interpreted as follows:

- if the viral antigen is present, it binds to the antibodies, forming an enzyme-linked immune complex. Following the addition of the enzyme-specific substrate, hydrolysis of the substrate takes place, a reaction indicated by the appearance of a characteristic colour;
- if the sample comes from a healthy plant, the immune complex does not form and no colour reaction occurs;
- if the sample is infected with a virus other than the one for which the antibodies used are specific, the antigen will not be recognized, so the immune complex will not form and no colour reaction will occur.

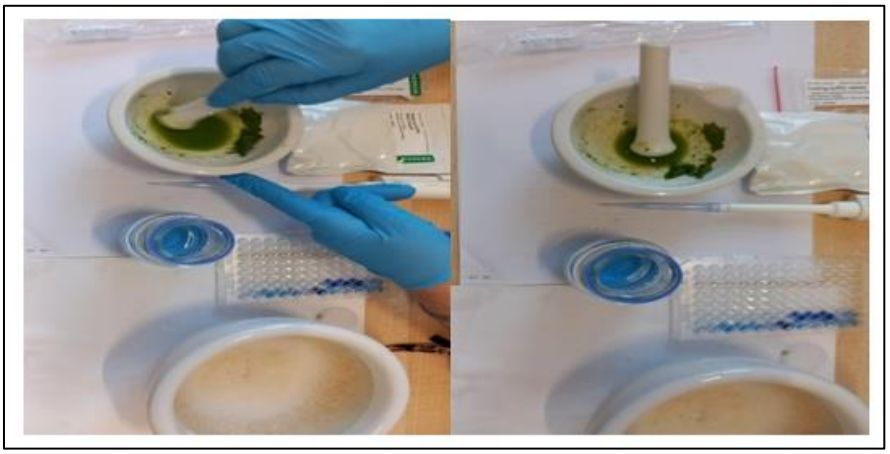

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Figure 3 and Figure 4 Elisa test

To carry out the ELISA test, the following are required: a polystyrene plate (Figure 2), which serves as a reaction substrate; antibodies specific to the virus under investigation (immunoglobulin G – IgG); the same antibodies conjugated to an enzyme; and the corresponding substrate (Figures 3 and 4).

The polystyrene plate consists of 96 wells, but those located at the edges are not normally used, leaving 60 wells available for carrying out the test. The analysis uses control samples taken from a healthy plant and an infected plant, as well as a blank sample. To ensure the accuracy of the results, samples must be taken from the same type of plant organ, and the plants from which they are taken must belong to the same species as the plant being tested. The samples collected are homogenised in a BPSTween extraction buffer solution, specific to the detection of *Plum pox virus* (PPV), after which they are filtered. The resulting filtrate is the material used in the actual test. To prevent cross-contamination, samples are prepared in a strict order: the sample from the healthy plant is processed first, followed by the sample taken from the infected plant. The ELISA test is carried out in four successive stages, each of which plays a vital role in detecting the pathogen. (Figures 5 and 6)

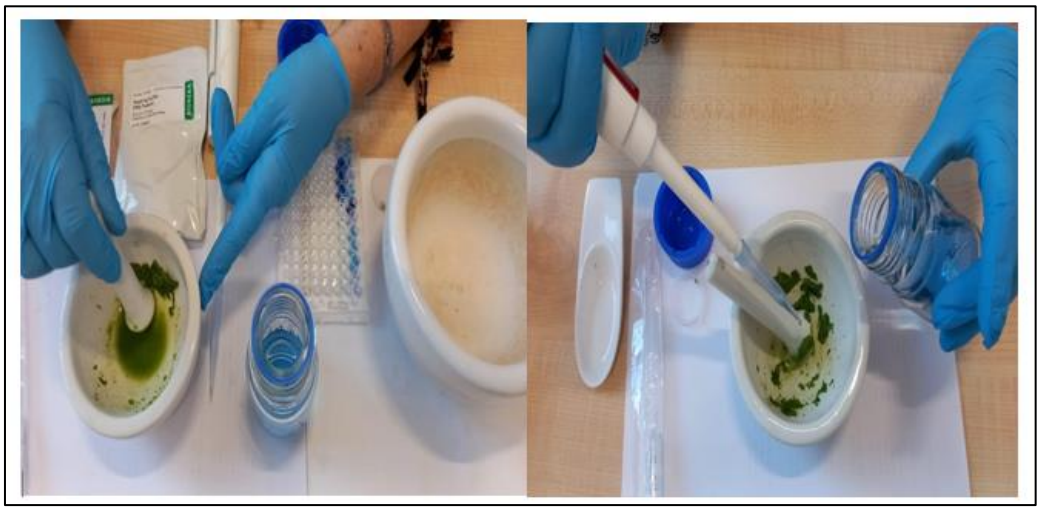

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Figure 5 and Figure 6 The stages of the ELISA test

- Stage 1 – Plate sensitization. The specific antibodies are diluted in the coating buffer, using 200 µl of antibodies per 20 ml of buffer. From this solution, 200 µl is dispensed into each well of the polystyrene plate. The plate is then covered, placed in a humid chamber and incubated overnight at refrigerator temperature. The following day, the plate is washed two or three times with wash buffer to remove unbound antibodies. (Figures 5 and 6)
- Stage 2 – Antigen addition. The plant extract, obtained by homogenizing the samples in extraction buffer, is dispensed into the wells in 200 µl volumes. The plate is covered again, placed in a humid chamber and incubated overnight at a temperature of 4–6°C to allow the viral antigen to bind to the antibodies fixed to the surface of the wells.
- Stage three – addition of the enzyme-conjugated antibody. After washing the plate again, 200 µl of the enzyme-conjugated antibody solution, prepared in the conjugate buffer, is added to each well. The plate is covered, placed in a humid chamber and incubated at 30°C for approximately 5 hours to allow the formation of the immune complex.
- Stage 4 – reaction with the substrate. The enzymatic substrate, p-nitrophenyl phosphate (pNPP), is dissolved in the substrate buffer and dispensed into each well in a volume of 200 µl. The plate is incubated at room temperature (20–25°C) in the dark to allow the colour reaction to develop. In the presence of the virus, the enzyme hydrolyses the substrate, causing a yellow colour to appear, the intensity of which is directly proportional to the amount of antigen present in the sample. Results may range from pale yellow to deep yellow, depending on the concentration of the virus.

3. Results and discussion

Following serological testing carried out on the peach varieties included in the study, interpretation of the colour reaction revealed that the **Armonie, Ștef, Dundi** and *Prunus davidiana* varieties tested negative, indicating the absence of infection with *Plum pox virus* (PPV). In contrast, the **Crevedia, Floarea, Nuca** and **Cardinal** varieties showed positive

reactions, confirming the presence of the virus, even though the symptoms observed on the plants were mild. The results are presented in Table 1.

Table 1 Serological results of the ELISA test

Nr crt.	The genotype studied	Serological results of the enzyme-linked immunosorbent assay Elisa
1	Crevedia	positive
2	Floarea	positive
3	Armonie	negative
4	Nuca	positive
5	Ștef	negative
6	Dundi	negative
7	*Prunus davidiana*	negative
8	Cardinal	positive
9	Negative probs	negative
10	Positive probs	positive

A one-step real-time PCR technique was used for the rapid detection of PPV infection in six local peach genotypes from private gardens. A total of six different peach genotypes from the national collection ("Crevedia", "Floarea", "Armonie", 'Nuca', 'Ștef', 'Dundi', etc.) including the controls and the positive control, were tested for their ability to sustain PPV infection under natural field infection conditions.

The varieties were tested under field conditions using the single-step Real-Time PCR technique; however, for greater certainty, the Romanian varieties were indexed against GF 305 (a PPV sensitivity indicator) and artificially infected under conditions of isolation from infection vectors. Samples were collected from both the varieties and the rootstocks, and these were analysed serologically and molecularly. (Table 2)

Table 2 Serological and molecular testing of certain peach genotypes artificially infected with PPV

Nr. Crt.	Genotyps	Serological results of the ELISA test 2024	Real Time PCR 2024	Serological results of the ELISA test 2025	Real Time PCR 2025
1	Crevedia	positive	++	positive	++
2	Floarea	positive	++	positive	++
3	Armonie	negative	--	negative	--
4	Nuca	positive	++	positive	++
5	Ștef	negative	--	negative	--
6	Dundi	negative	--	negative	--
7	*Prunus davidiana*	negative	--	negative	--
8	Cardinal	positive	++	positive	++
9	Negative probs	negative	--	negative	--
10	Positive probs	positive	++	positive	++

Not all peach varieties developed obvious symptoms, and PPV was detectable only through highly sensitive, single-step real-time PCR analyses. Given the infection rate and the results of the single-step real-time PCR analysis, it can be concluded that there are varieties within the germplasm collection that appear to exhibit stable resistance to PPV (Figures 7 and 8).

(source/original)

Figure 7 and Figure 8 Peach samples analysed serologically using the ELISA test

The ELISA assay has been optimised using the double sandwich antibody (DAS–ELISA) procedure, employing the certified Nunc MaxiSorp F96 immunoassay plate, with a volume of 200 µl per well. This test is suited to standard protocols in seed certification laboratories where large numbers of plants are tested each day. It is a more robust method used for potatoes, fruit trees and vines.

4. Conclusion

In this study, assessments carried out using serological and molecular methods revealed significant differences between the genotypes analysed in terms of their response to PPV infection. The genotypes **Armonie, Ştef, Dundi** and ***Prunus davidiana*** did not show positive reactions, indicating the absence of PPV infection under the experimental conditions analyzed. In contrast, the **Crevedia, Floarea, Nuca** and **Cardinal** genotypes tested positive, confirming the presence of the virus, even though the symptoms observed in the field were limited or mild. These results highlight the importance of using laboratory diagnostic methods, as assessment based solely on visible symptoms may lead to an underestimation of the incidence of infection. Advances in PPV research have contributed significantly to the development of modern diagnostic and detection methods, which are essential for the implementation of quarantine measures, the certification of planting material and the containment of the virus's spread. At the same time, numerous studies have sought to elucidate the mechanisms of the disease's epidemiology and dispersal, the interactions between the plant and the virus, and the development of breeding strategies based on both conventional genetic resistance and modern biotechnologies.

In the current context, continuing research through interdisciplinary collaboration is essential for capitalising on the accumulated knowledge regarding the biology and genetic variability of PPV. At the same time, the integration of molecular marker-assisted selection methods into breeding programmes can accelerate the development of *Prunus* varieties and rootstocks with a high and stable level of genetic resistance to PPV, thereby contributing to the development of sustainable fruit growing and to the reduction of economic losses caused by this virus.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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