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Study of Phenotypic and Molecular Approaches of *Potyvirus plumpoxi* Resistance Evaluation in New Czech Apricot Hybrids

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Abstract. **This study investigates the resistance of 17 apricot hybrids to *Potyvirus plumpoxi* (PPV) with the use of phenotyping, serological assays [double-antibody-sandwich enzyme-linked immunosorbent assay (DAS-ELISA)], and molecular marker analyses. The expression of symptoms expression was evaluated on four occasions throughout the course of the experiment, while the presence of the virus in the plant was diagnosed by DAS-ELISA. In addition, the genotyping with simple sequence repeat (SSR) markers (PGS1.21, PGS1.23) and ParPMC2 allele-specific polymerase chain reaction (PCR) for the purpose of evaluating PPV resistance. A total of five new apricot hybrids were evaluated as resistant to PPV, while five hybrids exhibited resistance despite the presence of some PPV symptoms. In addition, three hybrids demonstrated genotype-phenotype incongruences (GPIs). The statistical analysis revealed a significant relation between PPV-positive plants and the absence of resistance-associated alleles, confirming that allelic composition is strongly associated with phenotypic resistance. The average value of symptoms evaluation was 0.3 ± 0.1 for resistant hybrids and 1.7 ± 0.3 and 1.5 ± 0.4 for susceptible hybrids evaluated by PGS1.21 and PGS1.23, respectively. The Tukey's honestly significant difference (HSD) test showed the significant difference between genotypes evaluated by ParPMC2 marker as "RR" and "RS" (resistant) from "SS" (susceptible). However, discrepancies in hybrid resistance evaluation highlight the complexity of inheritance and the potential errors in genotyping. These results indicate that the most reliable method for evaluating PPV resistance is the time-consuming phenotyping method. Conversely, the use of SSR markers greatly improves the effectiveness of breeding.**

Apricot (*Prunus armeniaca* L.) belonging to the *Prunus* family Rosaceae, is one of the most favorite temperate stone fruits. The fruits have numerous health benefits and are rich in nutrients, including fiber, minerals, vitamins, and phenolics. Due to their delicious taste, flavor, and aroma, they may be consumed fresh, dried, or canned (Göttingerová et al. 2021; Rakida 2023a; Yilmaz et al. 2023). The global production of apricots in the world reached 3.8 million tonnes (Mt), the main part of it is produced in Asian countries, mainly Türkiye (0.85 Mt), which produces the same amount as all European countries combined (FAO 2022). In the Czech Republic, growers produced only 6500 tonnes (Němcová and Buchtová 2023) and due to deteriorated climatic conditions, late spring frost, and disease damage, the production of apricots is constantly decreasing, leading to selling imported fruit in the supermarkets. The breeding of new frost- and disease-resistant apricot cultivars has the potential to overcome the limiting factors currently facing apricot growing (Göttingerová and Nečas 2020).

One of the most important diseases affecting stone fruits, especially plums and apricots, is sharka. *P. plumpoxi* (PPV) belonging to *Potyvirus* was first described in 1932 (Atanasoff 1932). Following extensive research, it has been established that PPV can be transmitted by aphids or through vegetative propagation. In general, susceptible cultivars are severely damaged and exhibit the characteristic symptoms of ring-shaped chlorotic spots on leaves and fruits (Rubio et al. 2023). The most effective method of prevention is the breeding and growing of PPV-resistant apricot cultivars. It is also important to maintain the quality, taste, and attractiveness of the fruit (Asma 2012; Gürcan et al. 2019; Nečas et al. 2020; Nesheva et al. 2019; Rubio et al. 2023). The classical phenotyping method for PPV resistance is still desirable, although new molecular characterization using SSR markers and allele-specific PCR methods were developed (Polo-Oltra et al. 2020; Soriano et al. 2012; Zuriaga et al. 2018). The use of SSR markers has become a common practice in the field of PPV resistance, facilitating the acceleration of selection and breeding of new PPV-resistant genotypes (Gürcan et al. 2019; Nicolás-Almansa et al. 2023; Rakida et al. 2023b). It has been demonstrated that the results obtained from different methods for evaluating the PPV resistance level are highly correlated. However, in some cases, there are GPIs related to environmental conditions and/or gene-environment interaction (Polo-Oltra et al. 2020). Decroocq et al. (2014) concluded that the use of a marker colocalizing with the PPVres locus is not a fully reliable method. Nevertheless, the results of numerous studies indicate that it remains an effective tool for the preliminary selection of resistant material (Nicolás-Almansa et al. 2023; Passaro et al. 2017; Rakida et al. 2023b). The aim of this study was to compare different methods for PPV resistance evaluation on new apricot hybrids derived from the breeding program at the Faculty of Horticulture in Lednice, Mendel University in Brno.

Material and Methods

Site and plant material. The plant material consisted of 22 apricot genotypes, including 17 apricot hybrids (Table 1) and 5 PPV-resistant control cultivars (Candela, Harlayne, Goldrich, Orange Red, Sophinka). The symbol LEM represents the city of Lednice (Faculty of Horticulture in Lednice, Mendel University in Brno) and the Czech word for apricot "meruňka." The plants were grown in insect-proof conditions (plastic tunnels preventing the entry of insects) in containers in a 1:1 mixture of topsoil and peat, irrigated using a drip system, and regularly fertilized. The plants were pruned with sterilized tools always in the spring to encourage the growth of new shoots.

PPV phenotyping and detection. In 2020, 10 plants of each apricot genotype (Table 1) were prepared by grafting of tested apricot genotypes on 1-year-old GF-305 rootstocks. The Dideron type of *P. plumpoxi* (PPV-D) was used as inoculum, as it is the most prevalent type known to infect apricots under the prevailing conditions. An apricot plant, previously positively tested to PPV-D type and exhibiting pronounced sharka symptoms from the virus collection of the Department of Fruit Science was used as the inoculum.

In Summer 2021, the plants were graft-inoculated with PPV-D, when one bud was grafted onto the apricot genotype and one bud onto the shoot of a rootstock (Fig. 1). In Summer 2022, the plants were reinoculated by grafting a PPV-D bud onto the apricot genotype to increase the infection pressure of the virus. One plant of each genotype, which had not been inoculated, was maintained separately as a healthy control.

The PPV symptoms were evaluated for 2 years (2023 and 2024) in two evaluations for

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Table 1. Analyzed apricot genotypes and their origin.

Genotype	Origin	Genotype	Origin
LEM/2017/8	Orange Red × Poyer	LEM-139	Uncertain
LEM/2017/12	Orange Red × Poyer	LEM-151 (LE-2904)	Hungarian Best × SEO
LEM/2017/18	Poyer × Harlayne	LE-3246	Vestar × SEO
LEM/2017/20	Orange Red × Poyer	LE-3662	Uncertain
LEM/2017/25	Uncertain	H 1077	Uncertain
LEM/2017/26	Poyer × Orange Red	H 994	Uncertain
LEM-106	Uncertain	Candela	Hungarian Best × SEO
LEM-120	(Goldrich × SEO) × Betinka	Harlayne	
LEM-121	Uncertain	Goldrich	
LEM-122	(Goldrich × SEO) × Betinka	Orange Red	
LEM-123	Early Blush × Candela	Sophinka	Hungarian Best × SEO

SEO = Stark Early Orange.

both years. First, the symptoms were detected on the leaves of GF-305 peach seedlings at the initial observation point, followed by a subsequent examination 2 weeks later. The expressed symptoms were scored according to the following parameters: 0 = no symptoms, 1 = barely visible chlorotic spots on a few leaves, 2 = clearly visible chlorotic spots on a few leaves, 3 = clearly visible chlorotic spots on more than 50% of the leaves of the plant, and 4 = strong expression of chlorotic spots on the most of leaves and their deformation (Fig. 2) (Polák and Salava 2008).

The plants were tested for PPV in the apricot leaves by DAS-ELISA (Clark and Adams 1977) applying specific PPV antibodies and buffers from Bioreba (Reinach, Switzerland) in Spring and Autumn 2023 and Spring 2024.

DNA extraction and marker-assisted selection. DNA extraction was performed by Qiagen Dneasy Plant Mini Kit (Qiagen, Germantown, MD, USA). The concentration of extracted DNA eluted in elution buffer (AE, Qiagen) was measured spectrophotometrically on SPECTROstar Nano (BMGLabtech, Ortenberg, Germany) in 2 µL of volume. Final concentration of DNA was adjusted to 2 ng·µL^{-1} for marker-assisted selection.

Three markers, PGS1.21, PGS1.23 (Soriano et al. 2012), and ParPMC2 (Polo-Oltra et al. 2020; Zuriaga et al. 2018), associated with PPV resistance were analyzed in all tested genotypes (Table 1). As a control, the parental cultivars were analyzed.

For markers PGS1.21 and PGS1.23, the 25-µL PCR mix consisted of sterile nuclease-free H_2O, 1x GoTaq Buffer (Promega, Madison, WI, USA), 1.5 mM $MgCl_2$ (Promega), 0.2 mM dNTP (Promega), 0.2 µM forward primer, 0.2 µM reverse primer, 1 U GoTaq polymerase G2 Flexi (Promega), and 10 ng DNA. The cycling conditions were as follows: an initial denaturation at 95 °C for 2 min, 35 cycles at 95 °C for 30 s, 45 °C/51 °C (PGS1.21/PGS1.23) for 30 s, 72 °C for 30 s, and final extension of 72 °C for 5 min. PCR products were separated in the capillary of the genetic analyzer ABI Prism 310 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA).

For marker ParPMC2, the 20-µL PCR reaction mix consisted of sterile nuclease-free H_2O, 1x GoTaq Buffer (Promega), 2 mM $MgCl_2$ (Promega), 0.125 mM dNTP (Promega), 0.5 µM forward primer, 0.5 µM reverse primer, 1 U GoTaq polymerase G2 Flexi (Promega), and 4 ng DNA. The cycling conditions were as follows: an initial denaturation at 95 °C for 2 min, 35 cycles at 95 °C for 30 s, 55 °C for 45 s, 72 °C for 45 s, and final extension of 72 °C for 5 min. PCR products were electrophoresed in 1% agarose gel.

Statistical analysis. The data were statistically analyzed using Statistica 14 software (TIBCO Software Inc., Palo Alto, CA, USA). The linear correlation was used for the relation analysis of number of PPV-positive plants and their scaled symptoms. The significance of data was analyzed by analysis of variance (ANOVA) and post hoc Tukey's HSD test ($P < 0.05$) was used for differentiation of homogeneous groups of number of PPV-positive plants, scaled symptoms, and analyzed SSR markers.

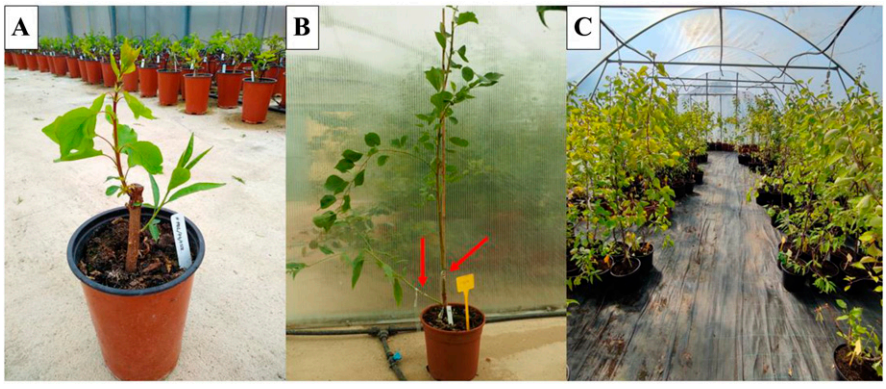

Fig. 1. (**A**) Apricot plant prepared for phenotyping with sprouted GF-305 rootstock in Spring 2021. (**B**) Inoculated apricot plant in Summer 2021, the red arrows show the Dideron type of *Potyvirus plumpoxi*–positive buds. (**C**) Apricot plants during the phenotyping experiment in technical isolation.

Results

Phenotyping and PPV detection The symptom expression on rootstock GF-305 confirmed the successful PPV inoculation of the plants, despite that the level between genotypes was different (Table 2).

In general, the data demonstrated an increasing trend of PPV symptoms on GF-305 in our evaluation throughout the experiment, with the exception of the rootstocks of hybrids LEM/2017/26 and LEM-106. In the initial evaluation in Spring 2024, the rootstocks exhibited the highest level of PPV symptoms and then decreased. For hybrids LEM/2017/26, LEM-106, and LEM/2017/8, the highest level of PPV symptoms on GF-305 was observed in the first cycle of both years. The hybrids LEM-139 and LEM-120 exhibited a higher level of PPV symptoms on GF-305 in Spring 2023 than in 2024.

The PPV symptoms on the apricot leaves increased over time and they were scaled lower than 3 on all plants (Table 2). The highest level of PPV symptoms was observed in Spring 2024 - 1. evaluation (2.4 ± 0.1), when high deformation and chlorotic spots were detected on GF-305 leaves. The hybrids LE-3662, LEM/2017/12, and H 1077 were scaled as 0 in all seasons. The hybrids LE-3246, LEM-123, LEM/2017/26, LEM-151, and LEM-139 exhibited symptoms that were scaled below 1 on average. Thus, these hybrids were evaluated to be resistant to PPV based on the results of the phenotyping.

The symptoms on leaves of the hybrids H 994, LEM/2017/20, and LEM/2017/18 were scaled as 0 in 2023 and they increased to 1 in 2024. The symptoms of hybrid LEM 2017/25 were scaled as 2 in the second cycle in 2023 and in 2024 they decreased to 1. Thus, the resistance of these five hybrids is uncertain after 2 years of evaluation by phenotyping.

The symptoms of hybrids LEM-122, LEM-120, LEM-106, and LEM/2017/8 were scaled higher than 2. Thus, they were evaluated as susceptible to PPV according to these results.

The results from DAS-ELISA test (Fig. 3) showed eight hybrids to be PPV negative in all plants. The number of positive plants of other hybrids increased in the time, with the exception of LEM-121 and LEM-151, where the highest number of positive plants was observed in Autumn 2023. All plants of the hybrids H 1077 and LEM-139 were negative in 2023 and a low number of plants (6.3% and 24%, respectively) were PPV positive in 2024.

We assume that the symptoms expression is related to the PPV susceptibility of the apricot plants. All plants of the asymptomatic hybrids LEM/2017/12, LEM/2017/26, LEM-123, LE-3246, and LE-3662 were negative by DAS-ELISA. A low number of the

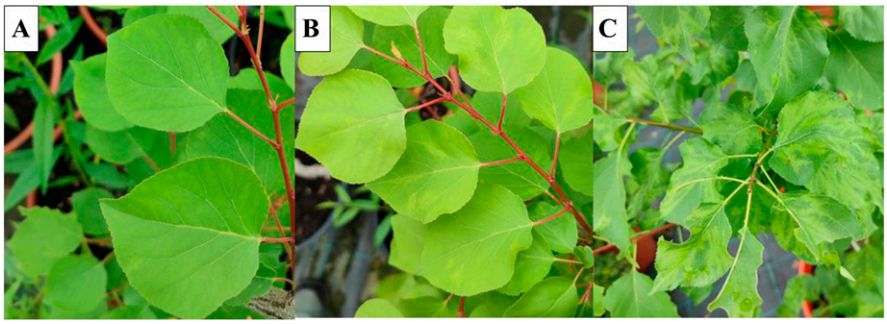

Fig. 2. Typical symptoms of *Potyvirus plumpoxi* on apricot leaves. (**A**) Barely visible ring-shape spots (1 on the scale). (**B**) Clearly visible chlorotic spots around the vein of leave (2 to 3 on the scale). (**C**) Strong expression of chlorotic spots and deformation of the leaves (4 on the scale).

asymptomatic hybrid H 1077 was found to be PPV positive, while the asymptomatic hybrid LEM-151 was evaluated as positive in more than 50% of the plants. The hybrids LEM/2017/20, LEM/2017/18, and H 994 exhibited symptoms and all plants were determined to be PPV negative by DAS-ELISA. The remaining hybrids were symptomatic and were diagnosed as PPV positive by DAS-ELISA.

The statistical ANOVA demonstrated a statistically significant result of symptoms monitoring. In Spring 2023 ($P < 10^{-5}$), the negative plants exhibited symptoms rated on a scale as 0.48 ± 0.06, whereas the positive plants exhibited symptoms rated at 1.5 ± 0.2. In Spring 2024 ($P < 10^{-4}$), the negative plants were rated on a scale 0.59 ± 0.06, the positive plants were rated at 2.2 ± 0.1. A linear correlation was identified between the data of symptoms and percentage (%) of positive plants detected by ELISA (Fig. 4). The correlation coefficient was higher for data from Spring 2024. Thus, the data showed the importance of the timing of PPV resistance analysis in plants, when the virus is detectable in the plant.

ParPMC2 marker analysis. The ParPMC2 genotyping enables detection of the R-allele and S-allele in separated PCR reactions and can be used for identification of homozygotes ("RR"-resistant and "SS"-susceptible) and heterozygotes ("RS") (Fig. 5). Two resistant control cultivars (Candela and Sophinka) and one parent cultivar (Betinka) were categorized as "RR" and two resistant control cultivars Harlayne and Orange Red as "RS."

Two apricot hybrids (LE-3662 and LE/2017/25) were evaluated as "RR." Apricot hybrids LEM-121, LEM-106, LEM-139, and LEM/2017/8 and parent cultivars Strepet, Early Blush, and Poyer were evaluated as "SS." The rest of the apricot hybrids and parent cultivars (Vestar, Goldrich, SEO, and Hungarian Best) were categorized as "RS."

PGS1.21 and PGS1.23 marker analysis. The data of SSR markers PGS1.21 and PGS1.23 associated with PPV resistance are summarized in Table 3. All control resistant cultivars (Sophinka, Candela, Orange Red, Goldrich, and Harlayne) and two parent cultivars (Betinka and SEO) had a "resistant" allele of 142 base pairs (bp) analyzed by the PGS1.23 marker and 222 bp analyzed by the PGS1.21 marker. In total, 10 hybrids had "resistant" alleles in both analyzed markers. Different results were evaluated for three hybrids (LEM-122, LEM-151, and LEM-120) that were evaluated to be PPV resistant only by the PGS1.23 marker but not by the PGS1.21 marker. Five parent cultivars (Poyer, Vestar, Early Blush, Strepet, and Hungarian Best) and seven hybrids were evaluated to be susceptible by both analyzed markers.

Statistical analysis and final comparison of used methods. In summary, the results of the phenotyping, ELISA testing, and SSR marker analysis are summarized in Table 3. The evaluation of PPV resistance according to the symptom expression scaled 1 was noted as uncertain. Five apricot hybrids (LE-3662, LEM/2017/12, LEM/2017/26, LEM-123, and LE-3246) and all resistant control cultivars were evaluated as PPV resistant and two apricot hybrids (LEM/2017/8 and LEM-106) were evaluated as susceptible by all methods. The symptoms evaluation of the hybrids LEM-121 and LEM-139 and results from DAS-ELISA were uncertain; however, the hybrids were evaluated as susceptible by other methods. The hybrids LEM 2017/25, H 1077, LEM/2017/20, LEM/2017/18, and H 994 were evaluated as PPV resistant, despite that the results from phenotyping or DAS-ELISA were uncertain (Table 3). The symptomatic hybrids LEM-122, LEM-151, and LEM-120 expressed GPIs, when they were classified as resistant by PGS1.23 marker.

In total, four hybrids originated from crossing between Orange Red (resistant) and Poyer (susceptible) cultivars, where LEM/2017/12, LEM/2017/20, and LEM/2017/26 were classified as PPV resistant and LEM/2017/8 as susceptible. Two hybrids from the crossing of Vestar (S) and SEO (R) (Betinka and LE-3246) and two hybrids from crossing Hungarian Best (S) and SEO (R) were evaluated as PPV resistant.

ANOVA revealed significant differences between resistant and susceptible genotypes

Table 2. Results from *Potyvirus plumpoxi* (PPV) phenotyping of apricot hybrids and control cultivars grafted on GF-305. In parentheses is a mean PPV symptoms intensity from a group of the plants of the hybrid or cultivar scaled according to Polák and Salava (2008).

	Spring 2023 - 1. evaluation		Spring 2023 - 2. evaluation		Spring 2024 - 1. evaluation		Spring 2024 - 2. evaluation	
Hybrid/Cultivar	GF-305	Apricot	GF-305	Apricot	GF-305	Apricot	GF-305	Apricot
LE-3246	1 (1.0 ± 0.6)	0 (0.0 ± 0.0)	1 (1.3 ± 0.3)	0 (0.3 ± 0.3)	3 (3.3 ± 0.6)	1 (0.5 ± 0.3)	3 (2.5 ± 0.5)	0 (0.0 ± 0.0)
LE-3662	2 (1.5 ± 0.5)	0 (0.0 ± 0.0)	2 (1.5 ± 0.5)	0 (0.0 ± 0.0)	2 (2.0 ± 0.0)	0 (0.0 ± 0.0)	2 (2.0 ± 0.0)	0 (0.0 ± 0.0)
LEM/2017/12	2 (1.9 ± 0.3)	0 (0.2 ± 0.1)	3 (2.8 ± 0.5)	0 (0.1 ± 0.1)	2 (1.7 ± 0.2)	0 (0.4 ± 0.2)	1 (1.3 ± 0.2)	0 (0.1 ± 0.1)
LEM-123	1 (1.4 ± 0.2)	1 (0.5 ± 0.3)	2 (2.1 ± 0.4)	0 (0.1 ± 0.1)	2 (1.9 ± 0.4)	0 (0.1 ± 0.1)	2 (2.1 ± 0.3)	0 (0.3 ± 0.2)
H 1077	3 (2.5 ± 0.2)	0 (0.2 ± 0.1)	3 (2.5 ± 0.2)	0 (0.1 ± 0.1)	3 (2.9 ± 0.3)	0 (0.0 ± 0.0)	1 (1.3 ± 0.2)	0 (0.3 ± 0.1)
LEM/2017/26	2 (1.5 ± 0.2)	0 (0.0 ± 0.0)	1 (1.1 ± 0.2)	0 (0.4 ± 0.2)	2 (2.2 ± 0.3)	1 (1.0 ± 0.2)	2 (1.7 ± 0.1)	0 (0.3 ± 0.1)
LEM-151	1 (1.2 ± 0.2)	1 (0.6 ± 0.2)	2 (1.8 ± 0.4)	1 (1.2 ± 0.6)	2 (2.0 ± 0.3)	0 (0.2 ± 0.2)	2 (2.0 ± 0.3)	0 (0.4 ± 0.2)
LEM-139	3 (2.8 ± 0.3)	1 (0.7 ± 0.2)	3 (3.0 ± 0.3)	0 (0.2 ± 0.2)	2 (2.2 ± 0.5)	1 (0.7 ± 0.2)	2 (1.7 ± 0.3)	1 (0.7 ± 0.3)
H 994	2 (1.8 ± 0.3)	0 (0.0 ± 0.0)	2 (2.0 ± 0.4)	0 (0.0 ± 0.0)	3 (2.8 ± 0.3)	1 (1.3 ± 0.3)	2 (2.0 ± 0.4)	1 (0.8 ± 0.5)
LEM-121	2 (1.8 ± 0.2)	1 (0.5 ± 0.2)	2 (2.1 ± 0.2)	1 (0.9 ± 0.2)	4 (3.7 ± 0.2)	1 (1.0 ± 0.2)	2 (2.3 ± 0.2)	1 (0.8 ± 0.3)
LEM/2017/20	2 (1.8 ± 0.3)	0 (0.0 ± 0.0)	2 (2.3 ± 0.5)	0 (0.0 ± 0.0)	3 (2.8 ± 0.6)	1 (1.0 ± 0.0)	3 (2.8 ± 0.6)	1 (1.0 ± 0.0)
LEM/2017/25	2 (1.9 ± 0.1)	1 (1.1 ± 0.4)	3 (2.6 ± 0.3)	2 (1.5 ± 0.3)	4 (3.5 ± 0.2)	1 (0.5 ± 0.2)	3 (2.6 ± 0.3)	1 (1.1 ± 0.1)
LEM/2017/18	1 (1.0 ± 0.4)	0 (0.3 ± 0.3)	2 (2.3 ± 0.6)	0 (0.0 ± 0.0)	4 (3.8 ± 0.8)	1 (1.0 ± 0.4)	3 (2.5 ± 0.3)	1 (1.1 ± 0.3)
LEM-122	1 (0.9 ± 0.2)	0 (0.3 ± 0.2)	2 (1.9 ± 0.3)	1 (1.0 ± 0.2)	3 (3.4 ± 0.2)	1 (1.3 ± 0.2)	2 (2.4 ± 0.2)	2 (1.6 ± 0.2)
LEM-120	3 (2.5 ± 0.5)	1 (0.8 ± 0.3)	3 (2.5 ± 0.3)	1 (0.8 ± 0.3)	2 (1.8 ± 0.8)	2 (1.5 ± 0.5)	2 (2.0 ± 0.4)	2 (2.3 ± 0.3)
LEM-106	2 (2.0 ± 0.3)	1 (1.3 ± 0.3)	1 (1.4 ± 0.2)	2 (1.5 ± 0.3)	2 (2.0 ± 0.4)	3 (3.0 ± 0.4)	1 (1.4 ± 0.2)	2 (2.4 ± 0.4)
LEM/2017/8	3 (2.5 ± 0.2)	1 (0.7 ± 0.2)	2 (2.1 ± 0.3)	2 (1.8 ± 0.3)	4 (3.8 ± 0.1)	2 (2.2 ± 0.3)	3 (2.6 ± 0.2)	3 (2.5 ± 0.3)
Orange Red	1 (1.2 ± 0.3)	0 (0.0 ± 0.0)	3 (2.8 ± 0.4)	1 (0.7 ± 0.5)	1 (1.0 ± 0.0)	0 (0.0 ± 0.0)	3 (2.8 ± 0.2)	0 (0.0 ± 0.0)
Candela	1 (1.0 ± 0.0)	0 (0.0 ± 0.0)	3 (2.8 ± 0.6)	1 (0.7 ± 0.5)	1 (1.0 ± 0.0)	0 (0.0 ± 0.0)	3 (3.0 ± 0.0)	0 (0.0 ± 0.0)
Sophinka	1 (1.2 ± 0.1)	0 (0.0 ± 0.0)	3 (2.6 ± 0.5)	0 (0.3 ± 0.5)	1 (1.2 ± 0.3)	0 (0.0 ± 0.0)	3 (2.5 ± 0.5)	0 (0.0 ± 0.0)
Goldrich	1 (1.0 ± 0.0)	0 (0.0 ± 0.0)	3 (2.5 ± 0.7)	0 (0.4 ± 0.5)	1 (1.4 ± 0.1)	0 (0.0 ± 0.0)	2 (2.4 ± 0.7)	0 (0.0 ± 0.0)
Harlayne	1 (1.0 ± 0.0)	0 (0.0 ± 0.0)	3 (2.7 ± 0.5)	0 (0.2 ± 0.4)	1 (1.0 ± 0.0)	0 (0.0 ± 0.0)	3 (2.8 ± 0.4)	0 (0.0 ± 0.0)

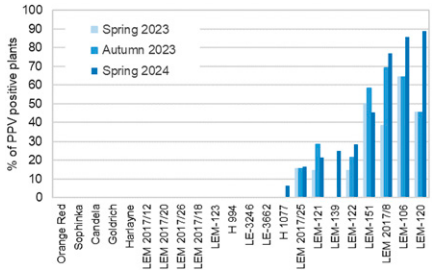

Fig. 3. Number of *Potyvirus plumpoxi* (PPV)-positive hybrids diagnostic by double-antibody-sandwich enzyme-linked immunosorbent assay expressed in % for different seasons.

based on the evaluation of symptoms and the percentage of PPV-positive plants. The differences were observed in accordance with the results obtained from the ParPMC2, PGS1.23, and PGS1.21 markers (Table 4). According to the Tukey's HSD test the genotypes categorized as "RR" and "RS" did not exhibit the significant differences in symptoms and the number of PPV-positive plants (Table 3), but "RR" and "RS" groups differed significantly from "SS" genotypes. The significant difference was evaluated from results of % of PPV-positive plants of resistant and susceptible genotypes according to the PGS1.21 analysis.

Discussion

The evaluation of the advantages and disadvantages of the methods used is a complex process, where different factors must be taken into account. These include the authenticity of the plant material, the PPV isolate, the impact of environmental factors, and the infective pressure on the plant or greenhouse conditions (Martínez-Gómez et al. 2000; Rubio et al. 2014).

According to Moustafa et al. (2001) phenotyping of the plants for PPV resistance based on a biological test using GF-305 is the most reliable method. In comparison with the method of molecular marker analysis, this approach is the most time-consuming. The evaluation of symptoms on the plants required 3

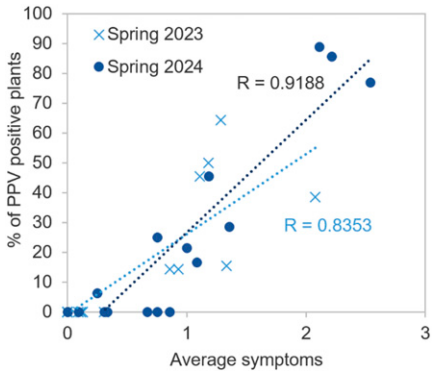

Fig. 4. Linear correlation between number of *Potyvirus plumpoxi* (PPV)-positive plants and average scaled symptoms, where R is a correlation coefficient.

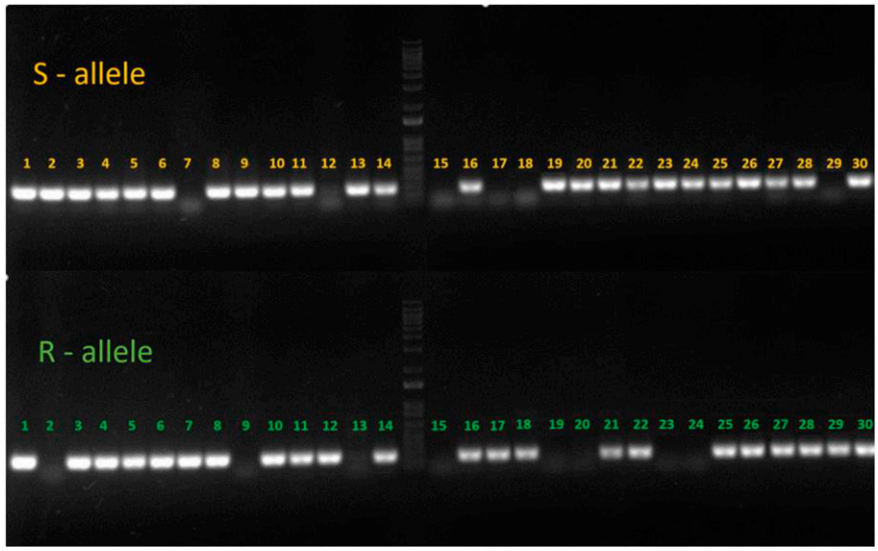

Fig. 5. ParPMC2 genotyping by allele-specific polymerase chain reaction for R-allele and S-allele amplifications in 1% agarose gel electrophoresis for different apricot genotypes [1: LEM/2017/12, 2: LEM-121, 3: LEM/2017/20, 4: Harlayne, 5: Vestar, 6: LEM/2017/26, 7: LEM/2017/25, 8: LEM-120, 9: LEM-106, 10: LEM-151 (LE-2904), 11: Goldrich, 12: Candela, 13: Strepet, 14: SEO, 15: BLANK, 16: Orange Red, 17: Sophinka, 18: Betinka, 19: Early Blush, 20: Poyer, 21: Hungarian Best, 22: LEM-123, 23: LEM-139, 24: LEM/2017/8, 25: H 1077, 26: LE-3246, 27: H 994, 28: LEM/2017/18, 29: LE-3662, 30: LEM-122].

years. The duration of the different methods was compared by Polo-Oltra et al. (2020), who found that phenotyping could be reduced from 8 months to just 4 by placing the plants in a cold chamber. In addition, some studies have indicated a gradual decrease of the symptom expression over the course of years of evaluation (Dondini et al. 2011; Karayiannis et al. 2008). Thus, the level of resistance of the young symptomatic apricot cultivar can be evaluated as tolerant or resistant after more years of study (Gürcan et al. 2016). In our study, the low expression of specific symptoms was evaluated on some hybrids (Table 2), which were later diagnosed as PPV negative. This indicates that the virus was eradicated through a hypersensitive response (Ilardi and Tavazza 2015), although this statement must be confirmed by further research. In natural conditions of Central Europe, the phenotyping method takes at least 2 years, and from our results, a multiyear experiment provides more reliable results.

Except for the time, it is necessary to have sufficient space protected against insect ingress for an extended period and the plants must be sufficiently mature to allow inoculation and subsequent symptom evaluation (Polo-Oltra et al. 2020). In addition, the results may be influenced by success or failure of the inoculation (Rubio et al. 2009) or during the phenotyping and the chlorosis of the leaves may be incorrectly evaluated as PPV symptoms. A further disadvantage is the higher cost of ELISA and reverse-transcription PCR diagnostic methods, which are used to confirm the presence of PPV.

The most significant advancement in the evaluation of PPV resistance has been the development of SSR molecular markers and allele-specific PCR methods (Polo-Oltra et al. 2020; Soriano et al. 2012; Zuriaga et al. 2018), which provide a cost-effective, simple, and rapid method of selecting promising apricot hybrids from the population during the breeding process.

Some reports mentioned that the ParPMC2-resistant allele is not sufficient to confer PPV resistance (Zuriaga et al. 2018). The GPIs can be evaluated because the PPVres locus could be modulated by other additional genes (Decroocq et al. 2014; Rubio et al. 2014). However, the finding of the resistant allele could be the result of a decrease in susceptibility, which is linked to a mechanism of gene silencing of the virus (Nicolás-Almansa et al. 2023; Rubio et al. 2014). In our study, the reliability of the SSR markers associated with PPV resistance was different for used markers (PGS1.21 and PGS1.23) and allele-specific PCR (ParPMC2). Although ParPMC2 provided the most rapid results, its reliability was considerably lower than that of the results obtained from the PGS1.23 and PGS1.21 marker analysis. A comparison of PGS1.23 and PGS1.21 marker analysis yielded disparate results in three hybrids (LEM-122, LEM-120, and LEM-151), with only the findings from the PGS1.21 marker analysis aligning with those of phenotyping. Consequently, following the completion of the phenotyping process, the PGS1.21 marker analysis was identified as the second-most reliable method for assessing PPV resistance in apricot hybrids. Nevertheless, the statistical analysis confirmed significant results for all employed methods.

In some cases, the genotypes that have been genetically classified as resistant may in fact be susceptible (Gürcan et al. 2019; Nicolás-Almansa et al. 2023). In our study, the GPI was observed on three hybrids (Table 3). The summary results from all methods and ANOVA indicate that the hybrids

Table 3. The comparison of the results of *Potyvirus plumpoxi* (PPV) resistance evaluation by different methods, where R represents a resistant genotype and S represents a susceptible genotype. The results of ParPMC2 are mentioned by specific alleles evaluated by the analysis. The evaluation of the symptom expression scaled 1 and number of the PPV-positive plants by double-antibody-sandwich enzyme-linked immunosorbent assay (DAS-ELISA) lower than 50% were noted as uncertain. Resistant alleles of simple sequence repeat markers PGS1.23 and PGS1.21 are in bold and italics.

Hybrid/Cultivar	Pedigree	Symptoms evaluation	DAS-ELISA	ParPMC2	PGS1.23	PGS1.21
LEM/2017/12	Orange Red × Poyer	R	R	RS	***142/142***	202/***222***
LEM/2017/26	Poyer × Orange Red	R	R	RS	***142/142***	202/***222***
LE-3662	Uncertain	R	R	RR	130/***142***	180/***222***
LEM-123	Early Blush × Candela	R	R	RS	136/***142***	176/***222***
LE-3246	Vestar × SEO	R	R	RS	130/***142***	192/***222***
LEM/2017/20	Orange Red × Poyer	Uncertain	R	RS	***142/142***	202/***222***
LEM/2017/18	Poyer × Harlayne	Uncertain	R	RS	***142/142***	202/***222***
H 994	Uncertain	Uncertain	R	RS	***142/142***	176/***222***
LEM/2017/25	Uncertain	Uncertain	Uncertain	RR	136/***142***	176/***222***
H 1077	Uncertain	Uncertain	Uncertain	RS	***142/142***	202/***222***
LEM-122	(Goldrich × SEO) × Betinka	S	Uncertain	RS	130/***142***	180/182
LEM-120	(Goldrich × SEO) × Betinka	S	S	RS	130/***142***	180/182
LEM-151	Hungarian Best × SEO	Uncertain	S	RS	136/***142***	176/190
LEM/2017/8	Orange Red × Poyer	S	S	SS	136/136	176/202
LEM-106	Uncertain	S	S	SS	148/164	182/192
LEM-121	Uncertain	Uncertain	Uncertain	SS	154/154	176/192
LEM-139	Uncertain	Uncertain	Uncertain	SS	140/148	176/182
Sophinka	Hungarian Best × SEO	R	R	RR	130/***142***	180/***222***
Candela	Hungarian Best × SEO	R	R	RR	130/***142***	208/***222***
Orange Red		R	R	RS	136/***142***	202/***222***
Goldrich		R	R	RS	140/***142***	182/***222***
Harlayne		R	R	RS	136/***142***	176/***222***
Betinka	Stark Early Orange × Vestar		R[i]	RR	130/***142***	180/***222***
SEO			R[ii]	RS	136/***142***	176/***222***
Vestar			S[iii]	RS	130/130	180/192
Early Blush			R[iv]	SS	136/140	176/182
Poyer			Uncertain	RS	136/136	176/202
Strepet			Uncertain	SS	134/154	196/208
Hungarian Best			S[v]	RS	130/136	174/190

[i] Krška and Vachůn (2016).
[ii] Audergon et al. (1994).
[iii] Gallois et al. (2018).
[iv] Faggioli and Barba (1996).
[v] Balan and Stoian (1995).

with 0.3 ± 0.1 scaled symptoms and 1.6% ± 1.2% of positive plants on average are PPV resistant (Table 4). Therefore, it can be concluded that the hybrid H 1077 is PPV resistant and the hybrids LEM/2017/25, LEM-151, and LEM-121 are PPV susceptible.

This study serves to confirm the resistance of hybrids LE-3246 and LE-3662, which were initially described in Polo-Oltra et al. (2020). The hybrids were evaluated as resistant by phenotyping and from ParPMC2 allele-specific PCR and were evaluated as "RS" and "RR," respectively. In addition, according to PGS1.21 and PGS1.23 markers, resistant alleles were found.

Table 4. Results of analysis of variance and post hoc Tukey's honestly significant difference test of evaluated symptoms, *Potyvirus plumpoxi* (PPV)-positive plants (%), and the results from ParPMC2 allele-specific polymerase chain reaction and simple sequence repeat marker analysis.

	Ø symptoms	Ø PPV-positive plants (%)
ParPMC2	$P = 0.009$	$P = 0.021$
RR	0 b	0 b
RS	0.6 ± 0.2 b	12 ± 6 b
SS	1.6 ± 0,4 a	52 ± 17 a
PGS1.23	$P = 1.4 \times 10^{-5}$	$P = 0.0001$
R	0.3 ± 0.1 a	2 ± 1 a
S	1.5 ± 0.4 a	50 ± 15 a
PGS1.21	$P = 4.5 \times 10^{-5}$	$P = 2 \times 10^{-6}$
R	0.29 ± 0.09 a	1.5 ± 1.2 a
S	1.7 ± 0.3 a	56 ± 7 b

Letters a, b differ from the statistically significant homogeneous group ($\alpha = 0.05$). R and S represent a PPV-resistant/susceptible genotype. The symbol Ø represents the average value.

Conclusion

Although the phenotyping method using the biological indicator under natural conditions is considered the most reliable method for PPV resistance evaluation, it is also the most time-consuming and isolation space–demanding method. Nowadays, the use of SSR molecular markers and the method of allele-specific PCR offer a relatively simple and rapid alternative evaluation, which could be used to select potentially PPV-resistant hybrids in breeding. From our results, the use of PGS1.21 offered the second-most reliable results after phenotyping. In addition, it was assumed, that using multiple different methods in evaluation of PPV resistance increases the credibility of the obtained results.

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