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Cost-Effective and Time-Efficient Molecular Assisted Selection for Ppv Resistance in Apricot Based on ParPMC2 Allele-Specific PCR

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Received: 31 July 2020; Accepted: 25 August 2020; Published: 31 August 2020

Abstract: Plum pox virus (PPV) is the most important limiting factor for apricot (Prunus armeniaca L.) production worldwide, and development of resistant cultivars has been proven to be the best solution in the long-term. However, just like in other woody species, apricot breeding is highly time and space demanding, and this is particularly true for PPV resistance phenotyping. Therefore, marker-assisted selection (MAS) may be very helpful to speed up breeding programs. Tightly linked ParPMC1 and ParPMC2, meprin and TRAF-C homology (MATH)-domain-containing genes have been proposed as host susceptibility genes required for PPV infection. Contribution of additional genes to PPV resistance cannot be discarded, but all available studies undoubtedly show a strong correlation between ParPMC2-resistant alleles (ParPMC2res) and PPV resistance. The ParPMC2res allele was shown to carry a 5-bp deletion (ParPMC2-del) within the second exon that has been characterized as a molecular marker suitable for MAS (PMC2). Based on this finding, we propose here a method for PPV resistance selection in apricot by combining high-throughput DNA extraction of 384 samples in 2 working days and the allele-specific genotyping of PMC2 on agarose gel. Moreover, the PMC2 genotype has been determined by PCR or by using whole-genome sequences (WGS) in 175 apricot accessions. These results were complemented with phenotypic and/or genotypic data available in the literature to reach a total of 325 apricot accessions. As a whole, we conclude that this is a time-efficient, cost-effective and straightforward method for PPV resistance screening that can be highly useful for apricot breeding programs.

Keywords: apricot; MAS; breeding; MATH; PPV resistance; agarose; ParPMC; ParPMC2-del

1. Introduction

Most cultivated apricots belong to the Prunus armeniaca L. species, a member of the Rosaceae family, Prunus genus and section Armeniaca (Lam.) Koch [1]. World apricot production reached 3.84 million tonnes in 2018, with Turkey, Uzbekistan and Iran as the main producers (http://www.fao.org/faostat/). This means an increase of about 45% since 1998 mainly due to Asian countries. By contrast, European production in this period has just increased slightly while the cultivated area declined up to 19%. Despite its wide geographical spread, apricot has very specific ecological requirements. Consequently, each region usually grows locally adapted cultivars. For this reason, significant breeding efforts have been undertaken since the first apricot breeding program started in 1925 at the Nikita Botanical Garden

Agronomy 2020, 10, 1292; doi:10.3390/agronomy10091292 www.mdpi.com/journal/agronomy
in Yalta (Crimea, Ukraine) [2]. However, apricot breeding based on biparental controlled crosses and subsequent selection of the best new allelic combinations is hardly limited by the capacity to evaluate trees in the field [3]. On one side, fruit trees show high space requirements to be grown. On the other, their juvenile phase is quite long and reliable pomological phenotyping requires several cropping seasons, which means that at least ten years are needed to release a new variety. Therefore, the implementation of marker-assisted selection (MAS) has a great potential to improve breeding efficiency in fruit trees, including apricot.

Sharka disease, caused by Plum pox virus (PPV), is currently the most important viral disease affecting stone fruit trees (Prunus spp.) [4]. To date, nine PPV strains (D, M, C, EA, W, Rec, T, CR and An) are identified [5]. However, PPV genetic diversity may be even bigger, as observed by Chirkov et al. [6], who recently described the new Tat isolates affecting sour cherry (Prunus cerasus). PPV-D and M are the most widespread and economically important strains [5,7]. A clear host preference is observed: PPV-D/plum/apricot and PPV-M/peach. However, underlying genetic determinants are still unknown [8].

Particularly in apricot, PPV-D has severely hindered production in the last three decades, especially in endemic areas. In this context, development of PPV-resistant varieties is the main objective of apricot breeding programs. However, resistant sources are scarce. Just a handful of North American PPV-resistant cultivars have been identified to date, and they are commonly used as donors in all apricot resistance breeding programs currently in progress [9]. Several independent works aimed at dissecting the genetic control of PPV resistance in apricot have identified the major dominant PPVres locus in the upper part of linkage group 1 [10–17]. According to the pedigree and fine mapping data, a single common ancestor carrying PPVres has been suggested for all PPV-resistant cultivars [16,18–20]. Moreover, other minor loci contributing to PPV resistance have been suggested [13–16], but their role has not yet been well defined. More recently, transcriptomic and genomic analyses of PPVres locus have pointed out ParPMC1 and ParPMC2, two members of a cluster of meprin and TRAF-C homology domain (MATHd)-containing genes, as host susceptibility paralogous genes required for PPV infection [21]. The ParPMC2 allele linked in coupling with PPV resistance (ParPMC2res) accumulates 15 variants, including a 5 nt deletion (ParPMC2-del) that results in a premature stop codon. Moreover, cultivars carrying the ParPMC2res allele show that ParPMC2 and especially ParPMC1 genes are downregulated. As a result, this ParPMC2res was proposed to be a pseudogene that confers PPV resistance by silencing functional homologs, the non-mutated ParPMC2 allele and/or ParPMC1. Another plausible scenario involves epigenetic modifications to explain ParPMC silencing in the resistant cultivars [22].

In spite of evidence supporting linkage with the PPVres locus, some genotype-phenotype incongruencies (GPIs) have been detected in biparental populations segregating for PPV resistance [17,23,24]. In other words, some phenotypically susceptible individuals carrying ParPMC2res were classified as genetically resistant. Possible causes underlying these discrepancies, including other loci contributing to PPV resistance, are still unresolved. However, the potential benefit of using a ParPMC2 allele-specific marker (PMC2) for MAS is still very high since sharka resistance phenotyping is a major bottleneck in apricot breeding programs. The most reliable method for apricot PPV resistance phenotyping is based on a biological test that uses GF-305 peach rootstocks as woody indicators and graft-inoculation with PPV [25]. This procedure is time-consuming and requires visual inspection during two to four growing seasons in several replicates per genotype followed by ELISA [26] and RT-PCR tests [27]. It should be noted that the plant to be tested must be of a significant size in order to have enough buds for grafting replicates, so it takes a couple of years from the time of crossing. As a result of a genetic mapping approach, Soriano et al. [18] reported the first successful MAS application for PPV resistance using 3 SSRs within the PPVres locus resolved by capillary electrophoresis. Afterwards, these SSRs were combined with a single sequence length polymorphism marker (ZP002) interrogating the ParPMC2-del resolved by capillary or acrylamide electrophoresis [24] and by high resolution
melting [28]. However, specialized DNA testing services are needed to adopt these MAS approaches, and together with the economic costs, this could be a challenge [29].

Here, we report a method combining high-throughput DNA extraction of 384 samples in 2 days and PMC2 genotyping by allele-specific PCR amplification and agarose gel electrophoresis. This method is proven to be an easily implemented tool for MAS of PPV-resistant seedlings in almost any apricot breeding program. Therefore, bioassays for PPV resistance evaluation will be needed to confirm the phenotype in selected materials. Moreover, PMC2 genotype has been determined and/or revised for 325 worldwide cultivated apricot accessions providing useful information for breeders to select parental genotypes.

2. Materials and Methods

2.1. High-Throughput DNA Isolation in 96-Well Plate

The genomic DNA extraction protocol was optimized from the original Doyle and Doyle method [30] to manage 384 samples per isolation using 8-well 1.2-mL strip tubes (VWR International). For each accession, 2 leaf discs were collected and placed into a tube with 3 glass beads (VWR International). The strips were frozen in liquid N2 and stored at −20 °C before DNA isolation. Frozen tissue was ground for 1 min with a frequency of 26/s using a Qiagen TissueLyser 85210 (Qiagen, Hilden, Germany). Then, 340 µL of preheated CTAB isolation buffer (with 0.2% 2-mercaptoethanol) was added to the ground tissue and incubated at 65 °C for 40 min, shaking gently every 10 min. After a short spin, 340 µL of chloroform-isoamyl alcohol (24:1) was added and mixed inverting the plates. Tubes were centrifuged for 10 min at 3000 rpm and 4 °C. The clean aqueous phase was transferred to new strip tubes, and 1.5 vol of 100% ethanol and 15 mM ammonium acetate were added and mixed gently. After overnight incubation at −20 °C, tubes were centrifuged for 10 min at 3000 rpm at 4 °C. The supernatant was discarded and finally 75 µL of TE was added. DNA at 1:10 dilution was used for PCR. Some random DNA samples from each plate were subjected to quality control. DNA integrity was checked on an agarose gel, and quantification was performed using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

2.2. PMC2 Genotype by Allele-Specific PCR Assay

PMC2 marker genotyping was performed using the allele-specific forward primer (PMC2-F-alleleR: 5’-GTCATTTTCATTGATGTCATTCA-3’ or PMC2-F-alleleS: 5’-GTCATTTTCATTGATGTCATTCA -3’) and one common reverse primer (PMC2-R: 5’- GTCATTTTCATTGATGTCATTCA -3’), as described by Zuriaga et al. [21]. PCRs were performed in a final volume of 20 µl containing 1 × DreamTaq buffer, 0.2 mM of each dNTP, 5 µM of each primer, 1 U of DreamTaq DNA polymerase (Thermo Fisher) and 2 µL of DNA extraction (diluted 1:10). Cycling conditions were as follows: an initial denaturing of 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 45 s and 72 °C for 45 s; and a final extension of 72 °C for 10 min. PCR products were electrophoresed in 1% (w/v) agarose gels.

Available DNA samples from 120 apricot cultivars and accessions were PCR screened in this work. Part of this collection is currently kept at the collection of the Instituto Valenciano de Investigaciones Agrarias (IVIA) in Valencia (Spain), while other samples were provided by the Departamento de Mejora y Patología Vegetal del CEBAS-CSIC in Murcia (Spain), the University of St. Istvan (Budapest, Hungary) or by SharCo project (FP7-KBBE-2007-1) partners.

2.3. WGS Mapping and PMC2 Screening

WGSs of 73 cultivars were used in this study. Twenty-four of these WGSs and the 454 sequenced BAC clones belonging to the “Goldrich” PPVres locus R-haplotype were already screened in our previous works [20–31]. The other 49 WGSs were downloaded from the SRA repository (https://www.
ncbi.nlm.nih.gov/sra). All raw reads were processed using the “run_trimmomatic_qual_trimming.pl” script from the Trinity software [32]. After removing the low-quality regions as well as vector and adaptor contaminants, cleaned reads were aligned to the peach genome v.2.0.a1 [33] using Bowtie2 v.2.2.4 software [34]. The presence/absence of the ParPMC2-del was visually inspected using IGV v.2.4.16 [35].

3. Results and Discussion

3.1. High-Throughput DNA Extraction and ParPMC2-del Genotyping for MAS

MAS offers great advantages over traditional seedling selection based just on phenotypic evaluations in fruit breeding [36]. DNA tests in segregating populations can improve the cost efficiency and/or the genetic gain for each seedling selection cycle [29], allowing to identify a few seedlings from among many thousands that have the genetic potential for desired performance levels [37]. As a result, agronomical evaluation in field trials is restricted to the promising selected materials. Implementation of MAS is especially valuable for traits that are difficult and/or expensive to phenotype as PPV resistance. As previously explained, the most reliable PPV resistance phenotyping is based on a biological test that uses graft-inoculated GF-305 peach seedlings [25] (Figure 1A). This protocol requires several replicates per genotype and visual symptoms inspection during 2–4 growing seasons, which entails the main bottleneck in apricot breeding programs. For instance, following this method at the IVIA’s greenhouse and cold chamber facilities, we can phenotype no more than 3000 plants per year, which equals 500 seedlings (i.e., 6 replicates are needed for each seedling).

In this work, we present a new strategy to speed up while reducing costs of the current application of MAS for PPV resistance in apricot [18,24,28]. Here, we combine a high-throughput DNA extraction protocol that does not need sophisticated robotic systems and can be implemented in any regular laboratory, with PMC2 allele-specific PCR amplification using previously described primers [21] and agarose electrophoresis (Figure 1B). Both forwards primers differ at the 3’-end, allowing to easily discriminate the presence/absence of the 5-bp ParPMC2-del (Figure 2). With this DNA extraction method, one person can easily process up to 384 samples (four 96-well sample plates) in 2 working days, enabling high throughput sample preparation. This is 4 times more samples than a standard CTAB method using individual tubes, while the cost of reagents and consumables is similar in both cases (around 0.29–0.30 € per sample) (Table S2). DNA obtained has enough quantity and quality to ensure subsequent regular PCRs. A 1:10 dilution of the DNA obtained was directly used for PCR amplification, without any additional purification step. In contrast, commercial kits are much more expensive in terms of reagents and consumables with costs around 4€ per sample. Then, using this DNA, 3 different methods could be applied for PPV MAS in apricot: the fluorescent labelling of PCR fragments that are resolved using capillary electrophoresis [18], the high-resolution melting (HRM) approach [28], and the use of standard PCR resolved by agarose gel electrophoresis [21]. It should be noted that the first two methods require the use of special equipment that could not be available for some laboratories and that also make the protocol more expensive. For instance, just the capillary electrophoresis costs around 1.5–2€ per sample (PCR not included) and the fluorescently labelled primers needed for PCR (136€ 10 nm) are much more expensive than the non-labelled ones (4€ 20 nm). On the other hand, commercial kits for HRM are not very expensive (around 1€ per sample) but requires the use of real-time PCR machines specially calibrated for this type of experiments and the analysis software. As a resume, although prices differ between laboratories or countries, our rough estimate of the cost points to first and second approaches as 13 and 8 times more expensive, respectively, in terms of reagents and consumables than the protocol proposed in this work (Table S2).

Practical advantages of PMC2 genotyping over classical phenotyping may be illustrated by the following example (Figure 1). The estimated time needed for evaluating 1000 samples at the IVIA’s facilities using bioassays is about 16 months (500 samples/8 months), taking into account that plants should be big enough to be ready-to-graft (approximately 2 years old). In contrast, just about 4 weeks
are needed to conduct PMC2 genotyping just after seed germination. This estimated time was calculated assuming a 40-h workweek. As 1000 samples could be distributed into 10.4 96-well plates, ideally the DNA extraction would need 5.2 days (4 plates each 2 days), the 2 allele-specific PCRs would need 7.8 days (3 h each plate) and the agarose electrophoresis would last 2.6 days (2 PCR 96-well plates and 2 h per gel). In total, we would need 15.6 working days to genotype 1000 samples. This improvement removes the phenotyping bottleneck since all seedlings obtained from a particular cross can be PCR screened that same year. Hence, this quick and high-throughput method for DNA testing is expected to have an important effect on the cost efficiency of MAS, as suggested by Edge-Garza et al. [37].

Figure 1. Comparison between traditional Plum pox virus (PPV) resistance phenotyping (A) and high-throughput marker-assisted selection (MAS) based on PMC2 allele-specific PCR (B). (*) Estimated duration based on Instituto Valenciano de Investigaciones Agrarias (IVIA) facilities.
One of the main pillars of plant breeding relies on skilful parental selection to create new genetic variation by controlled crossing. Usually, breeders just connect the concept of DNA-informed breeding with the use of molecular markers for seedling selection, but it also can be very helpful for parental selection when available. The PPV strain used for phenotyping was also included because differences in severity of the induced symptoms have been observed [10,16]. As a result, after screening 120 accessions by PCR and other 49 by WGS and reviewing the available literature, PMC2 genotype was determined in a total of 325 apricot cultivars or accessions that represent a wide range of geographic origins (Figure 3). A significant part of the materials come from European countries directly involved in PPV resistance research during the last decades, such as Italy (20.9%), Spain (15.7%) or France (14.8%) [38–42]. Regarding viral strain, PPV-M was more frequently used for phenotyping except for PPV-D in Spain and PPV-T in Turkey (Figure 3), in agreement with the prevalence of these two strains in every country [5,43].
Supposedly corresponding to this accession do not have the ParPMC2-del. Something similar occurs with Mirlo Naranja, classified as resistant [48], that was found to carry one copy of the ParPMC2-del by PCR in this work but not in that of Passaro [49]. Detailed accession documentation may be helpful to resolve these discrepancies, but 13 of the 16 identified GPIs have no pedigree data available. This information would be very valuable to increase the efficiency of apricot breeding programs and germplasm management.

In total, 110 accessions were considered phenotypically resistant (Table 1), 108 were susceptible (Table 2) and 11 showed uncertain phenotype against the same or different PPV strains (Table 3). ParPMC2-del highly correlates with PPV resistance, as evidenced by its presence in 92.8% of the resistant accessions (Table 1) and its absence in 92.6% of the susceptible accessions (Table 2). Only 16 out of 219 (7.3%) accessions phenotypically classified as resistant or susceptible showed genotype-phenotype incongruences (GPIs). GPIs were previously reported mainly when using segregating populations [18,23,24,28,44], but clarifying reasons underlying GPIs was found difficult, as quite different factors may be involved. These factors include complex phenotyping protocols, loci other than PPVres contributing to PPV resistance, environmental conditions and/or gene–environment interactions. Additionally, putative misclassifications could also explain some genotypic discrepancies observed in this work. For instance, Sunglo, the resistant donor parent of Goldrich, has been phenotyped as resistant by several authors using PPV-M [15,45,46] and PPV-D [47] and genotypically showed the SSR-resistant alleles targeting the PPVres locus [18]. However, WGS data (SRR2153157) supposedly corresponding to this accession do not have the ParPMC2-del. Something similar occurs with Mirlo Naranja, classified as resistant [48], that was found to carry one copy of the ParPMC2-del by PCR in this work but not in that of Passaro [49]. Detailed accession documentation may be helpful to resolve these discrepancies, but 13 of the 16 identified GPIs have no pedigree data available. This information would be very valuable to increase the efficiency of apricot breeding programs and germplasm management.
### Table 1. Apricot PPV-resistant accessions genotyped for PMC2.

| Name                  | Country | Origin                        | Pedigree                                | PPV Resistance Phenotype | PPV Strain Used | First Phenotype Ref | PMC2 Genotype | PMC2 Genotype Ref |
|-----------------------|---------|-------------------------------|-----------------------------------------|--------------------------|----------------|--------------------|---------------|-------------------|
| A4316                 | IT      |                               |                                        | R                        | M              | [15]               | RS            | WGS               |
| A4804                 | IT      |                               |                                        | R                        | M              | [15]               | RS            | WGS               |
| Adriana (= Le-3241)   | CR      | Horticulture Faculty, Lednice | Vestar × SEO [50]                      | R                        | M              | [51]               | RR            | [24]              |
| Alfred (= NY345)      | USA     | Geneva, NY State Expt Sta, by Robert C. Lamb | OP seedling of selection from (Doty × Geneva) | R                        | M              | [53]               | RS            | WGS               |
| Andswee               | IR      |                               |                                        | R                        | M              | [15]               | RS            | WGS               |
| Aneget                | FR      | INRA, CEP Innovation          |                                        | R                        | M/D            | [54]               | RS            | [49]              |
| Bergarouge (= Avrine A2914) | FR      | INRA                         | Bergeron × Orange Red [55]             | R                        | D              | [23]               | RS            | [49]              |
| Bergeval (= Aviculo, A3950) | FR      | INRA                         |                                        | R                        | M              | [56]               | RS            | [49]              |
| BO03615011            | IT      |                               | Goldrich × Harlayne [28]               | R                        | M*             | [49]               | RS            | [49]              |
| BO03615025            | IT      |                               | Goldrich × Harlayne [28]               | R                        | M*             | [49]               | RR            | [49]              |
| BO03615034            | IT      |                               | Goldrich × Harlayne [28]               | R                        | M*             | [28]               | RR            | [28]              |
| BO03615049            | IT      |                               | Goldrich × Harlayne [28]               | R                        | M*             | [28]               | RR            | [28]              |
| BO03615053            | IT      |                               | Goldrich × Harlayne [28]               | R                        | M*             | [28]               | RS            | [28]              |
| BO03615070            | IT      |                               | Goldrich × Harlayne [28]               | R                        | M*             | [49]               | RR            | [49]              |
| BO04624031            | IT      |                               | Portici × Goldrich [28]                | R                        | M*             | [28]               | RS            | [28]              |
| BO04624039            | IT      |                               | Portici × Goldrich [28]                | R                        | M*             | [49]               | SS            | [49]              |
| BO05636034            | IT      |                               | Kyoto × Priscilla [28]                 | R                        | M*             | [28]               | RS            | [28]              |
| BO06690012            | IT      |                               | Silvercot × Bora [28]                 | R                        | M*             | [49]               | RS            | [49]              |
| BO06690013            | IT      |                               | Silvercot × Bora [28]                 | R                        | M*             | [49]               | RS            | [49]              |
| BO06690024            | IT      |                               | Silvercot × Bora [28]                 | R                        | M*             | [49]               | RS            | [49]              |
| BO06690033            | IT      |                               | Silvercot × Bora [28]                 | R                        | M*             | [49]               | RS            | [49]              |
| BO06690036            | IT      |                               | Silvercot × Bora [28]                 | R                        | M*             | [49]               | RS            | [49]              |
| BO06690037            | IT      |                               | Silvercot × Bora [28]                 | R                        | M*             | [49]               | RS            | [49]              |
| BO06690039            | IT      |                               | Silvercot × Bora [28]                 | R                        | M*             | [49]               | RS            | [49]              |
| BO06690045            | IT      |                               | Silvercot × Bora [28]                 | R                        | M*             | [49]               | RS            | [49]              |
Table 1. Cont.

| Name                  | Country | Origin                                                                 | Pedigree                                | PPV Resistance Phenotype | PPV Strain Used | First Phenotype Ref | PMC2 Genotype | PMC2 Genotype Ref |
|-----------------------|---------|------------------------------------------------------------------------|-----------------------------------------|--------------------------|------------------|--------------------|---------------|-------------------|
| BO06609048            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [28]                | RS            | [28]              |
| BO06609055            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [28]                | RS            | [28]              |
| BO06609060            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609068            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609074            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609079            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609083            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609087            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609099            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609104            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609113            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609129            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609133            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO06609136            | IT      | Silvercot × Bora [28]                                                  | R                                       | R                        | M*               | [49]                | RS            | [49]              |
| BO96621002            | IT      | Goldrich × Lito [28]                                                  | R                                       | R                        | M               | [57]                | RR            | [28]              |
| BO96621030            | IT      | Goldrich × Lito [28]                                                  | R                                       | R                        | M               | [57]                | RS            | [28]              |
| Bora (BO90610010)     | IT      | University of Bologna and Milan, by D. Bassi                          | Early Blush × PA 705-2 [58]             | R                        | M/D             | [58]                | RS            | [21,28]           |
| Candela (= LE-2927)   | CR      | Horticulture Faculty, Lednice                                          | Hungarian Best × SEO [59]               | R                        | M               | [60]                | RR            | [49]              |
| Cebir                 | TU      |                                                                       |                                         | R                        | T               | [61]                | RS            | [61]              |
| Congat                | FR      | INRA, CEP Innovation                                                  |                                         | R                        | -               | [62]                | RS            | [49]              |
| Early Blush (= RUTBHART, NJA53, Aurora46) | US | Rutgers Horticultural Research Farm, New Brunswick, N.J. | RR17–62 × NJA-13 [63] | R | M | [65] | RS | PCR; [21,28,61] |
| Farlis                | FR      | Marie-France BOIS, France (IPS)                                        | R                                       | M*                      | [28,49]         | RS            | [28]              |
| Farmingdale (=NY346)  | USA     | Geneva, NY State Expt Sta, by Robert C. Lamb                          | OP seedling of selection from (Doty × Geneva) [66] | R | M | [53] | RS | [28] |
|Name| Country | Origin | Pedigree | PPV Resistance Phenotype | PPV Strain Used | First Phenotype Ref | PMC2 Genotype | PMC2 Genotype Ref |
|---|---|---|---|---|---|---|---|---|
|Flavor cot (=Bayoto)| USA| Washington State University Research, by Tom Toyama| | R | M | [57] | RS | [28] |
|Floproia| FR| PSB Produccion Vegetal S.L.| Goldrich × Ginesta [18]| R | M* | [28] | RS | PCR; [28] |
|GG9310| SP| IVIA, Moncada, Valencia| Goldrich × Ginesta [18]| R | D | IVIA | RS | PCR |
|GG9318| SP| IVIA, Moncada, Valencia| Goldrich × Ginesta [18]| R | D | IVIA | RS | PCR |
|GG937| SP| IVIA, Moncada, Valencia| Goldrich × Ginesta [18]| R | D | IVIA | RS | PCR |
|GG941| SP| IVIA, Moncada, Valencia| Goldrich × Ginesta [18]| R | D | IVIA | RS | PCR |
|GG979| SP| IVIA, Moncada, Valencia| Goldrich × Ginesta [18]| R | D | IVIA | RS | PCR |
|GG9869| SP| IVIA, Moncada, Valencia| Goldrich × Ginesta [18]| R | D | IVIA | RS | PCR |
|Gilgat| FR| INRA / CEP INNOVATION| | R | M* | [28]; [49] | RS | [28] |
|GP9817| SP| IVIA, Moncada, Valencia| Goldrich × Palau [18]| R | D | IVIA | RS | PCR |
|Dama Rosa (GG9871)| SP| IVIA, Moncada, Valencia| Goldrich × Ginesta [18]| R | D | IVIA | RS | PCR; [49] |
|Dama Taronja (GK988)| SP| IVIA, Moncada, Valencia| Goldrich × Katy [18]| R | D | IVIA | RS | PCR; [49] |
|Dulcinea| IT| Pisa University| Moniqui OP [67]| R | D | [64] | SS | PCR; [49] |
|Fracasso| IT| Agr. Canada, Res. Station, Harrow, Ontario, by REC Layne| V51092 ((Reliable × OP) × Sun Glo) [66]| R | D | M | [68] | RS | PCR; [20,21,24,28,61] |
|Harlayne| C| Agr. Canada, Res. Station, Harrow, Ontario, by REC Layne| Veecot × HW435 (Rouge du Roussillon × NJA2 (Morden604 OP)) [66]| R | M | | | | |
|Harval (=HW437)| C| Agr. Canada, Res. Station, Harrow, Ontario, by REC Layne| | | | | | |
|Henderson| USA| Geneva, NY, by GW Henderson| Unknown [66]| R | M | | | |
|Kaniñ (=M2252)| TU| Unknown | | | | | | |
|Karum| TU| Unknown | | | | | | |
|Lady cot (=HYB 3-3)| FR| COT International| V51092 ((Reliable o.p.) o.p.) × NJA1 [71]| R | M* | | | |
|Laycot| C| | | | | | WGS |
Table 1. Cont.

| Name         | Country * | Origin                      | Pedigree                   | PPV Resistance Phenotype b | PPV Strain Used | First Phenotype Ref | PMC2 Genotype c | PMC2 Genotype Ref |
|--------------|-----------|-----------------------------|----------------------------|----------------------------|-----------------|---------------------|-----------------|-----------------|
| LE-2904      | CR        | Horticulture Faculty, Lednice | Velkopavlovická × SEO [19] | R                          | M               | [72]                | RS              | [49]            |
| LE-3205      | CR        | Horticulture Faculty, Lednice |                            | R                          | M*              | [49]                | RR              | [49]            |
| LE-3246      | CR        | Horticulture Faculty, Lednice | Vestar × SEO [51]         | R                          | M               | [51]                | RS              | [24]            |
| LE-3662      | CR        | Horticulture Faculty, Lednice |                            | R                          | M               | [72]                | RR              | [49]            |
| Lifos        | TU        |                            |                            | R                          | T               | [61]                | RS              | [61]            |
| Lillycot     | FR        | SDR Fruit Llc (US)          | Unknown [73]               | R                          | M*              | [28]                | RS              | [28]            |
| Lito         | GR        |                            | SEO × Tirynthos [18]      | R                          | M               | [74]                | RS              | PCR; [24,28]    |
| Mediabel (=Mediabell) | FR | Newcot and IPS            |                            | R                          | M*              | [28]                | RS              | [28]            |
| Mirlo Naranja (= Mirlo anaranjado) | SP | CEBAS-CSIC, Murcia        | Rojo Pasión × Búida Precoz [48] | R                          | D               | [48]                | RS              | PCR             |
| Mirlo Blanco | SP        | CEBAS-CSIC, Murcia         | Rojo Pasión × Búida Precoz [48] | R                          | D               | [48]                | RS              | [28]            |
| Mirlo Rojo   | SP        | CEBAS-CSIC, Murcia         | Rojo Pasión × Búida Precoz [48] | R                          | D               | [48]                | RS              | PCR; [49]       |
| Mogador      | SP        | PSB Producción Vegetal S.L. |                            | R                          | M*              | [28,49]             | RS              | PCR; [28]       |
| Moixent (=GM961) | SP | IVIA, Valencia            | Goldrich × Mitger [18]     | R                          | D               | IVIA                | RS              | PCR; [49]       |
| Murciana     | SP        | CEBAS-CSIC, Murcia         | Orange Red × Currot [73]   | R                          | M               | [75]                | RS              | WGS; PCR; [49]  |
| Nikitskii    | UKR       |                            |                            | R                          | M               | [15]                | RS              | WGS             |
| NJA42        | USA       | New Jersey                 | NJA12 × NJA13 [76]         | R                          | ?               | [77]                | RS              | PCR             |
| Orange Red (=Barth; NJA-32) | USA | New Jersey                 | Lasgerdi Mashhad × NJA2 (= Morden 604 OP) [78] | R                          | D               | [68]                | RS              | PCR; [21]       |
| Pandora      | GR        |                            | SEO × Tirynthos [18]      | R                          | M               | [74]                | RS              | PCR             |
| Name                      | Country | Origin                                    | Pedigree                                                                 | PPV Resistance Phenotype | PPV Strain Used | First Phenotype Ref | PMC2 Genotype | PMC2 Genotype Ref |
|---------------------------|---------|-------------------------------------------|---------------------------------------------------------------------------|--------------------------|------------------|---------------------|---------------|------------------|
| Pelese di Giovanniello    | IT      | Tolerant                                  | Goldrich × Pelese di Giovanniello [73]                                  | Tolerant                 | D                | [64]                | SS            | [49]             |
| Perla                     | SP      | Murcia                                    |                                                                          |                          | R                | [64]                | SS            | PCR             |
| Petra (BO88617102)        | IT      | University of Bologna and Milan, Italy, by D Bassi |                                                                          | Tolerant                 | D                | [64]                | SS            | WGS             |
| Precoce d’Imola           | IT      | tolerant                                  |                                                                          |                          | R                | [64]                | SS            | WGS             |
| Priboto (=Zebra)          | FR      | bud mutation of Goldrich [80]             |                                                                          | Tolerant                 | D                | [64]                | SS            | WGS             |
| Pricia                    | FR      | Marie-France BOIS, France (IPS)           |                                                                          | Tolerant                 | D                | [64]                | SS            | WGS             |
| Pseudo Royal              | USA     |                                          |                                                                          | Tolerant                 | D                | [64]                | SS            | WGS             |
| Robada (= K106-2)         | USA     | Partler, California                       | Orange Red × K113-40 (ancestry includes Blenheim, Blush and Perfection) [81] | Tolerant                 | D                | [64]                | SS            | WGS             |
| Rojo Pasión               | SP      | CEBAS-CSIC                                 | Orange Red × Currot [83]                                                 | Tolerant                 | D                | [64]                | SS            | WGS             |
| Rosa                      | SP      | CEBAS-CSIC, Murcia                        | Orange Red × Palsteyn [73]                                               | Tolerant                 | D                | [64]                | SS            | WGS             |
| Rubista                   | FR      | Marie-France BOIS, France (IPS)           |                                                                          | Tolerant                 | D                | [64]                | SS            | WGS             |
| Sabbatani (= Selezione Sabbatani?) | IT      |                                          |                                                                          | Tolerant                 | D                | [64]                | SS            | WGS             |
| Selene                    | SP      | CEBAS-CSIC                                 | Goldrich × A2564 (=Screara × SEO) [18]                                  | Tolerant                 | D                | [64]                | SS            | WGS             |
| SEOP934                   | SP      | IVIA                                       | SEO × Palau [18]                                                        | Tolerant                 | D                | [64]                | SS            | WGS             |
| Spring Blush (= EA3126TH) | FR      | Escande EARL                               |                                                                          | Tolerant                 | D                | [64]                | SS            | WGS             |
| Stark Early Orange (= SEO, Earle Orange) | USA     | Grandview, Washington, by WL Roberts     | Unknown [66]                                                             | Tolerant                 | D                | [64]                | SS            | WGS             |
| Stella                    | USA     | Unknown [18]                               |                                                                           | Tolerant                 | D                | [64]                | SS            | WGS             |
| Sunglo (= Sun Glo)        | USA     | Columbia & Okanogan Nursery Co.           |                                                                           | Tolerant                 | D                | [64]                | SS            | WGS             |
Table 1. Cont.

| Name                  | Country a | Origin            | Pedigree | PPV Resistance Phenotype b | PPV Strain Used | First Phenotype Ref | PMC2 Genotype c | PMC2 Genotype Ref |
|-----------------------|-----------|-------------------|----------|---------------------------|-----------------|--------------------|-----------------|------------------|
| Sunnycot (= 97-3-203) | USA       | SDR FRUIT LLC – USA | R        | [62]RS                    | [49]            |                    |                 |                  |
| Traian                | RO        |                   | R D      | [86]RS                    | PCR; [87]       |                    |                 |                  |
| Tsunami (= EA 5016)   | FR        | Escande EARL      | R M*     | [28]RS                    | [28]            |                    |                 |                  |
| Wonder Cot (= RM 7)   | USA       | SDR FRUIT LLC – USA | R M*     | [28]RS                    | [28]            |                    |                 |                  |
| Zard                  | CA        |                   | R T      | [61]RS                    | [61]            |                    |                 |                  |

M*: strain likely used for phenotyping by the Phytosanitary Service, Emilia-Romagna (Italy). a Countries: C: Canada, CA: Central Asia, CR: Czech Republic, FR: France, GR: Greece, IR: Iran, IT: Italy, RO: Romania, SP: Spain, TU: Tunisia, TR: Turkey, UKR: Ukraine, US: United States of America; b Phenotype: R: Resistant, S: Susceptible; c Genotype: RR: homozygous for PMC2 resistant allele, SS: homozygous for PMC2 susceptible allele, RS: heterozygous.

Table 2. Apricot PPV susceptible accessions genotyped for ParPMC2-del.

| Cultivar            | Country a | Origin            | Pedigree | PPV Resistance Phenotype b | PPV Strain Used | First Phenotype Ref | PMC2 Genotype c | PMC2 Genotype Ref |
|---------------------|-----------|-------------------|----------|---------------------------|-----------------|--------------------|-----------------|------------------|
| A3521               | IR        |                   | S        | M [15]SS                  | WGS             |                    |                 |                  |
| A3522               | IR        |                   | S        | M [15]SS                  | WGS             |                    |                 |                  |
| Amabile Vecchioni   | IT        | Seedling by Prof. F. Scaramuzzi | Unknown [67] | S M [45]SS | [49] |                    |                 |                  |
| Apricot             | TR        |                   | S        | M [88]SS                  | PCR             |                    |                 |                  |
| Arrogante           | SP        | Murcia            | S D      | [89]SS                    | [21]            |                    |                 |                  |
| Avikaline           | FR        |                   | S        | M [15]SS                  | WGS             |                    |                 |                  |
| Bebecu (Bebeco)     | GR        | Unknown [18]      | S        | M/D [90]SS                | PCR; [21,28]    |                    |                 |                  |
| Bella Di Imola      | IT        | Spontaneous seedling [23] | S D     | [64]SS                    | [28]            |                    |                 |                  |
| Bergeron            | FR        | Saint-Cyr-au-Mont-d'Or, Lyon | Spontaneous seedling [23] | S M [90]SS | PCR; [21] |                    |                 |                  |
| Big Red (EA4006)    | FR        | Escande EARL, France | S        | M [57]RS                  | [28]            |                    |                 |                  |
| BC04624042          | IT        | Portici × Goldrich [28] | S M*     | [28]SS                    | [28]            |                    |                 |                  |
| BC04624043          | IT        | Portici × Goldrich [28] | S M*     | [28]SS                    | [28]            |                    |                 |                  |
| BC06609003          | IT        | Silvercot × Bora [25] | S M*     | [49]RS                    | [49]            |                    |                 |                  |
| BC081604311         | IT        | San Castrese × Reale di Imola [73] | S D     | [91]SS                    | [24]            |                    |                 |                  |
| BC096621021         | IT        | Goldrich × Lito [28] | S M*     | [28]RS                    | [28]            |                    |                 |                  |
| Boucheran Boutard    | FR        |                   | S        | M [15]SS                  | WGS             |                    |                 |                  |
| Bulida              | SP        | Murcia            | Unknown [73] | S D [92]M | [93] | PCR; [21] |                    |                 |
Table 2. Cont.

| Cultivar               | Country a | Origin                  | Pedigree                                      | PPV Resistance Phenotype b | PPV Strain Used | First Phenotype Ref | PMC2 Genotype c | PMC2 Genotype Ref |
|------------------------|-----------|-------------------------|-----------------------------------------------|---------------------------|-----------------|--------------------|-----------------|------------------|
| Cafona                 | IT        | Vesuvian area           |                                               | S                         | M [94]          | SS                 |                 | WGS              |
| CAID AGDZ z2          | MO        |                         |                                               | S                         | M [15]          | SS                 |                 | WGS              |
| Canino                 | SP        | Valencia                | Unknown [18]                                  | S                         | D [95]          | SS                 | PCR [20,21]     |                  |
| Castlebrite (=R111-6) | USA       | USDA, Fresno, California| OP seedling of B60-12 (= Perfection × Castleton) [66] | S                         | M [45]          | SS                 | PCR             |                  |
| Cegledi Bíbor          | HU        | Cegled Horticultural Research Institute | Chance seedling [96] | S                         | M [46]          | SS                 | PCR             | [28]             |
| Colorado (Colorado 43-15) | SP   | PSB Producción Vegetal SL | Unknown                                      | S                         | M* [49]         | SS                 | PCR; [28]        |                  |
| Corbató                | SP        | Valencia                | Unknown [18]                                  | S                         | D [95]          | SS                 | PCR             |                  |
| Currot                 | SP        | Valencia                | Unknown [18]                                  | S                         | D [95]          | SS                 | PCR             |                  |
| Estrella               | SP        | CEBAS-CSIC              | Orange Red × Z211-18 (= Goldrich × Pepito del Rubio) [23] | S                         | D [23]          | SS                 | PCR; [49]        |                  |
| Faralia                | FR        | Marie-France BOIS, IPS  |                                               | S                         | M* [28]         | SS                 |                 | [28]             |
| Farco                  | FR        | Marie-France BOIS, IPS  |                                               | S                         | M [57]          | SS                 |                 | [28]             |
| Favorit                | RO        |                         |                                               | S                         | M [94,97]       | SS                 |                 | [49]             |
| Geç Abligoz           | TR        |                         |                                               | S                         | T [61]          | SS                 |                 | [61]             |
| Ginesta                | SP        | Valencia                | Unknown [18]                                  | S                         | D [95]          | SS                 | PCR             |                  |
| Dama Vermella (HG9869) | SP   | IVIA                    | Harcot × Ginesta [18]                         | S                         | D IVIA          | SS                 | PCR; [49]        |                  |
| Hachhaloğlu (HW410)   | TR        | Richard EC Layne, Agr. Canada, Res. Station | V51092 ((Reliable × OP) × OP) × NJA1 (Phelps × Perfection) [66] | S                         | M [45]          | SS                 |                 | [21]             |
| Hasanbey              | TR        |                         |                                               | S                         | M [45]          | SS                 | PCR             |                  |
| Hungarian Best = (Best of Hungary?) | HU/RO |                      |                                               | S                         | T [61]          | SS                 |                 | [61]             |
| Katy                   | USA       | Zaiger’s Genetics*     |                                               | S                         | D [18]          | SS                 | PCR; [21]        |                  |
| Krasnoschekii         | UKR       | Advanced/improved cultivar |                                               | S                         | D [20]          | SS                 |                 | [20,21]         |
Table 2. Cont.

| Cultivar               | Country a | Origin | Pedigree | PPV Resistance Phenotype b | PPV Strain Used | First Phenotype Ref | PMC2 Genotype c | PMC2 Genotype Ref |
|------------------------|-----------|--------|----------|---------------------------|-----------------|---------------------|-----------------|------------------|
| Kyoto (= Kioto)        | FR        | Escande| Unknown [72] | S                         | M*              | [28]                | SS              | [28]             |
| Lambertin-1            | USA       | USDA, Fresno, California | A95-45 × B60-85 (= Perfection × Royal) [98] | S               | M                | [45]             | SS              | [21]             |
| Larclyd (= F168 cv; Jenny Cot) | NZ    | Central Otago | Sundrop × Moorpark [99] | S               | M                | [15]             | SS              | WGS              |
| Le-3218                | CR        | Faculty of Horticulture in Lednice | Vestar × SEO [51] | S               | M                | [51]             | SS              | [24]             |
| Luizet (= Sachet; Hatif du clos; Abricot du Clos) | FR | | | S | M* | [93] | SS | WGS |
| Luna                   | IT        |        |          | S                         | M*              | [28]                | RS              | [28]             |
| Madarska Narjepsia     | SL        |        |          | S                         | M                | [15]                | SS              | WGS              |
| Magic cot (= BM 22)    | USA       | SDR FRUIT LLC - USA | Unknown [22] | S | D | [23] | SS | [49] |
| Manicot                | FR        |        |          | S                         | D               | [92,100]            | SS              | WGS              |
| Maravilla              | SP        | CEBAS-CSIC, Murcia | Orange Red × Z211-18 (= Goldrich × Pepito) [23] | S               | D                | [23]             | SS              | PCR              |
| Mari de Cenad          | RO        |        | Unknown | S                         | T                | [61]                | SS              | [61]             |
| Markešti               | TR        |        |          | S                         | T                | [61]                | SS              | [61]             |
| Marlen                 | CR        | Horticulture Faculty, Lednice | clone of Hungarian Best [59] | S               | M                | [15]             | SS              | PCR              |
| Marouch 14             | MO        |        | Local landrace | S               | M                | [15]             | SS              | WGS              |
| Marouch 4              | MO        |        |          | S                         | M                | [15]                | SS              | WGS              |
| Mei Hwang              | CH        |        | Traditional cultivar/landrace | S               | M                | [15]             | SS              | WGS              |
| Mektep                 | TR        |        |          | S                         | T                | [61]                | SS              | [61]             |
| Mektep 8               | TR        |        |          | S                         | T                | [61]                | SS              | [61]             |
| Mitger                 | SP        | Castellón [30] | Unknown [18] | S | D | [95] | SS | PCR |
| Monceto                | IT        | Murcia | Unknown | S | M | [97] | SS | WGS |
| Monchi                 | SP        | Murcia | Unknown | S | M | [98] | SS | PCR, [21,24] |
| Mono                   | USA       | Le Grand, California, by FW Anderson | Perfection OP [66] | S | M | [60] | SS | [49] |
| Moongold (= Moongola?) | USA       | University of Minnesota | | S | - | [77] | SS | PCR |
| Moorpark (= Moor Park) | USA       |        |          | S                         | M                | [46]                | SS              | WGS              |
| Morden 604             | C         | Morden, Manitoba, by Canada Dept. Agr. Res. Sta. | Scout × McClure [66] | S | M | [15] | SS | WGS |
| Ninfa (BO81602075)     | IT        | University of Bologna and Milan, by D. Bassi | Ouardy × Tyrinthos [55] | S | M* | [28] | SS | PCR, [28,61] |
| Cultivar   | Country   | Origin          | Pedigree       | PPV Resistance Phenotype | PPV Strain Used | First Phenotype Ref | PMC2 Genotype | PMC2 Genotype Ref |
|-----------|-----------|-----------------|----------------|--------------------------|----------------|---------------------|--------------|------------------|
| Olimp     | RO        |                 |                | S                        | M              | [45]                 | SS           | WGS, [49]        |
| Orange Rubis ( = Couloumine) | FR        | Mallard         |                | S                        | M              | [57]                 | SS           | [28]             |
| Ordubat B. | TR       |                 |                | S                        | T              | [61]                 | SS           | [61]             |
| Ouardi    | TU        | INRAT, Ariana   | Canino × Hamidi [101] | S                        | M              | [46]                 | SS           | [49]             |
| Palsteyn (Palstein) | SA      |                 | Blenheim × Canino [73] | S                        | M              | [102]                | SS           | WGS              |
| Palabras  | SP        |                 |                | S                        | D              | [85]                 | SS           | PCR              |
| Palau     | SP        | Unknown [18]    |                | S                        | D              | [85]                 | SS           | PCR              |
| Paviot    | FR        |                 |                | S                        | M              | [93]                 | SS           | WGS              |
| Peche De Nancy | FR    |                 |                | S                        | M              | [15]                 | SS           | WGS              |
| Perfection | USA       | Waterville, Washington | Unknown [66] | S                        | M              | [46]                 | SS           | [21]             |
| Piera     | USA       |                 |                | S                        | M              | [65]                 | RS           | PCR              |
| Poizat    | FR        |                 |                | S                        | M              | [15]                 | SS           | WGS              |
| Polonais  | FR        | Spontaneous seedling [23] | S                | M                        | [93]           | SS                   | PCR, [24]    |
| Poppy     | USA       | Zaiger Genetics, Inc., Modesto, CA | 75EB575 × 123GD161 [58] | S                        | D              | [23]                 | SS           | [49]             |
| Portici ( = Pertini) | IT      | Vesuvian area   | Unknown; Local selection [23] | S                        | M              | [46]                 | SS           | PCR, [28]        |
| Precoce Ampuis | FR    |                 |                | S                        | M              | [15]                 | SS           | WGS              |
| Reale d’Imola | IT     |                 | Luizet OP [23] | S                        | M              | [46]                 | [21,24,49] |
| Rojo de Carlet | SP    | Valencia        |                | S                        | D              | [95]                 | SS           | PCR              |
| Rouge Du Roussillon | FR    |                 |                | S                        | M              | [45]                 | SS           | WGS              |
| Rouge De Fournes | FR     |                 |                | S                        | M              | [15]                 | SS           | WGS              |
| Saturn    | RO        |                 |                | S                        | M              | [48]                 | SS           | WGS              |
| Screara   | FR        |                 |                | S                        | D              | [70]                 | SS           | WGS              |
| Şekerpare B. | TR       |                 |                | S                        | T              | [61]                 | SS           | [61]             |
| Shalakh ( = Terevani, Erevani) | AR    | Local selection [23] | S                | M                        | [93]           | SS                   | WGS, [20,21] |
| Silistra × Ananas (Marculesti 43/1) | RO  |                 |                | S                        | M              | [15]                 | SS           | WGS              |
## Table 2. Cont.

| Cultivar                  | Country | Origin                        | Pedigree                                      | PPV Resistance Phenotype | PPV Strain Used | First Phenotype Ref | PMC2 Genotype | PMC2 Genotype Ref |
|---------------------------|---------|-------------------------------|----------------------------------------------|--------------------------|------------------|--------------------|---------------|-------------------|
| Sucre De Holub            | HU      | Bohême, by M. Holub          | S                                            | M                        | [15]             | SS                 | WGS           |
| Sublime                   | SP      | CEBAS-CSIC                    | Orange Red × Z211-18 (= Goldrich × Pepito del Rubio) [103] | S                        | D                | [103]              | SS            | PCR; [48]         |
| Super Rouge               | FR      |                               | S                                            | M                        | [15]             | SS                 | WGS           |
| Sweet Red                 | FR      |                               | S                                            | M                        | [57]             | SS                 | [49]          |
| Szegedi mamut (=Szegadti Mamut?) | HU | Foki István and Kovács Imre | Hybrid of Cegledi orias, "Giant" group [96] | S                        | M                | [94]              | SS            | [49]          |
| Taddeo (= Taddeo)         | SP      | Valencia                      | S                                            | D                        | [55]             | SS                 | PCR           |
| Tardif De Bordaneil       | FR      | Unknown [23]                  | S                                            | M                        | [46]             | SS                 | WGS           |
| Tardif De Tain            | FR      |                               | S                                            | M                        | [15]             | SS                 | WGS           |
| Tonda di costigliole      | IT      | Piedmont                      | S                                            | M                        | [104]            | SS                 | [49]          |
| Trevatt                   | AU      |                               | S                                            | M                        | [45]             | SS                 | PCR           |
| Tyrinthos                 | GR      | Unknown [18]                  | S                                            | D                        | [70]             | SS                 | PCR; WGS; [49] |
| Uleanos                   | SP      | Ulea, Murcia                  | S                                            | D                        | [89]             | SS                 | [49]          |
| Velázquez                 | SP      | Murcia                        | S                                            | D                        | [89]             | SS                 | PCR; [21]     |
| Venuis (= Venus 14147?)   | RO      | (Umberto × Ananas) × (Luzet × Umberto) [96] | S                                            | M                        | [46]             | SS                 | [49]          |
| Vestar                    | CR      | Hungarian Best × mixture of pollen from Chinese cultivars [55] | S                                            | M                        | [105]            | RS                 | WGS; [24]     |
| Vivagold                  | C       | Vineland Station, Ontario     | Veecot × V49024 (= Geneva × Gibb) [66]       | S                        | M                | [15]              | SS            | WGS               |
| Xirivello (=Chirivello)   | SP      | Valencia                      | Unknown                                      | S                        | M                | [46]              | SS            | PCR               |
| Yilbat (=M2243)           | TR      |                               | S                                            | T                        | [61]             | RS                 | [61]          |

M*: strain likely used for phenotyping by the Phytosanitary Service, Emilia-Romagna (Italy). \* Countries: AR: Armenia, AU: Australia, C: Canada, CH: China, CR: Czech Republic, FR: France, GR: Greece, HU: Hungary, IR: Iran, IT: Italy, MO: Morocco, NZ: New Zealand, RO: Romania, SA: South Africa, SL: Slovakia, SP: Spain, TR: Turkey, UKR: Ukraine and US: United States of America; Phenotype: R: Resistant, S: Susceptible; Genotype: RR: homozygous for PMC2 resistant allele, SS: homozygous for PMC2 susceptible allele and RS: heterozygous.
Table 3. Apricot accessions with uncertain PPV resistance phenotype genotyped for ParPMC2-del.

| Cultivar                      | Country | Origin                                                                 | Pedigree                                                                 | PPV Resistance Phenotype | PPV Strain Used | First Phenotype Ref | PMC2 Genotype | PMC2 Genotype Ref |
|-------------------------------|---------|------------------------------------------------------------------------|--------------------------------------------------------------------------|--------------------------|-------------------|--------------------|---------------|-------------------|
| Badami                        | IR      | Origin Pedigree                                                      |                                                                         | S                        | M                 | [102] WGS          | SS            |                   |
| Farbaly                       | FR      | Marie-France BOIS, IPS                                               |                                                                         | S                        | M*                | [28] RS            | RS            | [28]              |
| Goldrich                      | USA     | USDA and Washington State University, Prosser, Washington            | Sun Glo × Perfection [73]                                               | R                        | D                 | [68] RS PCR; [20,21,24,28] | R D           | S M*              |
| Harcot                        | C       | Agr. Canada, Res. Station, Harrow, Ontario, by REC Layne             | (T2 (Geneva × Naramata) × Morden 604 (Scout × McClure)) × NJA1 (Phelps × Perfection) [66] | T?                       | -                 | [28] RS PCR; [21,24,28] | R M           | S D               |
| Incomparable de Malissard (= Valssard) | FR      | Malissard, Valence                                                  |                                                                         | R                        | M                 | [15] SS WGS         | S M           |                   |
| Pisana                        | IT      | ICAPI 2n/5 OP [55]                                                   |                                                                         | S                        | M*                | [28] PCR           | S            |                   |
| Pieve (BO0860815)             | IT      | University of Bologna and Milan, by D. Bassi                       | Harcot × Reale di Imola [73]                                            | S                        | M*                | [28] SS            | [28]          |                   |
| San Castrese                  | IT      | Naples                                                                | Unknown [73]                                                            | T                        | D                 | [64] SS WGS         | S M           | [49]              |
| Sulmona                       | RO      | (Luizet × Re Umberto) × (Ananas × Ananas) [71]                      |                                                                         | S                        | M                 | [45] SS             | S            | [49]              |
| Veecot                        | C       | Ontario Dept Agr Res Inst, Vineland Station, Ontario, by OA Bradt    | Reliable OP [18]                                                       | R                        | M                 | [105] RS PCR; [21] | R T           | S D               |
| Viceroy (=Viceroy_603_G7)     | RO      |                                                                         |                                                                         | R                        | -                 | [77] SS             | S             | PCR               |

*M*: strain likely used for phenotyping by the Phytosanitary Service, Emilia-Romagna (Italy). *a* Countries: C: Canada, FR: France, IR: Iran, IT: Italy and RO: Romania; *b* Phenotype: R: Resistant and S: Susceptible; and *c* Genotype: RR: homozygous for PMC2 resistant allele, SS: homozygous for PMC2 susceptible allele and RS: heterozygous.
Accurate evaluation of PPV resistance is a complex process, and results obtained by different researchers sometimes are contradictory, as exemplified by Farbaly and Pieve (Table 3), which may lead to GPIs. This problem is also observed in well-known accessions. For instance, Goldrich, usually classified as resistant against both PPV-D and M strains, has also been classified as uncertain or even as susceptible at least once (Table 3). Moreover, the effect of the PPV strain used [9,24] has also been observed, as at least 5 accessions showed different behaviour against PPV-M, D or T infection (Table 3). In addition, the environmental effect on symptoms and the different PPV detection techniques employed could also been involved in GPIs [9].

On the other hand, PPV resistance has been related with the downregulation of both ParPMC2 and, especially, ParPMC1, putatively due to an RNA silencing mechanism triggered by the pseudogenization of ParPMC2res [21]. Notwithstanding, the presence of epigenetic changes has also been suggested as a possible cause [22]. In any case, resistant cultivars show residual expression levels that could somehow be influenced by environmental conditions. This might explain sporadic symptoms that eventually lead to GPI classification. Moreover, the role of additional PPV resistance loci or genes may also contribute to GPIs. In this sense, Gallois et al. [105] pointed out that a large part of a resistant phenotype conferred by a given QTL depends on the genetic background due to frequent epistatic effects between resistance genes. In fact, other minor loci, linked or not to PPVres, have been suggested to underlie PPV resistance in apricot [13–16]. Altogether, the identification and/or confirmation of GPIs in this work pave the way for future studies to unravel the PPV resistance mechanism.

The handful of North American cultivars originally described as PPV resistant [9] have been extensively used as donors in all breeding programs currently in progress. As a result, the PPVres locus has been introduced in different genetic backgrounds. In order to complete our survey, genotypic information was compiled from other 96 accessions without available PPV phenotype data (Table S1, [107–113]). In summary, 152 accessions (46.8%) have at least one copy of the ParPMC2-del (Figure 3) and 15 out of them are homozygous for ParPMC2-del, including the North American PPV-resistant cultivar Stella [114]. Those materials derived from crosses with North American PPV-resistant cultivars represent an opportunity to accelerate the development of new varieties better adapted to the Mediterranean basin conditions [9]. In this context, it should be highlighted that MAS allows to improve cost efficiency and/or genetic gain in apricot breeding programs aimed to select PPV-resistant seedlings. This improvement is highly significant even if some PPV susceptible individuals among those with ParPMC2-del are dragged, since they will be later identified by PPV phenotyping. Similarly, Tartarini et al. [115] underlined the advantage of the identification of homozygous Rvi6 scab-resistant plants using MAS, despite segregating progenies showing at least 5% of GPIs.

4. Conclusions

Here, we present a high-throughput method to quickly perform DNA testing for PPV resistance that may greatly improve the efficiency of apricot breeding programs. The long-lasting PPV phenotyping process will only be performed with those advanced selections showing promising agronomic behaviour in advanced stages to guarantee the selection of PPV-resistant individuals. Additionally, a wide survey over 300 accessions has been made to identify PPV-resistant sources that could also be useful in apricot breeding programs.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/9/1292/s1, Table S1. PMC2 genotyped apricot accessions without phenotypic data against PPV infection; Table S2. Estimation cost of DNA extraction and PMC genotyping for PPV MAS in apricot.

Author Contributions: Conceptualization: C.R., M.L.B. and E.Z.; experimental procedures: Á.P.-O., I.L. and E.Z.; bioinformatics: E.Z.; funding acquisition: M.L.B.; writing—original draft, E.Z.; writing—review and editing, Á.P.-O., C.R., I.L., M.L.B. and E.Z. All authors have read and agreed to the published version of the manuscript.
Acknowledgments: The authors would like to express their gratitude to Bassi (University of Milan, Italy) for providing pedigree information from their apricot breeding program.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Funding: This research was funded by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)-FEDER (RTA2017-0011-C03-01). A.P.O. was funded by a fellowship cofinanced by the Generalitat Valenciana and European Social Fund (2014–2020) (DOCV 8426/19.11.2018).
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