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

Ángela Polo-Oltra [1], Carlos Romero [2], Inmaculada López [1], María Luisa Badenes [1] and Elena Zuriaga [1,*]

[1] Citriculture and Plant Production Center, Instituto Valenciano de Investigaciones Agrarias (IVIA), CV-315, km 10.7, 46113 Moncada, Valencia, Spain; polo_ang@externos.gva.es (Á.P.-O.); lopez_inmcap@gva.es (I.L.); badenes_mlu@gva.es (M.L.B.)

[2] Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas (CSIC)—Universidad Politécnica de Valencia (UPV), Ingeniero Fausto Elio s/n, 46022 Valencia, Spain; cromero@ibmcp.upv.es

* Correspondence: garcia_zur@gva.es; Tel.: +34-963424040

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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

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 inverting the tubes, and 300 µL of 70% ethanol was added. After centrifugation 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′-GTCGTTTTCATTGATGTCCAAAC-3′, respectively) and one common reverse primer (PMC2-R: 5′-GTGCTCTTTCACATTCTTGCTC-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].

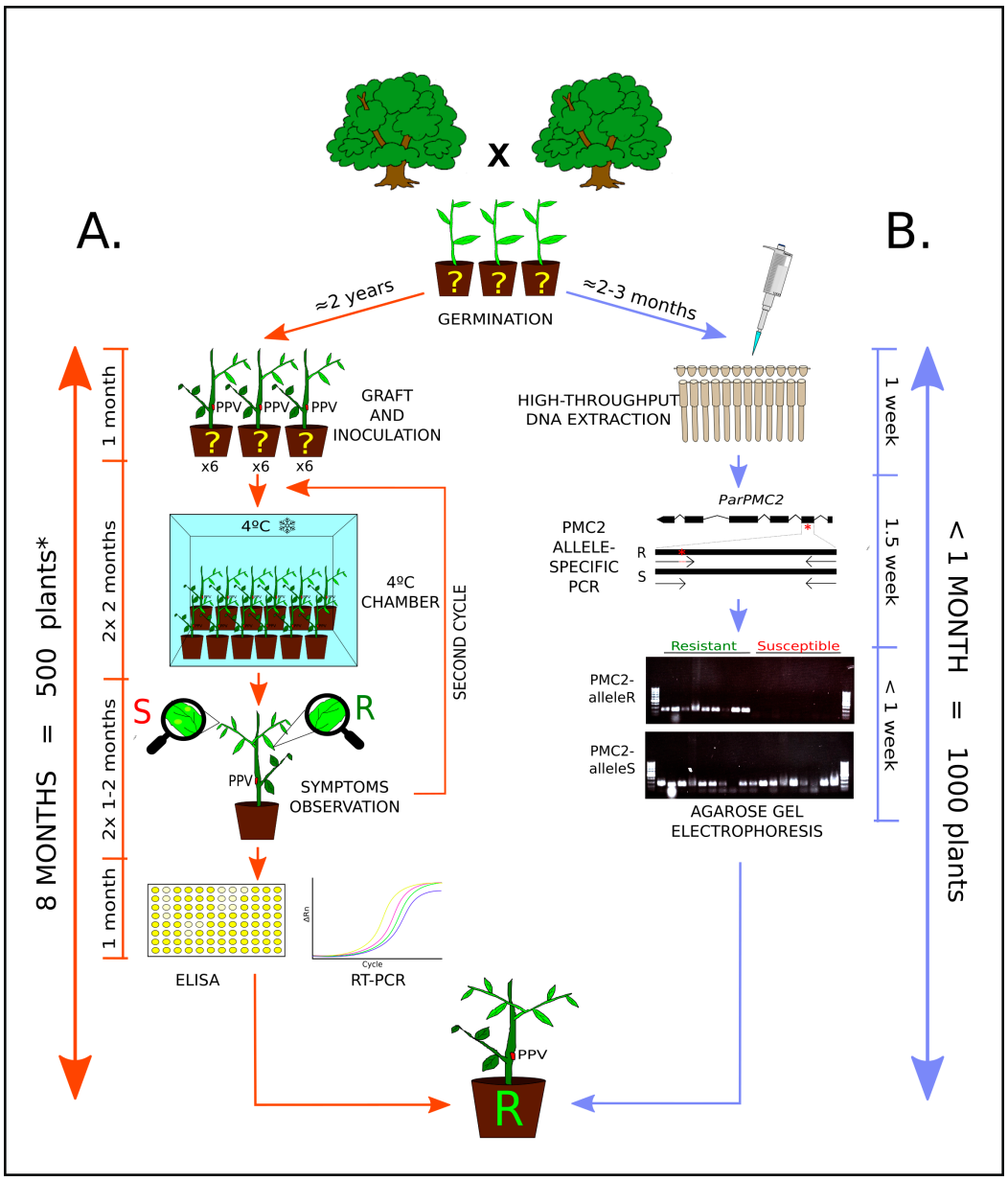

Figure 1. Comparison between traditional Plum pox virus (PPV) resistance phenotyping (**A**) and high-throughput marker-assisted selection (MAS) based on PMC2 allele-specific PCR (**B**). (*) Estimated duration based on Instituto Valenciano de Investigaciones Agrarias (IVIA) facilities.

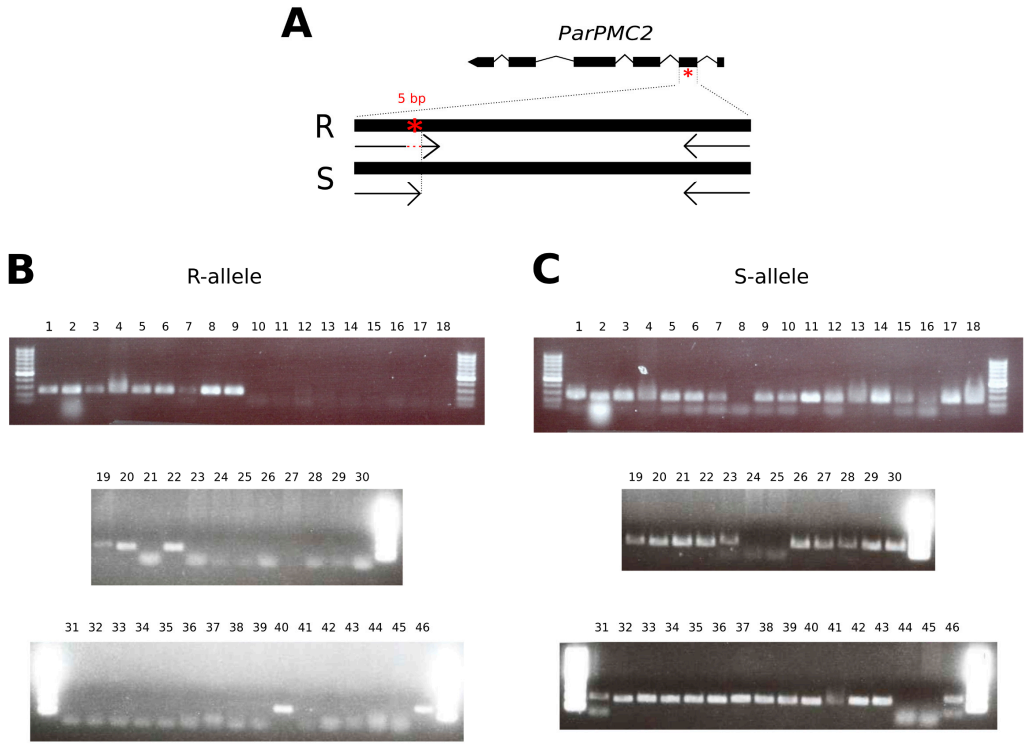

Figure 2. PMC2 genotyping by allele-specific PCR using forward primers differing at the 3′-end (**A**): R-allele (**B**) and S-allele (**C**) amplifications in 1% agarose gel electrophoresis for 46 apricot accessions (1: Goldrich, 2: Harlayne, 3: Henderson, 4: Lito, 5: Orange Red, 6: Pandora, 7: SEO, 8: Stella, 9: Veecot, 10: Bebeco, 11: Bergeron, 12: Canino, 13: Currot, 14: Ginesta, 15: Katy, 16: Mitger, 17: Palau, 18: Tyrinthos, 19: Piera, 20: Selene, 21: Colorao, 22: Moixent, 23: Perla, 24: Dama Vermella, 25: Maravilla, 26: Ninfa, 27: Palabras, 28: Sublime, 29: Dorada, 30: Castlebrite, 31: Martinet, 32: Corbató, 33: Gandía, 34: Cristalí, 35: Manri, 36: Gavatxet, 37: Pisana, 38: Xirivello, 39: Velazquez, 40: Mirlo Rojo, 41: Rojo Carlet, 42: Bulida, 43: ASP, 44: Silvercot, 45: Bora and 46: Roxana).

3.2. ParPMC2-del Highly Correlates with PPV Resistance in Apricot Germplasm

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 [36]. This is the case in apricot breeding for PPV resistance. Two decades ago, Martínez-Gómez et al. [9] reviewed phenotypic information regarding apricot cultivar behaviour against PPV. Similarly, here, we compile the PMC2 genotype of a wide set of apricot accessions to facilitate parental selection tasks incorporating also their resistance phenotype, pedigree and origin data from the literature 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].

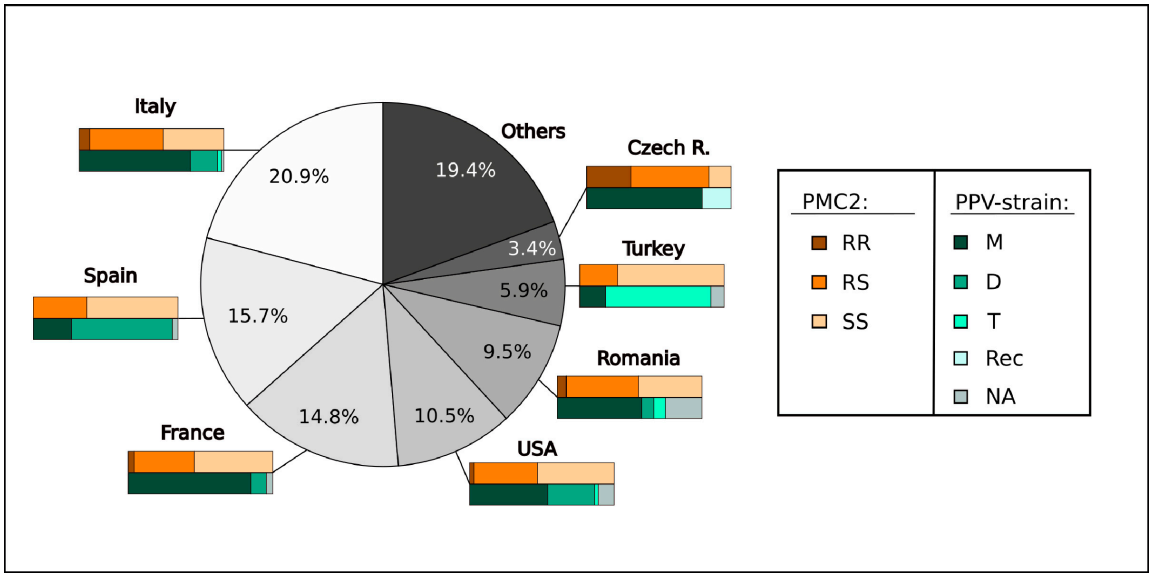

Figure 3. Geographic distribution of apricot accessions: PMC2 genotypes (RR: homozygous for the resistant allele; SS: homozygous for the susceptible allele; and RS: heterozygous) and PPV strain used for phenotyping are also indicated.

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 [a]	Origin	Pedigree	PPV Resistance Phenotype [b]	PPV Strain Used	First Phenotype Ref	PMC2 Genotype [c]	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]
					Rec	[52]		
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
Anegat	FR	INRA, CEP Innovation		R	M/D	[54]	RS	[49]
Bergarouge (= Avirine A2914)	FR	INRA	Bergeron × Orange Red [55]	R	D	[23]	RS	[49]
					M	[56]		
Bergeval (= Aviclo, 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]
BO06609012	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609013	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609024	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609033	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609036	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609037	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609039	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609045	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]

Table 1. *Cont.*

Name	Country [a]	Origin	Pedigree	PPV Resistance Phenotype [b]	PPV Strain Used	First Phenotype Ref	PMC2 Genotype [c]	PMC2 Genotype Ref
BO06609048	IT		Silvercot × Bora [28]	R	M*	[28]	RS	[28]
BO06609055	IT		Silvercot × Bora [28]	R	M*	[28]	RS	[28]
BO06609060	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609068	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609074	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609079	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609083	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609087	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609099	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609104	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609113	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609129	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609133	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO06609136	IT		Silvercot × Bora [28]	R	M*	[49]	RS	[49]
BO96621002	IT		Goldrich × Lito [28]	R	M	[57]	RR	[28]
BO96621030	IT		Goldrich × Lito [28]	R	M	[57]	RS	[28]
Bora (BO90610010)	IT	University of Bologna and Milan, by D. Bassi	Early Blush × PA 7005-2 [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	D	[64]	RS	PCR; [21,28,61]
					M	[65]		
					T	[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]

Table 1. *Cont.*

Name	Country [a]	Origin	Pedigree	PPV Resistance Phenotype [b]	PPV Strain Used	First Phenotype Ref	PMC2 Genotype [c]	PMC2 Genotype Ref
Flavor cot (=Bayoto)	USA	Washington State University Research, by Tom Toyama		R	M	[57]	RS	[28]
Flopria	FR	PSB Producción Vegetal S.L.		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			Tolerant	T	[61]	SS	[61]
Harlayne	C	Agr. Canada, Res. Station, Harrow, Ontario, by REC Layne	V51092 ((Reliable × OP) × OP) × Sun Glo [66]	R	D M	[68] [45]	RS	PCR; [20,21,24,28,61]
Harval (=HW437)	C	Agr. Canada, Res. Station, Harrow, Ontario, by REC Layne	Veecot × HW435 (Rouge du Roussillon × NJA2 (Morden604 OP)) [66]	R	M	[69]	RS	[28]
Henderson	USA	Geneva, NY, by GW Henderson	Unknown [66]	R	M D	[46] [70]	RS	PCR; [21]
Kaniş (=M2252)	TU			R	T	[61]	SS	[61]
Karum	TU			R	T	[61]	RS	[61]
Lady cot (=HYB 3-3)	FR	COT International		R	M*	[28]	RS	[28]
Laycot	C		V51092 ((Reliable o.p.) o.p.) × NJA1 [71]	R	M	[15]	RR	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
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]
					D	IVIA		
Mediabel (=Mediabell)	FR	Newcot and IPS		R	M*	[28]	RS	[28]
Mirlo Naranja (= Mirlo anaranjado)	SP	CEBAS-CSIC, Murcia	Rojo Pasión × Búlida Precoz [48]	R	D	[48]	RS	PCR
							SS	[49]
Mirlo Blanco	SP	CEBAS-CSIC, Murcia	Rojo Pasión × Búlida Precoz [48]	R	D	[48]	RS	[28]
Mirlo Rojo	SP	CEBAS-CSIC, Murcia	Rojo Pasión × Búlida 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	D	[75]	RS	WGS; PCR; [49]
					M	[15]		
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]
					M	[79]		
Pandora	GR		SEO × Tirynthos [18]	R	M	[74]	RS	PCR
					D	[47]		

Table 1. *Cont.*

Name	Country [a]	Origin	Pedigree	PPV Resistance Phenotype [b]	PPV Strain Used	First Phenotype Ref	PMC2 Genotype [c]	PMC2 Genotype Ref
Pelese di Giovanniello	IT			Tolerant	D	[64]	SS	[49]
Perla	SP	Murcia		R	D	[64]	SS	PCR
Petra (BO88617102)	IT	University of Bologna and Milan, Italy, by D Bassi	Goldrich × Pelese di Giovanniello [73]	R	M*	[28]	RS	[28]
Precoce d'Imola	IT			tolerant	D	[64]	SS	WGS
Priboto (=Zebra)	FR		bud mutation of Goldrich [80]	R	M	[15]	RS	WGS; [49]
Pricia	FR	Marie-France BOIS, France (IPS)		R	M*	[28,49]	RS	[28]
Pseudo Royal	USA			R	M	[15]	RS	WGS
Robada (= K106-2)	USA	Parlier, California	Orange Red × K113-40 (ancestry includes Blenheim, Blush and Perfection) [81]	R	M	[82]	RS	WGS
Rojo Pasión	SP	CEBAS-CSIC	Orange Red × Currot [83]	R	D	[83]	RS	PCR; [49]
Rosa	SP	CEBAS-CSIC, Murcia	Orange Red × Palsteyn [73]	R Tolerant	D	[41] [23]	RS	[49]
Rubista	FR	Marie-France BOIS, France (IPS)		R	M*	[28,49]	RS	[28]
Sabbatani (= Selezione Sabbatani?)	IT			R	D	[64]	SS	[49]
Selene	SP	CEBAS-CSIC	Goldrich × A2564 (=Screara × SEO) [18]	R	D	[84]	RS	PCR; [49]
SEOP934	SP	IVIA	SEO × Palau [18]	R	D	IVIA	RS	PCR
Spring Blush (= EA3126TH)	FR	Escande EARL		R	M*	[57]	RR	[49]
Stark Early Orange (= SEO, Earle Orange)	USA	Grandview, Washington, by WL Roberts	Unknown [66]	R	M D	[85] [70]	RS	PCR; [20,21,24,28,61]
Stella	USA		Unknown [18]	R	M D	[85] [70]	RR	PCR; [21]
Sunglo (= Sun Glo)	USA	Columbia & Okanogan Nursery Co.	Unknown [66]	R	M D	[45] [47]	RS SS	PCR 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 Vecchoni	IT	Seedling by Prof. F. Scaramuzzi	Unknown [67]	S	M	[45]	SS	[49]
Aprikoz	TR			S	M	[88]	SS	PCR
Arrogante	SP	Murcia		S	D	[89]	SS	[21]
Avikaline	FR			S	M	[15]	SS	WGS
Bebecou (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]
BO04624042	IT		Portici × Goldrich [28]	S	M*	[28]	SS	[28]
BO04624043	IT		Portici × Goldrich [28]	S	M*	[28]	SS	[28]
BO06609003	IT		Silvercot × Bora [28]	S	M*	[49]	RS	[49]
BO81604311	IT		San Castrese × Reale di Imola [73]	S	D	[91]	SS	[24]
BO96621021	IT		Goldrich × Lito [28]	S	M*	[28]	RS	[28]
Boucheran Boutard	FR			S	M	[15]	SS	WGS
Búlida	SP	Murcia	Unknown [73]	S	D	[92]	SS	PCR; [21]
					M	[93]		

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
					D	[64]		
CAID AGDZ n2	MO			S	M	[15]	SS	WGS
Canino	SP	Valencia	Unknown [18]	S	D	[95]	SS	PCR; [20,21]
					M	[90]		
Castlebrite (=K111-6)	USA	USDA, Fresno, California	OP seedling of B60-12 (= Perfection × Castleton) [66]	S	M	[45]	SS	PCR
Ceglédi Bíbor	HU	Cegléd Horticultural Research Institute	Chance seedling [96]	S	M	[46]	SS	[28]
Colorado (Colorao 43-15)	SP	PSB Producción Vegetal SL	Unknown	S	M*	[49]	SS	PCR; [28]
					D	[89]		
Corbató	SP	Valencia		S	D	[95]	SS	PCR
					M	[46]		
Currot	SP	Valencia	Unknown [18]	S	D	[95]	SS	PCR
					M	[46]		
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]
Farclo	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
					M	[46]		
Dama Vermella (HG9869)	SP	IVIA	Harcot × Ginesta [18]	S	D	IVIA	SS	PCR; [49]
Hacıhaliloğlu	TR			S	T	[61]	SS	[61]
Hargrand (= HW410)	C	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] [1]	SS	PCR
Hungarian Best = (Best of Hungary?)	HU/RO			S	T	[61]	SS	[61]
					M	[94]		
Katy	USA	Zaiger's Genetics[4]		S	D	[18]	SS	PCR; [21]
Krasnoshchekii	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 [73]	S	M*	[28]	SS	[28]
Lambertin-1	USA	USDA, Fresno, California	A95-45 × B69-85 (=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 (= Suchet; Hatif du clos; Abricot du Clos)	FR		Spontaneous seedling [71]	S	M	[93]	SS	WGS
Luna	IT			S	M*	[28]	RS	[28]
Madarska Narijlepsia	SL			S	M	[15]	SS	WGS
Magic cot (= RM 22)	USA	SDR FRUIT LLC - USA	Unknown [23]	S	D	[23]	SS	[49]
Manicot	FR			S	D	[97,100]	SS	WGS
					M	[15]		
Maravilla	SP	CEBAS-CSIC, Murcia	Orange Red × Z211-18 (= Goldrich × Pepito) [23]	S	D	[23]	SS	PCR; [49]
Mari de Cenad	RO		Unknown	S		[86]	RS	PCR
Markuleşti	TR			S	T	[61]	SS	[61]
Marlén	CR	Horticulture Faculty, Lednice	clone of Hungarian Best [59]	S	Rec	[14]	SS	PCR; [24]
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
					M	[46]		
Monaco Bello	IT			S	M	[97]	SS	WGS
Moniqui	SP	Murcia	Unknown	S	M	[90]	SS	PCR; [21,24]
Mono	USA	Le Grand, California, by FW Anderson	Perfection OP [66]	S	M	[93]	SS	[49]
Moongold (= Moongola?)	USA	University of Minessota		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]
					T	[61]		

Table 2. *Cont.*

Cultivar	Country [a]	Origin	Pedigree	PPV Resistance Phenotype [b]	PPV Strain Used	First Phenotype Ref	PMC2 Genotype [c]	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		Blenhein × Canino [73]	S	M	[102]	SS	WGS
Palabras	SP			S	D	[95]	SS	PCR
					M	[46]		
Palau	SP		Unknown [18]	S	D	[95]	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				S	M	[65]	RS	PCR
Poizat	FR			S	M	[15]	SS	WGS
Polonais	FR		Spontaneous seedling [23]	S	M	[93]	SS	[24]
Poppy	USA	Zaiger Genetics, Inc., Modesto, CA	78EB575 × 123GD161 [58]	S	D	[23]	SS	[49]
Portici (= Pertini)	IT	Vesuvian area	Unknown; Local selection [23]	S	M	[46]	SS	PCR; [28]
					D	[64]		
Precoce Ampuis	FR			S	M	[15]	SS	WGS
Reale d'Imola	IT		Luizet OP [23]	S	M	[46]	SS	[21,24,49]
					D	[64]		
Rojo de Carlet	SP	Valencia		S	D	[95]	SS	PCR
					M	[46]		
Rouge Du Roussillon	FR			S	M	[45]	SS	WGS
Rouge De Fournes	FR			S	M	[15]	SS	WGS
Saturn	RO			S	M	[45]	SS	WGS
Screara	FR			S	D	[70]	SS	WGS
					M	[45]		
Şekerpare B.	TR			S	T	[61]	SS	[61]
Shalakh (=Yerevani, 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 [a]	Origin	Pedigree	PPV Resistance Phenotype [b]	PPV Strain Used	First Phenotype Ref	PMC2 Genotype [c]	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; [49]
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]
Tabriz	TR			S		[86]	SS	PCR
Tadeo (= Taddeo)	SP	Valencia		S	D	[95]	SS	PCR
					M	[45]		
Tardif De Bordaneil	FR		Unknown [23]	S	M	[46]	SS	WGS
					D	[64]		
Tardif De Tain	FR			S	M	[15]	SS	WGS
Tonda di costigliole	IT	Piedmont		S		[104]	SS	[49]
Trevatt	AU			S	M	[45]	SS	PCR
Tyrinthos	GR		Unknown [18]	S	D	[70]	SS	PCR; WGS; [49]
					M	[97]		
Uleanos	SP	Ulea, Murcia		S	D	[89]	SS	[49]
Velázquez	SP	Murcia		S	D	[89]	SS	PCR; [21]
Venus (= Venus 1414?)	RO		(Umberto × Ananas) × (Luizet × 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). [a] 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; [b] Phenotype: R: Resistant, S: Susceptible; [c] 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 [a]	Origin	Pedigree	PPV Resistance Phenotype [b]	PPV Strain Used	First Phenotype Ref	PMC2 Genotype[c]	PMC2 Genotype Ref
Badami	IR			S	M	[102]	SS	WGS
				T	D			
Farbaly	FR	Marie-France BOIS, IPS		S	M*	[28]	RS	[28]
				R	M	[56]		
Goldrich	USA	USDA and Washington State University, Prosser, Washington	Sun Glo × Perfection [73]	R	D	[68]	RS	PCR; [20,21,24,28]
					M	[45]		
				uncertain	M	[106]		
					D	[68]		
				S	D	[64]		
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	[90]		
					D	[70]		
				S	T	[61]	SS	[61]
Incomparable de Malissard (= Valssard)	FR	Malissard, Valence		R	M	[15]	RS	WGS
				S		[57]		
Pisana	IT		ICAPI 26/5 OP [55]	S	M*	[28]	SS	PCR; [28]
				R	M	[65]		
				R	D	[64]		
				S	D	[28]		
Pieve (BO89608015)	IT	University of Bologna and Milan, by D. Bassi	Harcot × Reale di Imola [73]	S	M*	[28]	SS	[28]
				R	M	[65]		
San Castrese	IT	Naples	Unknown [73]	T	D	[64]	SS	WGS; [49]
				S	M	[46]		
Sulmona	RO		(Luizet × Re Umberto) × (Ananas × Ananas) [71]	S	M	[45]	SS	[49]
				R	-	[77]		
Veecot	C	Ontario Dept Agr Res Inst, Vineland Station, Ontario, by OA Bradt	Reliable OP [18]	R	M	[45]	RS	PCR; [21]
				S		[105]		
				T	D	[47]		
Viceroy (=Viceroy_603_G?)	RO			R	-	[77]	SS	PCR
				S	-	[86]		

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.

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