Scientia Horticulturae 318 (2023) 112131

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Phenotypic and molecular characterization of new interspecific Japanese plum × apricot hybrids (plumcots)

María Nicolás-Almansa [a,*], David Ruiz [a], Juan Alfonso Salazar [a], Alfonso Guevara [b], José Cos [b], Pedro Martínez-Gómez [a], Manuel Rubio [a]

[a] Department of Plant Breeding, CEBAS-CSIC, PO Box 164, Espinardo, Murcia E-30100, Spain
[b] Department of Hortofruticulture, IMIDA, Murcia, Spain

ARTICLE INFO

Keywords:
Prunus salicina
Prunus armeniaca
PPV resistance
Self-incompatibility
Floral biology
Fruit quality
Interspecific crosses

ABSTRACT

Japanese plum is a very important temperate tree fruit, ranking in the second position among the stone fruits, after peaches. In this species, there are very few self-compatible varieties and a lack of resistance to sharka disease (plum pox virus, PPV). In this context, interspecific crosses between Japanese plums and self-compatible, sharka-resistant apricot (*Prunus armeniaca* L.) cultivars have been performed in recent years to obtain resistant and self-compatible plumcots. The plumcot provides a new fruit typology combining the horticultural and market characteristics of these two *Prunus* species. In this work, new interspecific plumcots have been obtained and characterized. An initial screening of more than 600 seedlings was carried out for interspecific hybrid verification using Simple Sequence Repeat (SSR) markers. In addition, floral compatibility by S-allele genotyping and phenotypic characterization and PPV resistance evaluations in controlled conditions were performed in the interspecific genotypes. The results show some interspecific hybrids with the desired characteristics of self-compatibility alleles and sharka tolerance together with new fruit typologies with an attractive skin and flesh color and different valued organoleptic characteristics.

1. Introduction

Plum species, including Japanese plum (*Prunus salicina* Lindl.) and European plum (*Prunus domestica* L.), are temperate tree fruits of paramount importance. Plums are the second most important stone fruits after peaches [*Prunus persica* (L.) Batsch], with an average production of 11.5 Mt (FAO, 2022). Japanese plum originated in China, where it was first cultivated for several thousand years and then introduced into Japan more than two thousand years ago (Yoshida, 1987; Faust and Surányi, 1998). In the late 19th century, it was introduced into California (USA) from Japan, where Luther Burbank started modern plum breeding by crossing it with other diploid plums such as *P. americana* Marshall, *P. hortulana* L. H. Bailey, *P. munsoniana* W. Wight & Hedrick and *P. simonii* Carrière to enhance its adaptation to local environments. Burbank's efforts led to the creation of several cultivars, including 'Santa Rosa', 'Beauty', 'Eldorado', and 'Burbank', among others, which were spread from California to temperate zones around the world in the 20th century (Burbank, 1914; Faust and Surányi, 1998; Topp et al., 2012). Some of these cultivars were further intercrossed with other local plum species such as *P. americana, P. angustifolia* Marshall, *P. nigra* Aiton and *P. pumila* var. besseyi (L. H. Bailey) Waugh in the United States and *P. cerasifera* Ehrh. in South Africa and Australia. As such, the term "Japanese plum" thus refers to a heterogeneous group of interspecific hybrids derived from crosses involving up to 15 different *Prunus* species, rather than a pure species (Okie, 2006; Topp et al., 2012; Karp, 2015).

Burbank also bred hybrids between Japanese plum and apricot, called "plumcots" (Roeding, 1908). The creation of plumcots occurred more than 100 years ago (Hedrick, 1911; Howard, 1945; Topp et al., 2012), but the first ones were not commercially viable due to very low productivity and high acidity (Ledbetter et al., 1994; Okie, 2005; Jun and Chung, 2007). The first generation of hybrid seedlings exhibited characteristics such as pubescent skin from the apricot and a variable fruit size, shape, color, flavor and firmness (Ledbetter et al., 1994; Jun et al., 2009). Through backcrossing between plumcots, apricots and Japanese plums, varieties of greater commercial interest have been obtained (Topp et al., 2012). The Zaiger Genetics breeding program registered the terms "pluot" and "aprium" in 1991—the results of crossing plumcot × plum (75% plum and 25% apricot) and plumcot ×

* Corresponding author.
E-mail address: mnicolas@cebas.csic.es (M. Nicolás-Almansa).

https://doi.org/10.1016/j.scienta.2023.112131
Received 23 March 2023; Received in revised form 2 May 2023; Accepted 5 May 2023
Available online 10 May 2023

apricot (75% apricot and 25% plum), respectively (Brantley, 2004).

Japanese plum has certain problems that make it difficult to grow. Up to now, there have been very few self-compatible Japanese plum cultivars and a lack of resistance to sharka disease (plum pox virus, PPV) (Rubio et al., 2011a). These two traits (self-compatibility and sharka resistance) are two of the principal aims of Japanese plum breeding programs around the world, including the Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investigaciones Científicas (CEBAS-CSIC) and Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) program in Murcia (Spain) (Ruiz et al., 2019). Unlike the case of the Japanese plum, apricot breeding has achieved self-compatibility in many varieties as well as a significant number of varieties resistant to sharka (Rubio et al., 2014). In apricot species, marker-assisted selection for floral self-compatibility and resistance to sharka is used routinely. It is possible to determine the self-compatibility (SC) allele using the AprFBC8 marker (Halász et al., 2010). For sharka resistance, the PGS1.21 marker (Soriano et al., 2012; Rubio et al., 2019)—and more recently, the ParPMC2 marker (Zuriaga et al., 2018; Polo-Oltra et al., 2020)—make it possible to identify genetically resistant genotypes.

Interspecific hybridization is a potential strategy used in breeding programs with the aim of transferring interesting genes from one species to another. However, interspecific hybrids are more difficult to obtain within the *Prunus* genus (Perez and Moore, 1985). The great genetic distance between parents increases the genetic barriers to hybridization (Okie, 2005; Morimoto et al., 2019). This results in poor fruit set with many aborted fruits and a very low yield compared to standard intra-specific crosses. The compatibility of cross-breeding between Japanese plum, myrobalan plum, apricot and plumcot depends on the direction of the cross, and using *P. salicina* as the maternal parent presents more satisfactory results than other species (Yoshida et al., 1975; Jun and Chung, 2007; Szymajda et al., 2015; Yaman and Uzun, 2020, 2022).

Since the 1980s, the plumcot has provided a relatively new type of fruit that combines the horticultural and market characteristics of apricots and Japanese plum species (Okie et al., 1992). Over the last 40 years, new plumcot cultivars have been released and exhibit different fruit morphologies, including fruit size, skin and flesh color, and post-harvest characteristics. These new plumcot cultivars have been extensively developed from interspecific crosses in breeding programs in the US (Gómez and Ledbetter, 1993; Ledbetter et al., 1994; Okie and Ramming, 1999; Okie, 2005); Korea (Jun et al., 2011; Nam et al., 2016; Kwon et al., 2020); Bulgaria (Zhivondov, 2012); and China (Niu et al., 2015).

The previously released plumcot genotypes have been extensively characterized from a horticultural level, including at the phenological and pomological levels. In terms of molecular characterization and genetic diversity, however, previous works have primarily focused on Japanese plum cultivars (Boonprakob et al., 2001; Qiao et al., 2007; Klabunde et al., 2014; Merkouropoulos et al., 2017; Abdallah et al., 2019; Guerrero et al., 2021). Scarce work has been done on interspecific hybrids. Moreover, this paucity is compounded by the lack of information on their pedigree and hybrid classification (plumcot, pluot, aprium) due to the confidential nature of private breeding programs, which hinders study (Ahmad et al., 2004; Guerrero et al., 2022). As far as we know, no detailed molecular characterization and PPV evaluations of plumcots generated in breeding programs have been carried out. Despite the high number of plumcots already released, none of them has both sharka resistance and self-compatibility.

In this context, interspecific crosses between Japanese plums and self-compatible, sharka-resistant apricot (*P. armeniaca* L.) cultivars have been performed in recent years at the CEBAS-CSIC-IMIDA breeding program in Murcia (Spain) in order to obtain resistant and self-compatible interspecific plumcots. The objective of this work was to evaluate the phenotypic behavior of the newly obtained plumcot genotypes, including the phenological, floral and fruit quality traits. As an original contribution, this work also includes PPV-resistance phenotyping, as well as a molecular characterization and the determination of S-haplotypes through PCR analysis.

2. Materials and methods

2.1. Plant material

The plant material used in this work included seedlings obtained by interspecific crosses between Japanese plum cultivars and self-compatible, sharka-resistant apricot cultivars, and their progenitors (Table 1). Crosses were performed as part of the Japanese plum breeding program developed jointly by CEBAS-CSIC and IMIDA of Murcia (Spain). A total of 30 interspecific crosses have been evaluated in this work, obtaining a variable number of offspring per cross, clustered in 16 combinations using *P. salicina* Lindl. as mother parent and another 14 using *P. armeniaca* L. (Table 2). Seeds obtained from the crosses were germinated by embryo rescue following the protocol described by Perez-Jimenez et al. (2021). This technique is widely used to overcome post-zygotic barriers/incompatibilities in interspecific hybridizations in *Prunus* (Liu et al., 2007; Morimoto et al., 2019; Sallom et al., 2021). The seedlings were acclimatized in pots in a greenhouse before being planted in the field. The offspring and their genitors were cultivated in the same experimental orchard located in Calasparra (Murcia, southeastern Spain; lat. 38°16′N, long. 1°35′W; 350 m altitude) following standard Japanese plum orchard management.

2.2. DNA extraction and SSR characterization

Young leaves were collected in spring and stored at –80 °C before DNA isolation, which was carried out according to a modification of the CTAB method described by Doyle and Doyle (1987). A NanoDrop™ND-1000 spectrophotometer (Bio-Science, Hungary) was used to analyze the quantity and quality of DNA. SSR analysis was carried out to perform an initial screening and confirm the interspecific nature. The SSRs PGS1.21 (Soriano et al., 2012), CPSCT005 (Mnejja et al., 2004), and BPPCT010 (Dirlewanger et al., 2002) were selected for PCR, due to their high polymorphism in the parents involved in the crosses. In addition, a total of 17 SSR markers developed in peach, apricot and Japanese plum were used (Tables 3 and S1) to achieve a complete molecular characterization in the verified interspecific hybrids and their parents. SSR amplifications from most markers were analyzed in an ABI Prism 3130xl automated sequencer, and the size of the segregating alleles of some of the markers was analyzed using GeneTools gel analysis software from SYNGENE (Beacon House, Cambridge, UK). PCRs were performed in a SimpliAmp™ Thermal Cycler (ThermoFisher, USA) under the following conditions: an initial denaturation for 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 57 °C, 30 s at 72 °C, and a final extension of 5 min at 72 °C. PCR products were electrophoresed in 3% (w/v) Metaphor® Agarose gels (Fig. 1A), and DNA bands were visualized under UV transilluminator. The molecular sizes of amplified fragments were estimated using a 1 Kb Plus DNA Ladder (Invitrogen™, Life Technologies, Spain).

2.3. Genetic diversity evaluation and phylogenetic analysis

The data of alleles generated by SSR markers were used to estimate genetic diversity parameters. For each microsatellite locus, GENALEX software version 6.51 (Peakall and Smouse, 2012) was used to calculate the number of alleles per locus (N_A) and size range and to estimate the expected genetic heterozygosity (H_e), observed heterozygosity (H_o) and polymorphism information content (PIC). Expected heterozygosity for each marker was calculated as $H_e = 1\text{-}\sum p_i^2$ where, p_i is the frequency of the *i*th allele, $\mathrm{PIC} = 1\text{-}\sum p_i^2 \text{ - } \sum(2\, p_i^2 \cdot p_j^2)$ and observed heterozygosity (H_o) the number of heterozygous genotypes divided by the total number of genotypes. In addition, the power of discrimination (PD) of each SSR

Table 1
Pedigree, origin and main agronomic characteristics (self-compatibility, flowering and ripening time, skin and flesh color and PPV resistance) of the apricots and Japanese plum cultivars used in the interspecific crosses with verified interspecific hybrids. SC: self-compatible; SI: Self-incompatible; R: PPV resistant; S: PPV susceptible.

	Pedigree	Origin	Self-compat.	Flowering	Ripening	Skin colour	Flesh colour	PPV
Apricot								
Mirlo Anaranjado	Rojo Pasión × Búlida Precoz	Spain	SC	Early	Early	Light orange (Red)	Orange	R
Rojo Pasión	Orange Red × Currot	Spain	SC	Medium	Early-Medium	Orange (Red)	Light Orange	R
(1001) 5-26	(Orange Red × Currot) × OP	Spain	SC	Early	Early	Light Orange	Orange	R
Japanese plum								
Red Beaut	Eldorado × Burmosa	USA	SI	Early	Early	Red	Yellow	S
Black Splendor	Black Amber × OP	USA	SI	Very Early	Early	Black	Red	S
Honey Dawn	Unknown	South Africa	SI	Early	Early	Yellow	Yellow	S
0716-5	0112-11 × OP	Spain	SI	Early	Early	Red	Light Red	S
0112-11	Black Splendor × OP	Spain	SI	Very Early	Early	Black	Red	S
0614-5	Black Splendor × OP	Spain	SI	Very Early	Very Early	Red	Light Red	S

Table 2
Interspecific crosses performed and evolution of the plants obtained during the process of *in-vitro* germination, acclimatization in pots, planting in the field and verification of their interspecific nature by SSR markers. The percentage of interspecific success is shown in parentheses.

Crosses	Nº seeds obtained	Nº plants in field evaluated	Nº interspecific verified
Apricot × plum			
Rojo Pasión × 0716-5	64	41 (64)	0 (0)
Rojo Pasión × Black Splendor	244	41 (17)	0 (0)
Rojo Pasión × Santa Rosa	251	27 (11)	0 (0)
Rojo Pasión × Pioneer	69	21 (30)	0 (0)
Mirlo Anaranjado × 0716-5	128	6 (5)	0 (0)
Mirlo Anaranjado × Black Splendor	109	11 (10)	0 (0)
Mirlo Anaranjado × Pioneer	146	34 (23)	0 (0)
Mirlo Anaranjado × 0716-6	147	9 (6)	0 (0)
Mirlo Anaranjado × Santa Rosa Precoz	87	5 (6)	0 (0)
Mirlo Anaranjado × Honey Dawn	91	12 (13)	0 (0)
Mirlo Anaranjado × 0716-5	89	39 (44)	0 (0)
CEBAS-57 × Pioneer	15	0 (0)	0 (0)
CEBAS-57 × Santa Rosa Precoz	21	9 (43)	0 (0)
CEBAS-57 × 0716-6	20	2 (10)	0 (0)
CEBAS-57 × Honey Dawn	21	2 (10)	0 (0)
(1001) 5-26 × Black Splendor	2	1 (50)	0 (0)
Total	*1504*	*260 (17)*	*0 (0)*
Plum × apricot			
Red Beaut × Rojo Pasión	158	16 (10)	2 (13)
Red Beaut × Mirlo Anaranjado	460	81 (18)	5 (6)
Red Beaut × (1001) 5-26	123	18 (15)	1 (6)
Black Splendor × Mirlo Anaranjado	109	47 (43)	6 (13)
Black Splendor × Rojo Pasión	351	6 (2)	0 (0)
Black Splendor × (1001) 5-26	1	1 (100)	0 (0)
Black Splendor × CEBAS-57	34	19 (56)	0 (0)
Honey Dawn × CEBAS-57	131	92 (70)	0 (0)
Honey Dawn × Mirlo Anaranjado	43	37 (86)	2 (5)
0716-5 × Rojo Pasión	1	1 (100)	1 (100)
0413-1 × Mirlo Anaranjado	10	0 (0)	0 (0)
0614-5 × Mirlo Anaranjado	21	16 (76)	14 (88)
0112-11 × Mirlo Anaranjado	48	3 (6)	2 (67)
1112-166 × Mirlo Anaranjado	35	15 (43)	0 (0)
Total	*1525*	*352 (23)*	*33 (9)*

marker was calculated as PD = $1-\sum g_i^2$ as g_i^2 is the frequency of the ith genotype. The genetic relationships among plums, apricots and their hybrids were determined using a Neighbor-Joining (NJ) cluster analysis (Saitou and Nei, 1987). Molecular Evolutionary Genetics Analysis (MEGA) software (Tamura et al., 2021) was used to calculate the distance analysis and to construct NJ dendrograms. Relative support for the branches in each dendrogram was assessed with 2000 replicates of bootstrap. The genetic distance was computed using the p-distance method.

2.4. *Determination of S-haplotypes through PCR analysis and pollen viability*

S-allele amplification was carried out using three pairs of consensus primers. PaConsIF-PaConsIR2 (Sonneveld et al., 2006) and PruC2-PCER (Tao et al., 1999; Yamane et al., 2001) are specific for the S-RNase first and second introns respectively, while the specific primer pair AprFBC8-F-AprFBC8-R (Halász et al., 2010) allows for the identification of the Sc allele associated with self-compatibility in apricot, by amplifying a fragment of approximately 500 bp from the V2 and HVb variable regions of the *SFB* gene. A PCR amplification of S-RNase first intron (PaConsIF-PaConsIR2) was made in a total volume of 20 µl, containing 1X GoTaq® Flexi Buffer, 1 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each primer, 90 ng of genomic DNA and 1Ul of GoTaq® DNA Polymerase (Promega). The PCR conditions used had an initial step of 3 min at 95 °C, 35 cycles of 1 min at 95 °C, 30 s at 57 °C and 3 min at 72 °C, and a final step of 7 min at 72 °C. Amplified PCR products were separated by electrophoresis on 3% (w/v) Metaphor Agarose gel and analyzed on an ABI Prism 3130xl automated sequencer to identify the size of the alleles that had not been previously identified in other studies. A PCR amplification of the S-RNase second intron (PruC2-PCER) was done in a total volume of 10 µl, containing 1X PCR Buffer, 2 mM $MgCl_2$, 0.4 mM of each dNTP, 0.6 µM of each primer, 1X Q-Solution (Qiagen), 90 ng of genomic DNA and 5 U/µl of Qiagen® Taq DNA polymerase. The PCR conditions used had an initial step of 2 min at 94 °C, 10 cycles of 10 s at 94 °C, 2 min at 58 °C and 2 min at 68 °C, 25 cycles of 10 s at 94 °C, 2 min at 58 °C and 2 min at 68 °C with the addition of 10 s/cycle, and a final step of 10 min at 68 °C. Amplified PCR products were separated by electrophoresis on 0.7% (w/v) agarose gel. A PCR amplification of the *SFB* gene by AprFBC8-F-AprFBC8-R was done in a total volume of 15 µl containing 1X Reaction Buffer (Biotools, Spain) with the final concentrations of 2 mM $MgCl_2$, 0.5 mM of each dNTP, 0.2 µM of each primer, 90 ng of genomic DNA and 8 mU/µL of Biotools® DNA polymerase. The PCR conditions used had an initial step of 3 min at 95 °C, 35 cycles of 30 s at 95 °C, 1 min 30 s at 55 °C and 2 min at 72 °C, and a final step of 5 min at 72 °C. Amplified PCR products were separated by electrophoresis on 1% (w/v) agarose gel.

In addition, pollen grains from the assayed plumcot genotypes were germinated *in vitro* to test their viability. Pollen grains were obtained from flowers collected at the balloon stage, collecting the anthers, drying it and later spreading it on a germination medium formed by 15% sucrose and 1.2% agar. After being cultured for 6–8 h at 22 °C, the germinated pollen was counted and the germination percentage was calculated considering that pollen grains are viable when the length of the growing tube was higher than the pollen grain diameter.

Table 3
Genetic diversity parameters of 33 interspecific hybrid genotypes, 6 Japanese plum genotypes and 3 apricot genotypes using 17 SSR markers. Linkage Group (LG), Number of alleles (N_A), allele size, Polymorphism Information Content (PIC), Observed heterozygosity (Ho), Expected heterozygosity (He) and Power of Discrimination (PD).

Ref.	Species	SSR marker	LG	N_A	Alleles (pb)	*PIC*	*Ho*	*He*	*PD*
Cipriani et al., 1999	*P. persica*	UDP96-005	2	8	106-167	0.75	0.88	0.75	0.72
Dirlewanger et al., 2002	*P. persica*	BPPCT001	2	4	122-189	0.65	0.90	0.65	0.70
		BPPCT007	3	6	113-174	0.69	0.81	0.69	0.77
		BPPCT010	4	5	115-168	0.71	0.90	0.71	0.79
		BPPCT025	6	6	160-195	0.72	0.95	0.72	0.82
		BPPCT039	3	7	130-198	0.64	0.83	0.64	0.51
García-Gómez et al., 2018	*P. armeniaca*	CA-RISO	4	7	120-146	0.71	0.93	0.71	0.73
		GER-SIN	4	5	200-227	0.60	0.92	0.76	0.83
		MADS-BOX	3	8	201-238	0.77	0.95	0.77	0.87
		MET-T	4	6	131-172	0.68	0.93	0.68	0.70
		MYB-TF	3	7	239-263	0.73	0.90	0.73	0.81
		PruDest-2	8	3	230-280	0.29	0.90	0.54	0.34
		PSPD1	4	4	249-259	0.58	0.86	0.62	0.70
		UPDAR	3	7	219-270	0.79	0.93	0.79	0.86
Mnejja et al., 2004	*P. salicina*	CPSCT005	4	5	161-231	0.69	0.86	0.69	0.77
Soriano et al., 2012	*P. armeniaca*	PGS1.21	1	6	166-226	0.75	0.95	0.75	0.81
		PGS1.24	1	8	101-139	0.80	0.90	0.80	0.87
Mean				*6*		*0.68*	*0.90*	*0.71*	*0.74*

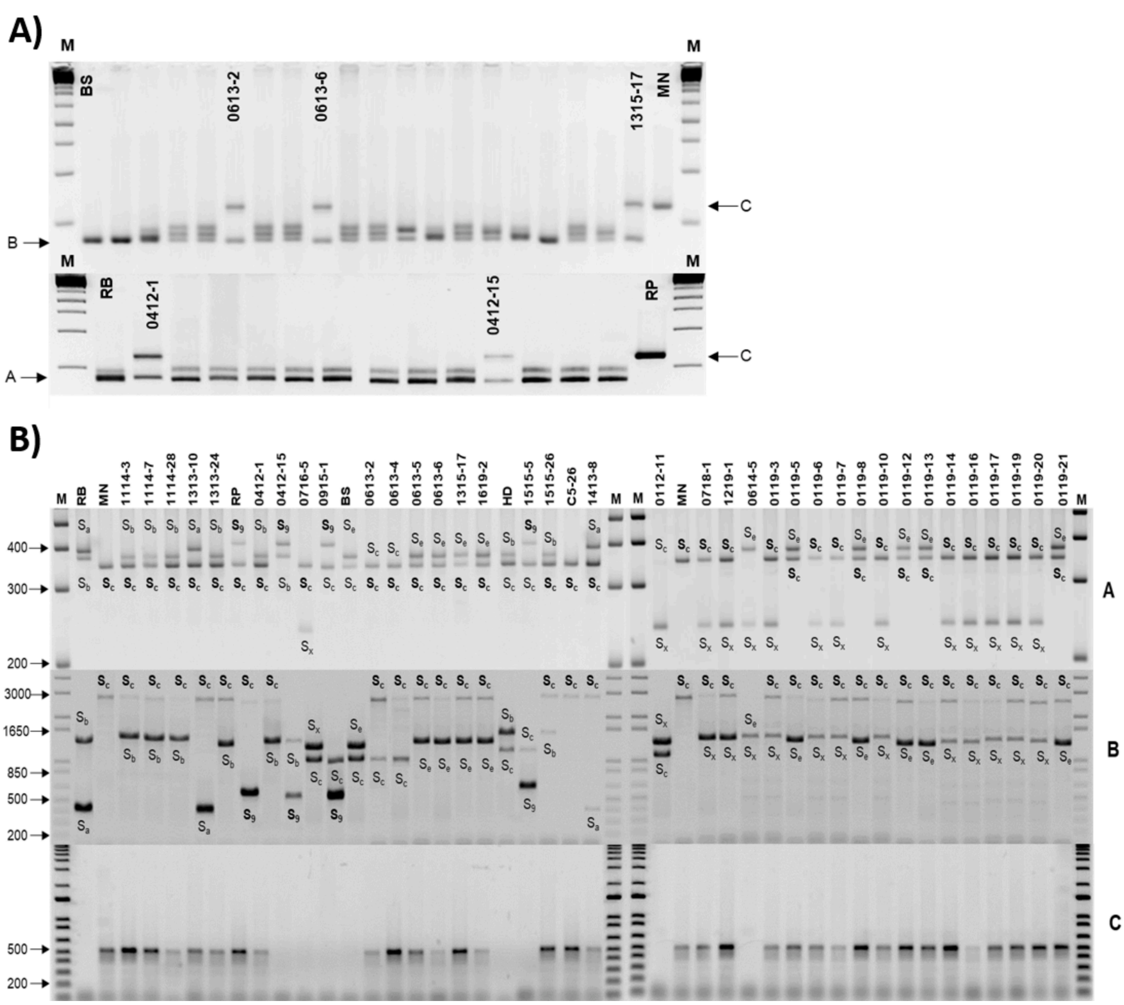

Fig. 1. (A) Metaphor® Agarose gel showing the allelic segregation of the microsatellite locus CPSCT005, sequenced from Japanese plum, in the intercross *P. salicina* × *P. armeniaca*. Some of the identified plumcots are shown in those gels (0412-1, 0412-15, 0613-2, 0613-6, 1315-17-). The arrows point to the parental alleles, and those are identified by letters (A, B and C). RB and BS are the female parent, while RP and MN are the male parent of the crossings. (B) *S*-PCR analysis of apricot, Japanese plum and plumcot genotypes by consensus primers amplifying the first (A) and second (B) introns of Prunus S-RNase gene and amplification of SFBc (C) from apricot using specific primers. M: Marker kb+ ladder; Red Beaut (RB), Mirlo Anaranjado (MN), Rojo Pasión (RP), Black Splendor (BS), Honey Dawn (HD), (1001) 5-26 (C5-26). Alleles in bold are from apricot.

2.5. Evaluation of horticultural traits

Phenological, morphological and fruit quality traits were evaluated on the verified interspecific hybrids for four consecutive years (2017–2020). Flowering time (FT) was evaluated every 3–4 days and recorded in the field in stage 65 according to the international BBCH scale (Meier et al., 1994), when 50% of flowers were opened (F_{50}). Flowering intensity (FI) was measured on a scale of 0 (null) to 3 (maximum). The ripening date (RD) was determinated when the fruit had an appropiate firmness and color, at physiological maturity stages, and the productivity (P) was registered with a score of 0 (null) and 5 (maximum). Both FT and RD were evaluated in Julian days (natural days from January 1). The fruit developmental period (FDP) was considered as the difference between FT and RD. The presence/absence of pubescence of gynoecium (PUB) and chalice color (CC) were evaluated by observation. Certain fruit quality traits were also analyzed, distinguishing between physical traits [fruit weight (FW), stone weight (SW), adherence of stone to flesh (AD), skin color (SC), flesh color (FC), and firmness (F)] and biochemical traits [soluble solids content (SSC), acidity (A) and pH]. FW and SW were measured using a Blauscal digital balance (model AH-600) with an accuracy of 0.01 g. Fruit color (SC and FC) was measured made using a Minolta Chroma Meter (CR-300; Minolta, Ramsey, NJ, USA) tri-stimulus color analyzer calibrated to a white porcelain reference plate using a CIELAB scale with color space coordinates L^*, a^* and b^*. To assess color, we used the Hue angle [H°= arctangent(b*/a*)] parameter, which was determined around the equatorial region (Brown and Walker, 1990). F was quantified in Newton (N) by a texture analyzer (TA.XT plus, Texture Technologies, Hamilton, MA, USA) using a 75 mm cylinder and compressing to a depth of 5 mm. SSC was determined using a digital ATAGO® hand-held refractometer calibrated as the percentage of sucrose at 20 °C. A was determined by acid-base titration using 2 g of sample diluted in 30 ml of distilled water as malic acid grams per 100 ml.

2.6. Plum pox virus (PPV) resistance evaluation

The molecular evaluation of PPV resistance was performed using the SSR marker PGS1.21, which is tightly linked to PPV resistance in apricot (Soriano et al., 2012) and previously described as inside the PPVres locus (Zuriaga et al., 2013). A gene with allelic variants linked in coupling to PPV resistance called *ParPMC2* has recently been identified by Zuriaga et al. (2018). *ParPMC2* resistant (R) and susceptible (S) alleles were amplified using two forward specific primers (PMC2-F-alleleR: 5′-GTCATTTTCATTGATGTCATTCA-3′, or PMC2-F-alleleS: 5′-GTCGTTTTCATTGATGTCCAAAC-3′, respectively) and one common reverse primer (PMC2-R: 5′-GTGCTCTTTCACATTCTTGCTC-3′). PCR amplification of the PGS1.21 marker was done as described above, and ParPMC2 PCRs were performed in a total volume of 20 μl containing 0.5X GoTaq® Flexi Buffer, 0.2 mM of each dNTP, 0.5 μM of each primer, 1.87 mM $MgCl_2$, 75 ng of genomic DNA and 1U of GoTaq® DNA Polymerase (Promega). The *ParPMC2* PCR conditions were as follows: initial denaturation step at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 45 s, followed by a final extension step at 72 °C for 10 min. The PCR products were electrophoresed on 1% (w/v) agarose gels. Due to the lack of knowledge of the mode of inheritance of PPV resistance in interspecific Japanese plum-apricot hybrids, and because the current set of markers based on the single resistance locus is not sufficient to predict resistance to PPV, resistance of the plumcot genotypes was evaluated for 3 phenotyping cycles in controlled conditions in a scaled greenhouse located in the experimental facilities of CEBAS-CSIC in Murcia (Spain).

The *Prunus insititia* L. 'Adesoto 101' was used as rootstock for grafting the plumcots to be evaluated. This rootstock was chosen because of its high susceptibility to PPV (Rubio et al., 2005). The PPV isolate used as inoculum was 3.30RB/GF-IVIA (GenBank: KJ849228.1), a Dideron Type (PPV-D) isolate, obtained from Japanese plum in Spain maintained in the PPV collection of the Instituto Valenciano de Investigaciones Agrarias (IVIA) in Valencia (Spain). This isolate is considered to be representative of the Spanish PPV population. Six replicates of each genotype were grafted onto 'Adesoto' rootstocks previously inoculated by grafting with a piece of bark (chip-budding) from another 'Adesoto' with strong sharka symptoms. The presence of symptoms in the rootstock and the plumcot leaves was scored from 0 (no symptoms) to 5 (maximum intensity of symptoms) two months after putting the plants in the greenhouse (Martínez-Gómez and Dicenta, 2000; Rubio et al., 2013). Only plants whose rootstocks showed sharka symptoms were considered for the evaluation process. In each cycle, plants without symptoms were reinoculated after symptom observation. An ELISA-DASI (double antibody sandwich indirect) test was applied to the leaves to detect PPV's coat protein (CP) using monoclonal antibodies 5B-IVIA® (Plant Print Diagnostics SL, Valencia, Spain) specific to the CP of PPV (Cambra et al., 1994). Optical densities (OD) were recorded at 405 nm after 60 min, and samples with an OD less than twice that of the healthy control were considered negatives (Sutula et al., 1986). For the detection of PPV RNA, an RT-PCR analysis was carried out using total RNA extracted from the same leaves of the ELISA-DASI using an adapted CTAB method for plants described in Tong et al. (2012). Two specific primers within the CP gene—VP337 (CTCTGTGTCCTCTTCTTGTG), complementary to positions 9487– 9508 of genomic PPV, and VP338 (CAATAAAGCCATTGTTGGATC), homologous to positions 9194–9216—were assayed (Sanchez-Navarro et al., 2005). Amplified products were electrophoresed on 1% (w/v) agarose gel. A 1 Kb Plus DNA Ladder (Invitrogen™, Life Technologies, Spain) was used as molecular weight standard.

3. Results

3.1. Interspecific hybrid verification using SSR markers

A total of 3029 seeds were obtained from interspecific crosses, including 1525 using Japanese plums as the female parent and 1504 using apricot as the female parent (Table 2). However, due to losses during germination, acclimatization, and subsequent planting in the field, only 612 genotypes (20.2%) could be evaluated. The verification of the interspecific nature of the offspring obtained using SSRs is shown in Table 2. The screening by SSR markers of the 612 genotypes generated by interspecific crossings clearly identified a total of 33 interspecific hybrids (Table 2). The 5.4% of verified interspecific hybrids shows the difficulty in obtaining plumcots, underscoring the technical challenges associated with this process. This finding highlights the need to improve the hybridization protocol. If we consider the total number of obtained seeds, the results are even worse, with only 1.1% plumcots obtained. All plumcots came from crosses in which Japanese plum was the female parent and apricot the male parent, suggesting that hybridization efficiency depends on the crossing-over direction. An example of the molecular identification of interspecific hybrids with the CPSCT005 marker is shown in Fig. 1A, where only the interspecific individuals showed the alleles from parents used in the cross, and the remaining genotypes were escapes.

The genetic profiles of each genotype are included in Supplementary Table S1. Most of the SSR markers used in this work allow for early marker-assisted selection (MAS) to distinguish interspecific hybrids from possible escapes resulting from controlled pollination. However, some inconsistencies were detected in some markers. Most of the plumcot hybrids only segregate apricot alleles, except for '0412-1', '0412-15', '1413-8' and '1515-5' for BPPCT039 and '0915-1' for UDP96-005, which also have plum alleles. On the other hand, '0613-4', '0915-1' and '1515-5' genotypes have an allele of 181 bp for the PGS1.21 marker that does not match with any alleles of their apricot genitor.

3.2. Plumcot genetic diversity and cluster analysis

All the SSR markers used in this study showed a correct amplification and turned out to be polymorphic in the analysis of 33 interspecific Japanese plum × apricot hybrids and their nine genitors. A total of 102 alleles were amplified using 17 SSR primers, ranging from 3 (PruDest-2) to 8 (MADS-BOX, PGS1.24 and UDP96-005) alleles per locus, with an average value of 6. All the SSR loci were polymorphic. The smallest allele was obtained with the SSR marker PGS1.24 (101 bp) and the largest one with PruDest-2 (280 bp). Polymorphism Information Content (PIC) values ranged between 0.29 (PruDest-2) and 0.80 (PGS1.24), with an average of 0.68 per locus; the PruDest-2 marker was thus slightly informative and the rest highly informative (Botstein et al., 1980). The observed heterozygosity values (Ho) ranged from 0.81 (BPPCT007) to 0.95 (BPPCT025, MADS-BOX and PGS1.21), with an average value of 0.81, whereas the expected heterozygosity (He) ranged from 0.54 (PruDest-2) to 0.80 (PGS1.24), with a mean value of 0.71 (Table 3).

Fig. 2 shows the phenotypic relationships among the different genotypes assayed. The NJ dendrogram showed two main clusters: one big cluster (I) with all the interspecific hybrids and apricots, and another cluster (II) with plums. The results showed significant differences between the plumcots and the species from which they originated, although a greater similarity of the plumcots with the apricots was found than with the plums. Plumcots from the same cross are grouped together.

3.3. Identification of self-compatibility alleles and pollen viability

PCR analysis using primers from conserved regions of S-RNase of sour cherry (*P. cerasus*) and sweet cherry (*P. avium*), and a specific primer of SFBc in apricot, was used to identify the S-haplotypes of plumcot hybrids and their genitors (Fig. 1B). Cultivars with known S-

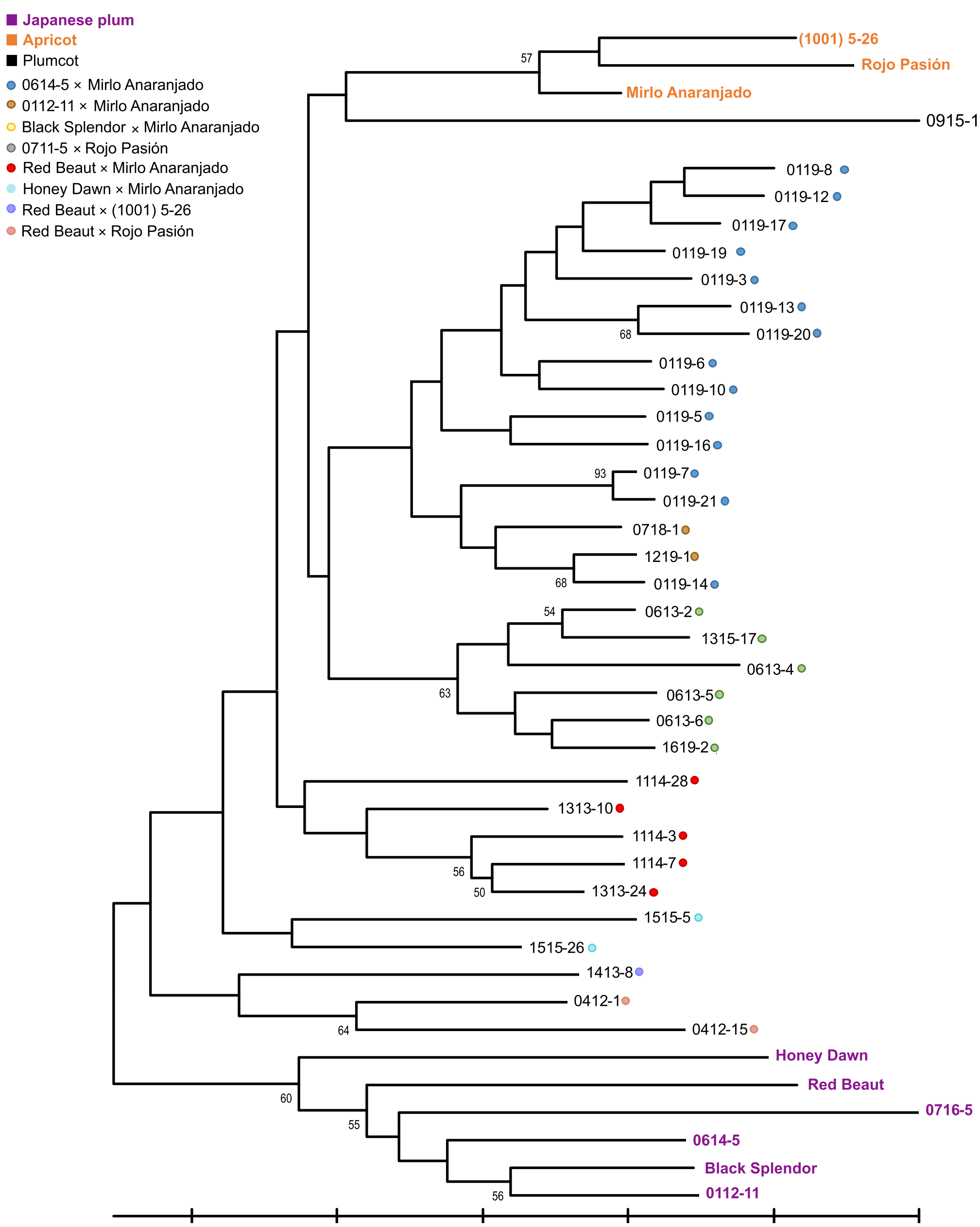

Fig. 2. NJ cluster analysis of plum, apricot and interspecific plumcot hybrids based on the SSR characterization created from 2000 bootstrap replications. Bootstraping values >50% were placed on the branches. Colored points indicated the different interspecific families assayed.

alleles were used to confirm the size of S-RNase alleles previously identified (S_a-S_e in plums and Sc-S_9 in apricots) using primer pairs PaConsI-PaConSIR2 and PruC2-PCER. Seven different alleles were amplified from the genitors, two from apricot (Sc and S_9) and five from Japanese plum (S_a, S_b, S_c, S_e and S_x) (Table 4). The plum genitors '0716-5', '0614-5' and '0112-11', and some genotypes of their offspring, showed fragments with 237 and 1166 sizes for the first and second intron, respectively, that have not yet been identified. Such fragments of different sizes from those already described in Japanese plum have been considered as a new allele, named S_x in this work (Table S2). Using PaConSI-PaConsIR2 primers, we were able to distinguish between the S_e allele and the new S_x allele in Japanese plum, since amplification of the second intron produced a band of the same size between them (Table S2). The plumcots were placed in incompatibility groups distinct from those described in both Japanese plum and apricot based on their mixture of alleles from both species. From the 33 interspecific genotypes evaluated, seven new haplotypes were identified in plumcots (S_aSc, S_bSc, S_bS_9, S_cSc, S_cS_9, S_eSc and S_xSc) (Table 4). Three plumcots did not segregate the apricot Sc allele (Table 4). The rest of the interspecific hybrids inherited the Sc allele, which confers self-compatibility in apricot (Vilanova et al., 2006), so a priori they should be self-compatible genotypes.

The studies of pollen viability determined the androsterility of all plumcot genotypes. These results suggest that interspecific hybridization causes androsterility in the offspring.

Table 4
S-genotype of the apricots, plums and plumcots generated in this study. S alleles in bold are from apricot.

Pedigree	Genotype	*S*-genotype
Prunus armeniaca L.		
Rojo Pasión × Búlida precoz	Mirlo Anaranjado	$\mathbf{S_c}$ $\mathbf{S_c}$
Orange Red × Currot	Rojo Pasión	$\mathbf{S_c}$ $\mathbf{S_9}$
(Orange Red × Currot) × OP	(1001) 5-26	$\mathbf{S_c}$ $\mathbf{S_c}$
Prunus salicina Lindl.		
Eldorado × Burmosa	Red Beaut	S_a S_b
Black Amber × OP	Black Splendor	S_c S_e
Unknown	Honey Dawn	S_c S_b
0112-11 × OP	0716-5	S_c S_x
Back Splendor × OP	0112-11	S_c S_x
	0614-5	S_e S_x
P. salicina × *P. armeniaca*		
Red Beaut × Mirlo Anaranjado	1114-3	S_b $\mathbf{S_c}$
	1114-7	S_b $\mathbf{S_c}$
	1114-28	S_b $\mathbf{S_c}$
	1313-10	S_a $\mathbf{S_c}$
	1313-24	S_b $\mathbf{S_c}$
Red Beaut × Rojo Pasión	0412-1	S_b $\mathbf{S_c}$
	0412-15	S_b $\mathbf{S_9}$
Black Splendor × Mirlo Anaranjado	0613-2	S_c $\mathbf{S_c}$
	0613-4	S_c $\mathbf{S_c}$
	0613-5	S_e $\mathbf{S_c}$
	0613-6	S_e $\mathbf{S_c}$
	1315-17	S_e $\mathbf{S_c}$
	1619-2	S_e $\mathbf{S_c}$
Honey Dawn × Mirlo Anaranjado	1515-5	S_c $\mathbf{S_9}$
	1515-26	S_b $\mathbf{S_c}$
Red Beaut × (1001) 5-26	1413-8	S_a $\mathbf{S_c}$
0716-5 × Rojo Pasión	0915-1	S_c $\mathbf{S_9}$
0112-11 × Mirlo Anaranjado	0718-1	S_x $\mathbf{S_c}$
	1219-1	S_x $\mathbf{S_c}$
0614-5 × Mirlo Anaranjado	0119-3	S_x $\mathbf{S_c}$
	0119-5	S_e $\mathbf{S_c}$
	0119-6	S_x $\mathbf{S_c}$
	0119-7	S_x $\mathbf{S_c}$
	0119-8	S_e $\mathbf{S_c}$
	0119-10	S_x $\mathbf{S_c}$
	0119-12	S_e $\mathbf{S_c}$
	0119-13	S_e $\mathbf{S_c}$
	0119-14	S_x $\mathbf{S_c}$
	0119-16	S_x $\mathbf{S_c}$
	0119-17	S_x $\mathbf{S_c}$
	0119-19	S_x $\mathbf{S_c}$
	0119-20	S_x $\mathbf{S_c}$
	0119-21	S_e $\mathbf{S_c}$

3.4. Evaluation of horticultural traits

The data obtained about phenological and floral traits are shown in Table 5. Only 12 of the 33 obtained plumcots had been able to flower so far, and two of them did not present any production. The plumcots that flourished had a gynoecium with pubescence on the ovary, characteristic of apricot fruit, and pink or pinkish-green variegated sepals, unlike plum, which have green sepals. A greater number of abnormal multi-pistil flowers were detected in the hybrids, with up to four pistils. The hybrids had leaves with an intermediate shape between apricot and plum, with some leaves more oval and similar to apricot and others with a more elliptical shape typical of plum (Fig. 3). In contrast to their parents, all interspecific genotypes showed a lower and more erratic level of flowering and productivity (Table 5). The genotype with the highest flowering intensity was '1413-8' with a mean value of 1.4 (scale 0-3), and the hybrid with the highest productivity was '1313-10'. On the other hand, the hybrid '0613-4' had the earliest flowering time, although it had the lowest flowering intensity and has not produced fruit yet. Compared to their genitors, most interspecific hybrids showed delayed flowering time and intermediate values for ripening, resulting in a shorter fruit development period than their genitors.

As for the physical characteristics of the fruit (Table 6), the genotypes showed a wide variability in size and skin and flesh color (Fig. 3). Fruit size varied from 77.1 g for '0613-5'— the only genotype that exceeded its parents in size—to 20.7 g for '1515-5', which had the smallest fruit. All plumcot hybrid fruits had pubescent skin. The majority of plumcot hybrids exhibited stone adherence to the flesh, a characteristic inherited from their Japanese plum progenitors: four plumcots were clingstone and three were semi-clingstone. The only freestone genotypes, like apricot, were '0412-15', '0613-5' and '0613-6'. Most of the plumcots obtained had a purplish-reddish skin color, characteristic of Japanese plum, except for genotypes '1114-28' and '1515-5', which were orange and yellow, respectively. The plumcot with the most striking flesh color was '0613-5', which had an intense red color similar to its genitor 'Black Splendor'. The maximum fruit firmness was in genotype '1114-7', with 54.5 N, and '0613-5' had the highest soluble solids content, with 15.6 °Brix. All plumcots, except '1515-5', showed transgressive values for acidity, i.e., higher than their genitors had.

3.5. Plum pox virus (PPV) resistance evaluation

Table 7 shows the PPV phenotyping of 20 interspecific hybrids and their genitors grafted onto infected 'Adesoto' plum rootstocks over three vegetative cycles in greenhouse conditions. According to the symptoms observation and the ELISA-DASI and RT-PCR results, genotypes were classified into the following three groups: resistant (without symptoms and ELISA/RT-PCR negative); tolerant (symptomless and ELISA or RT-PCR positive); or susceptible (with symptoms and ELISA/RT-PCR positive).

The results obtained confirm the already-described resistance of apricot cultivars 'Rojo Pasión' and 'Mirlo Anaranjado' and the advanced selection '(1001) 5-26' used in interspecific crosses as a source of PPV resistance. On the other hand, Japanese plum genitors 'Red Beaut', 'Black Splendor', '0614-5' and '0112-11' showed susceptibility, while selection '0716-5' was considered tolerant: it was asymptomatic and only tested positive by RT-PCR in two of eleven observations in the first cycle (Table 7).

The total number of replicates correctly evaluated (rootstock infected and cultivar sprouted) was 106 out of 168 (63%), with a mean symptom intensity on the rootstocks of 2.2.

Among the twenty plumcots assayed, fifteen showed sharka symptoms (Fig. 3) and were ELISA-DASI and/or RT-PCR positive, so have been classified as susceptible (Tables 7 and 8). Four plumcots behaved as

Table 5
Phenological and floral traits of the assayed plumcots and their genitors. Flowering time (FT), flowering intensity (FI), gynoecium pubescence (PUB), chalice color (CC), ripening date (RD), fruit development period (FDP) and productivity (P). FT, RD and FDP were in Julian days (JD).

	FT (JD)	FI (0-3)	PUB	CC	RD (JD)	FDP (JD)	P (0-5)
Apricot							
Mirlo Anaranjado	51 ± 2	2.00 ± 0.0	+	Pink	133 ± 3	82 ± 1	3.5 ± 0.5
Rojo Pasión	62 ± 1	2.50 ± 0.0	+	Pink	148 ± 3	87 ± 2	4.5 ± 0.5
(1001) 5-26	52 ± 4	1.50 ± 0.5	+	Pink	133 ± 5	81 ± 5	2.1 ± 1.3
Plum							
Red Beaut	49 ± 4	2.00 ± 0.0	–	Green	152 ± 12	103 ± 13	3.5 ± 0.0
Black Splendor	47 ± 4	3.00 ± 0.0	–	Green	168 ± 1	121 ± 4	3.7 ± 0.6
Plumcot							
0412-1	68 ± 13	1.00 ± 0.5	+	Pink	136 ± 11	69 ± 21	0.4 ± 0.4
0412-15	65 ± 3	1.00 ± 0.0	+	Pinkish-green	155 ± 0	92 ± 0	0.5 ± 0.0
0613-4	48 ± 0	0.20 ± 0.0	+	Pinkish-green			
0613-5	58 ± 8	1.30 ± 0.9	+	Pink	165 ± 3	106 ± 10	0.5 ± 0.5
0613-6	66 ± 0	0.50 ± 1.0	+	Pink	162 ± 0	96 ± 0	0.3 ± 0.3
1313-10	59 ± 7	1.13 ± 0.2	+	Pink	143 ± 2	82 ± 3	1.6 ± 1.3
1413-8	60 ± 8	1.40 ± 0.5	+	Pinkish-green	142 ± 13	82 ± 14	1.0 ± 0.0
1114-3	56 ± 2	1.00 ± 1.0	+	Pinkish-green	146 ± 4	90 ± 4	1.0 ± 1.0
1114-7	58 ± 12	0.20 ± 0.0	+	Pink	146 ± 7	88 ± 5	0.5 ± 0.5
1114-28	52 ± 3	1.17 ± 0.3	+	Pink	141 ± 4	90 ± 2	0.4 ± 0.3
1315-17	52 ± 6	0.35 ± 0.2	+	Pinkish-green			
1515-5	59 ± 8	0.50 ± 0.0	+	Pink	155 ± 0	101 ± 0	0.5 ± 0.0

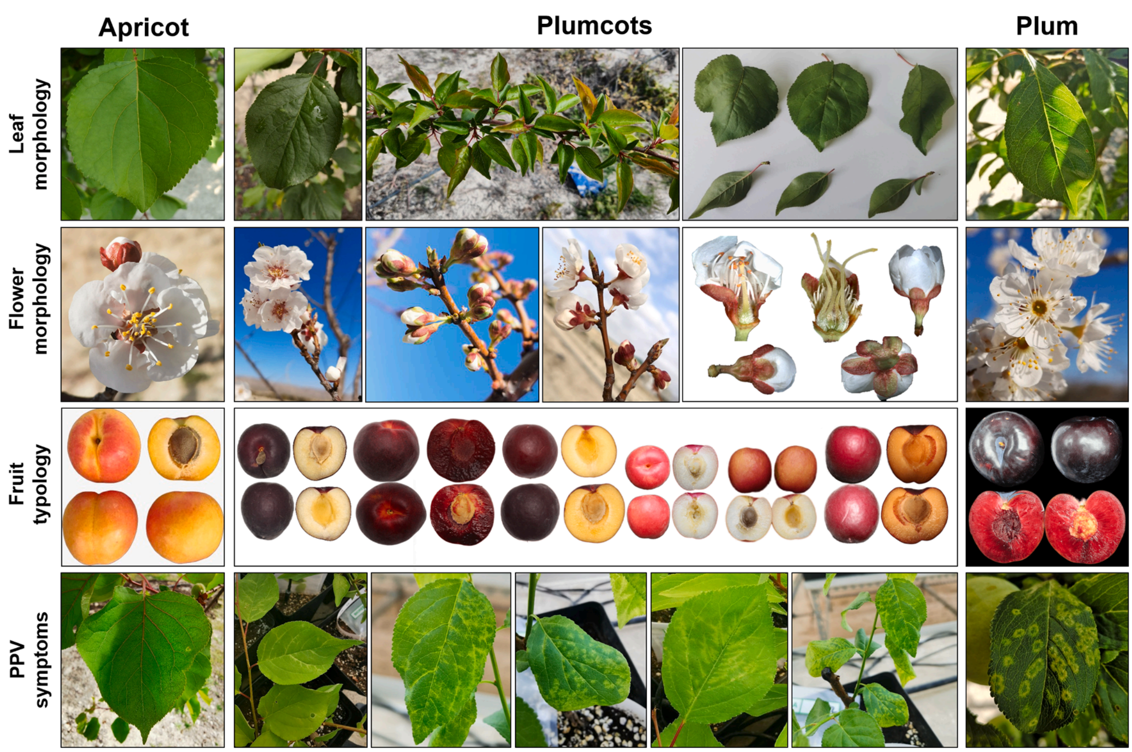

Fig. 3. General morphological characteristics of leaves, flowers and fruits, as well as PPV symptomatology of apricot, Japanese plum and interspecific plumcot hybrids generated in this work. The fruit typology from left to right correspond to 'Mirlo Anaranjado' apricot, plumcots '1114-3', '0613-5', '0613-6', '0412-1','1114-28', '1313-10' and 'Black Splendor' Japanese plum.

tolerant, i.e., they did not display sharka symptoms but were RT-PCR positive in at least one replicate. The only plumcot that showed resistance was the genotype '0718-1', which has not presented symptoms and has not tested ELISA or RT-PCR positive. However, it has not been possible to perform a complete evaluation of '0718-1', since only one plant was evaluated during one of the cycles due to high replicate mortality, thus providing inconclusive results. A total of 60% of the susceptible plumcots showed sharka symptoms, and the overall symptom intensity ranged from 0.8 to 2.5; more than 70% tested RT-PCR positive. Regarding the tolerant plumcots, among 27 observations, only 25% were positive by RT-PCR (Table 8).

The results of the molecular characterization of PPV resistance by markers linked to PPV resistance in apricot are shown in Table 8. The presence of the resistant allele (226 bp) of the PGS1.21 marker matches with the genotypes that show a PMC2-resistant genotype (RR and RS), except for the individual '0119-16', which shows a sensitive genotype (SS) even with the 226 allele. Hybrids '0613-4', '1515-5' and '0915-1' show an allele of 181 bp that none of their parents display, which could be due to a genetic recombination phenomenon/event during meiosis. The data obtained did not match the expected resistance, since most of the plumcots genetically classified as resistant were susceptible to PPV. However, three out of four tolerant plumcots carry the resistant alleles.

Table 6
Physical and fruit quality traits of the assayed plumcots and their genitors. Fruit weight (FW), stone weight (SW), adherence of stone to flesh (AD), skin color, flesh color, firmness (F), soluble solid content (SSC), pH and acidity. Blush color of skin is given in parentheses.

Genotypes	FW (g)	SW (g)	AD	Skin Color		Flesh Color		F (N)	SSC (°Brix)	pH	Acidity (%)		
				Visual	Hue angle	Visual	Hue angle				Citric	Malic	Tartaric
Apricot													
Mirlo Anaranjado	63.9 ± 4.3	2.9 ± 0.1	Free	Light orange (red)	70.0 ± 2.8	Orange	73.4 ± 1.5	38.6 ± 13.0	12.7 ± 0.8	3.55 ± 0.09	1.38 ± 0.10	1.45 ± 0.10	1.62 ± 0.11
Rojo Pasión	63.1 ± 14.9	2.4 ± 0.3	Free	Orange (red)	80.8 ± 3.2	Light orange	75.8 ± 2.9	26.9 ± 13.4	11.6 ± 0.1	3.58 ± 0.01	1.44 ± 0.03	1.51 ± 0.03	1.69 ± 0.04
(1001) 5-26	71.6 ± 8.9	2.3 ± 0.1	Free	Light orange	71.0 ± 2.8	Orange	77.3 ± 2.1	28.4 ± 8.6	16.6 ± 1.7	3.75 ± 0.05	1.33 ± 0.07	1.39 ± 0.07	1.56 ± 0.08
Plum													
Red Beaut	56.5 ± 8.4	1.2 ± 0.3	Cling	Red	19.7 ± 2.9	Yellow	89.0 ± 4.0	27.2 ± 2.7	12.7 ± 1.6	3.30 ± 0.04	1.46 ± 0.22	1.53 ± 0.23	1.71 ± 0.26
Black Splendor	87.6 ± 17.8	1.3 ± 0.2	Cling	Black	8.6 ± 1.9	Red dark	23.5 ± 2.0	41.1 ± 5.0	13.8 ± 0.7	3.17 ± 0.03	1.89 ± 0.13	1.98 ± 0.14	2.21 ± 0.16
Plumcot													
0412-1	44.6 ± 28.8	2.2 ± 2.3	Cling	Red	26.9 ± 1.3	Light yellow	90.8 ± 6.3	33.2 ± 15.0	13.6 ± 1.7	3.22 ± 0.11	1.81 ± 0.58	1.90 ± 0.61	2.13 ± 0.68
0412-15	20.7 ± 2.6	0.7 ± 0.2	Free	Violet	18.2 ± 0.3	Light yellow	81.9 ± 2.3	27.6 ± 5.3	14.1 ± 2.1	3.45 ± 0.00	1.79 ± 0.00	1.87 ± 0.00	2.10 ± 0.00
0613-5	77.1 ± 6.0	2.3 ± 0.3	Free	Red	27.6 ± 4.5	Red dark	32.4 ± 8.9	23.4 ± 8.5	15.6 ± 0.3	3.22 ± 0.07	1.68 ± 0.29	1.76 ± 0.30	1.97 ± 0.33
0613-6	54.8 ± 14.7	1.9 ± 0.4	Free	Violet	17.3 ± 3.4	Yellow	87.9 ± 2.0	33.1 ± 9.6	14.3 ± 0.1	3.07 ± 0.04	2.41 ± 0.35	2.52 ± 0.37	2.82 ± 0.41
1313-10	53.2 ± 5.9	1.8 ± 0.1	Cling	Violet	16.3 ± 1.0	Orange	89.4 ± 1.6	34.8 ± 3.1	14.7 ± 4.0	3.13 ± 0.11	2.46 ± 0.15	2.58 ± 0.15	2.89 ± 0.17
1413-8	50.3 ± 18.1	1.9 ± 1.5	Cling	Violet	16.1 ± 3.4	Orange	71.6 ± 2.8	25.3 ± 9.6	13.9 ± 1.6	3.36 ± 0.04	1.56 ± 0.12	1.60 ± 0.11	1.79 ± 0.12
1114-3	50.59 ± 7.8	2.9 ± 0.7	Semi-cling	Dark violet	19.6 ± 5.5	Light yellow	90.1 ± 4.6	41.1 ± 17.4	11.7 ± 1.5	3.15 ± 0.10	2.27 ± 0.18	2.37 ± 0.19	2.65 ± 0.21
1114-7	73.7 ± 19.3	3.4 ± 1.5	Semi-cling	Dark violet	16.2 ± 2.8	Orange	69.3 ± 0.5	54.4 ± 21.0	11.5 ± 0.7	3.08 ± 0.03	2.21 ± 0.06	2.31 ± 0.06	2.59 ± 0.06
1114-28	57.7 ± 1.7	2.2 ± 0.1	Semi-cling	Orange (red)	57.9 ± 14.4	Light yellow	85.8 ± 2.7	44.3 ± 13.1	14.7 ± 0.9	3.27 ± 0.12	2.17 ± 0.40	2.28 ± 0.42	2.55 ± 0.47
1515-5	47.9 ± 10.6	1.3 ± 0.2	Cling	Yellow	103.4 ± 1.4	Yellow	96.2 ± 5.4	10.4 ± 7.3	10.1 ± 1.7	3.51 ± 0.00	1.29 ± 0.00	1.35 ± 0.00	1.51 ± 0.00

The genotype '0412-15' showed susceptibility, but we were only able to evaluate it in the first cycle, so we do not know if it would have behaved like other genotypes with several observations in the following cycles without symptoms and negative ELISA and RT-PCR results.

4. Discussion

The present study demonstrates the challenge of generating plumcot hybrids. The difficulty in obtaining interspecific hybrids within the genus *Prunus* has been widely reported in many studies (Yoshida et al., 1975; Perez and Moore, 1985; Ahmad et al., 2004; Jun and Chung, 2007; Jun et al., 2009; Yaman and Uzun, 2020). Such crossings results in very few fruits, seeds, and hybrids in relation to the number of pollinated flowers due to the genetic distance between *Prunus* species.

Hybridization was only successful in this study when Japanese plum was used as the female parent. These findings are consistent with those reported in previous works (Yoshida et al., 1975; Singh et al., 1997; Claverie et al., 2004; Jun and Chung, 2007; Szymajda et al., 2015). These studies have noted that hybridization success rates are influenced by the direction of the cross and that crosses involving *P. salicina* as the maternal parent generally prove more fruitful than those involving *P. armeniaca*. The reason for the higher fruit production in the *P. salicina* × *P. armeniaca* crosses compared to *P. armeniaca* × *P. salicina* may be due to the ability of apricot pollen tubes to grow faster than those of *P. salicina*, as well as the shorter pistil of the plum, providing a shorter path for apricot pollen to reach the ovary (Perez and Moore, 1985). Additionally, the Japanese plum's compatibility as the female parent has also been confirmed in crosses with peach (Morimoto et al., 2019). Furthermore, Szymajda et al. (2022) observed that the crosses made between Japanese plum × apricot were the least successful when compared to Japanese plum × myrobalan plum and myrobalan plum × apricot.

In addition, the loss of a significant number of hybrid seeds obtained from controlled pollinations has also been reported by Jun et al. (2009) and Szymajda et al. (2022), where around 65% of the fruits obtained in the pollinations were lost during the processes of stratification, germination and acclimatization. This loss may be due to genetic disorders resulting from the combination of two incompatible genomes in the hybrid seedling, as suggested by Szymajda et al. (2022).

The interspecific nature of plumcot hybrids has been successfully verified using molecular markers, as demonstrated in previous studies (Byrne and Littleton, 1989; Ahmad et al., 2004; Jun et al., 2009; Szymajda et al., 2022). However, most of the genotypes considered interspecific, because they came from seeds of fruits resulting from controlled crosses, were shown not to be interspecific based on validation using SSR markers (33 interspecific plumcots from 612 genotypes verified). This could be due to escapes from uncontrolled pollination, as well as possible cleistogamy in the case of crosses with a self-compatible female apricot parent. To date, there have been very few studies that have confirmed the interspecific nature of plumcot hybrids by SSR. As a result, many hybrids considered as plumcots may actually be Japanese plum-type hybrids (Ahmad et al., 2004; Okie, 2005; Jun et al., 2009; Guerrero et al., 2022).

The SSR polymorphism results highlight the high transferability of the SSR markers used in this study among *Prunus* species, as previously observed in other works (García-Gómez et al., 2018; Guerrero et al., 2022). The expected and observed heterozygosity values are similar to those observed in other studies of putative advanced selections of interspecific plumcot-type hybrids (He=0.76; Ho=0.70) (Guerrero et al., 2022) and different species of plum such as *P. salicina* (He=0.67-0.68; Ho=0.70-0.65) (Abdallah et al., 2019; Guerrero et al., 2021); *P. domestica* (He=0.69; Ho=0.73); and *P. insititia* (He=0.70;

Table 7
PPV phenotyping of apricots, Japanese plums and plumcots to the type D isolate 3.30RB/GF-IVIA of plum pox virus, grafted onto infected 'Adesoto' plum rootstocks, during three vegetative cycles. N: Number of replicates correctly evaluated; Symp: Number of replicates showing sharka symptoms, in brackets (), mean intensity of symptoms scored from 0 to 5; ELISA: number of replicates positive by ELISA-DASI in brackets (), mean optical density recorded at 405 nm after 60 min; RT+: number of positive RT-PCRs.

		Cycle 1				Cycle 2				Cycle 3			
		Adesoto	Genotype			Adesoto	Genotype			Adesoto	Genotype		
Genotype	N	Symp	Symp	ELISA	RT+	Symp.	Symp.	ELISA	RT+	Symp.	Symp.	ELISA	RT+
Apricot													
Mirlo Anaranjado	4	4 (2.0)	0 (0.0)	0 (0.079)	0	4 (2.2)	0 (0.0)	0 (0.068)	0	4 (2.2)	0 (0.0)	0 (0.091)	0
Rojo Pasión	5	5 (2.2)	0 (0.0)	0 (0.106)	0	5 (2.6)	0 (0.0)	0 (0.065)	0	5 (1.8)	0 (0.0)	0 (0.085)	0
(1001) 5-26	3	3 (3.3)	0 (0.0)	0 (0.053)	0	3 (2.7)	0 (0.0)	0 (0.107)	0	3 (1.7)	0 (0.0)	0 (0.063)	0
Plum													
Red Beaut	6	6 (3.0)	6 (2.7)	6 (1.540)	6	6 (2.7)	6 (2.8)	6 (1.060)	6	5 (2.2)	5 (2.1)	5 (1.370)	5
Black Splendor	4	4 (1.5)	4 (1.5)	2 (0.750)	4	1 (1.0)	0 (0.0)	0 (0.067)	0	3 (1.7)	1 (3.0)	1 (1.386)	2
0112-11	3	3 (2.3)	2 (2.0)	2 (0.357)	2	1 (2.0)	0 (0.0)	0 (0.073)	0	2 (2.0)	0 (0.0)	1 (0.211)	1
0614-5	4	2 (1.5)	4 (1.7)	4 (0.982)	4	1 (2.0)	1 (1.0)	1 (0.890)	1	1 (2.0)	0 (0.0)	0 (0.112)	1
0716-5	4	4 (1.5)	0 (0.0)	0 (0.149)	2	3 (2.7)	0 (0.0)	0 (0.062)	0	4 (1.0)	0 (0.0)	0 (0.078)	0
Plumcot													
0412-1	3	2 (2.0)	3 (2.3)	3 (0.671)	3	1 (1.0)	1 (2.0)	1 (0.134)	1	2 (1.5)	1 (3.0)	1 (1.981)	2
0412-15	3	1 (3.0)	3 (2.0)	3 (0.333)	3	–	–	–	–	–	–	–	–
0613-2	2	1 (1.0)	0 (0.0)	1 (0.161)	1	2 (4.0)	2 (1.5)	2 (1.455)	2	1 (3.0)	1 (1.0)	1 (0.106)	1
0613-5	4	2 (1.5)	4 (1.7)	4 (0.982)	4	1 (2.0)	1 (1.0)	1 (0.890)	1	1 (2.0)	0 (0.0)	0 (0.112)	1
0613-6	3	2 (2.0)	3 (1.7)	3 (0.667)	3	2 (2.0)	0 (0.0)	0 (0.067)	0	2 (3.0)	0 (0.0)	1 (0.214)	1
1313-10	5	4 (2.5)	4 (3.5)	5 (1.403)	5	3 (1.3)	2 (2.5)	4 (0.694)	4	3 (1.6)	3 (1.6)	3 (1.934)	3
1313-24	4	4 (2.0)	3 (2.3)	4 (1.737)	4	3 (1.3)	2 (2.0)	2 (1.124)	2	2 (2.0)	1 (1.0)	1 (0.744)	2
1413-8	2	2 (2.0)	0 (0.0)	1 (1.812)	1	3 (2.5)	1 (1.0)	1 (2.738)	1	2 (1.0)	0 (0.0)	0 (0.079)	0
1114-3	4	4 (2.3)	0 (0.0)	2 (0.848)	2	3 (2.0)	0 (0.0)	0 (0.071)	0	2 (1.0)	0 (0.0)	1 (1.832)	1
1114-7	5	5 (1.7)	5 (2.2)	4 (0.306)	5	3 (1.3)	0 (0.0)	0 (0.071)	0	3 (2.0)	0 (0.0)	0 (0.081)	0
1114-28	5	4 (1.8)	4 (1.5)	4 (1.209)	5	3 (1.7)	0 (0.0)	0 (0.072)	0	1 (1.0)	0 (0.0)	0 (0.084)	0
1315-17	3	2 (1.5)	0 (0.0)	0 (0.073)	0	2 (3.0)	0 (0.0)	0 (0.080)	1	3 (1.3)	0 (0.0)	0 (0.078)	0
1515-5	6	5 (1.8)	6 (1.8)	6 (0.957)	6	3 (3.3)	3 (1.7)	2 (1.135)	2	3 (3.3)	2 (1.5)	2 (0.752)	2
1515-26	5	3 (2.3)	4 (2.2)	4 (0.777)	4	2 (1.5)	0 (0.0)	0 (0.075)	2	–	–	–	–
0915-1	5	5 (2.0)	3 (1.70)	4 (0.507)	4	4 (1.7)	1 (1.0)	1 (0.206)	3	3 (2.0)	0 (0.0)	1 (1.005)	2
0718-1	1	1 (2.0)	0 (0.0)	0 (0.128)	0	–	–	–	–	–	–	–	–
0119-5	1	1 (4.0)	0 (0.0)	0 (0.117)	1	1 (4.0)	0 (0.0)	0 (0.103)	0	2 (4.0)	0 (0.0)	0 (0.084)	0
0119-6	3	3 (2.7)	0 (0.0)	0 (0.121)	1	2 (2.0)	0 (0.0)	0 (0.104)	1	2 (1.5)	0 (0.0)	0 (0.096)	0
0119-10	5	5 (3.0)	5 (1.2)	3 (3.095)	5	3 (3.0)	1 (1.0)	1 (0.251)	1	2 (2.5)	0 (0.0)	0 (0.087)	0
0119-16	4	4 (2.5)	2 (1.5)	1 (0.230)	3	2 (3.5)	0 (0)	0 (0.100)	0	3 (2.3)	0 (0.0)	0 (0.092)	0

Ho=0.74) (Abdallah et al., 2019).

The genetic relationships obtained in the present study show higher similarity between plumcots and apricots than with plum, placing plumcots in a distinct and differentiated cluster. This contrasts with previous results: Ahmad et al. (2004) positioned the only evaluated plumcot between the clusters generated by apricot and plum. On the other hand, Guerrero et al. (2022) found significant differentiation between apricots and the rest of the evaluated genotypes, placing the putative interspecific plumcot hybrids indiscriminately with plums, pluots and other types of diploid plums (*P. cerasifera* and *P. simonii*). This suggests that the hybrid accessions evaluated are probably not plumcot-type hybrids, but rather resulted from repeated backcrossing with plum.

S-alleles were successfully genotyped in all hybrids and their parents using consensus primers, which showed good transferability between species (Tao et al., 1999; Yamane et al., 2001; Sonneveld et al., 2006). All the interspecific hybrids exhibited one allele derived from the Japanese plum and another from the apricot, thus confirming that all the studied interspecific hybrids are true plumcots. These genotypes do not belong to the incompatibility groups described to date due to the combination of compatibility alleles from two different species (Guerra et al., 2012; Herrera et al., 2018). Guerrero et al. (2021) genotyped the S-alleles of putative plumcot hybrids, and the identified alleles only corresponded to S genotypes previously described in Japanese plum (Beppu et al., 2002; Halász et al., 2010; Guerra et al., 2012) and not in apricot (Herrera et al., 2018).

Furthermore, most hybrids have segregated the Sc allele from apricot, which confers apricot self-compatibility (Vilanova et al., 2006; Halász et al., 2007), so they should a priori be self-compatible. However, pollen viability studies have shown androsterility in all the plumcots that flourished. This is consistent with the results obtained in various studies in which the majority of plumcots do not produce pollen or their pollen is not viable (Okie, 2005; Jun et al., 2009, 2011; Nam et al., 2016), suggesting that interspecific hybridization causes androsterility in the offspring, which could explain why most plumcots were not very productive.

The phenotypic characterization of the plumcot hybrids also revealed their interspecific nature. They showed characteristics acquired from apricot such as leaf morphology and pistil and fruit pubescence (present in apricot, absent in plum). The presence of pubescence on the ovaries and fruits of plumcot hybrids has been reported in numerous studies (Ledbetter et al., 1994; Okie, 2005; Jun et al., 2009; Zhivondov and Uzundzhalieva, 2012; Nam et al., 2016). Considered a dominant trait in interspecific crosses between Japanese plums and apricots (Jun et al., 2009), this could serve as a physical characteristic to distinguish a true plumcot from other types of hybrids. Hakoda et al. (1998) also obtained all of their offspring from interspecific hybrids between Japanese apricot (*P. mume*) and Japanese plum with fruit that had pubescence. Our results confirm that the presence of pubescence on ovaries and fruit skin is an unequivocal characteristic for verifying the "real" plumcots.

Transmission of horticultural traits from apricot and Japanese plum to plumcot has been studied since the 1980s. The first studies evaluating different plumcot progenies clearly showed this transmission (Ledbetter et al., 1994).

Ledbetter et al. (1994) also studied full flowering, vegetative bud break and fruit harvest dates in addition to pollen viability and fruit weight in the obtained plumcot descendants, showing that plumcot ripening dates and fruit weights were influenced by the parental apricot. Once again, this suggests the dominance of apricot genes in confirmed plumcots. The freestone trait is considered dominant in *P. domestica, P. persica, P. armeniaca* and recessive in *P. salicina* (Okie and Weinberger,

Table 8
Overall behavior against PPV-D of the apricots, plums and plumcots, according to the number of observations correctly phenotyped (N); number of observations displaying sharka symptoms, in () mean intensity of symptoms; percentages of infected observations, ELISA and RT-PCR positive, after three phenotyping cycles. Molecular characterization by two markers (PGS1.21 and PMC2) linked to PPV resistance (marked in italics). Plumcots only evaluated in the first cycle are marker with an asterisk (*).

Genotype	N	Symptoms	Percentages Infected	ELISA +	RT-PCR +	Markers PGS1.21	PMC2
Susceptible							
Red Beaut	17	17 (2.5)	100	100	100	168/170	SS
0412-15*	3	3 (2.0)	100	100	100	168/*226*	*R*S
1313-10	12	9 (2.5)	75	100	100	168/207	SS
0613-2	4	3 (0.8)	75	100	100	168/207	SS
0614-5	6	5 (0.9)	83	83	100	168/170	SS
0412-1	6	5 (2.4)	83	83	100	168/207	SS
0613-5	6	5 (0.9)	83	83	100	168/170	SS
1515-26	6	4 (1.1)	67	67	100	168/207	SS
1313-24	9	6 (1.8)	67	78	89	168/207	SS
1515-5	12	11 (1.7)	92	92	83	168/181	SS
0915-1	12	4 (0.9)	33	50	75	168/181	SS
Black Splendor	8	5 (1.8)	63	38	75	168/170	SS
0119-10	10	6 (1.1)	60	40	60	168/*226*	*R*S
0613-6	7	3 (1.4)	43	57	57	168/207	SS
1114-28	9	4 (1.5)	44	44	56	168/*226*	*R*S
0112-11	6	2 (2.0)	33	50	50	168/170	SS
1114-7	11	5 (2.2)	45	36	45	168/*226*	*R*S
0119-16	9	2 (1.5)	22	11	33	170/*226*	SS
1413-8	7	1 (1.0)	14	14	28	170/*226*	*R*S
Tolerant							
1114-3	9	0 (0.0)	0	33	33	168/207	SS
0119-6	7	0 (0.0)	0	0	28	168/*226*	*R*S
0119-5	4	0 (0.0)	0	0	25	170/*226*	*R*S
0716-5	11	0 (0.0)	0	0	18	166/170	SS
1315-17	7	0 (0.0)	0	0	14	168/*226*	*R*S
Resistant							
Mirlo Anaranjado	12	0 (0.0)	0	0	0	207/*226*	*R*S
Rojo Pasión	15	0 (0.0)	0	0	0	207/*226*	*R*S
(1001) 5-26	9	0 (0.0)	0	0	0	*226*/*226*	*RR*
0718-1*	1	0 (0.0)	0	0	0	170/*226*	*R*S

1996; Scorza and Sherman, 1996; Okie and Hancock, 2008). The results obtained regarding stone adherence to the flesh in plumcots are consistent with those obtained previously, which have suggested that clingstone is dominant in interspecific crosses between Japanese plum and apricot (Jun et al., 2009). Higher levels of acidity in plumcots compared to apricot and Japanese plum have also been reported by Bae et al. (2014) and Jun et al. (2009).

Regarding fruit quality, aromatic profiles from plumcot accessions more closely resemble apricot than plum profiles (Gómez et al., 1993; Gómez and Ledbetter, 1993). Gómez and Ledbetter (1997) found important differences in the volatile constituent profiles, and that the characteristic compounds of apricot aroma were much higher in apricot than plumcot.

Regarding PPV phenotyping in plumcots, there are a very few studies that deal with sharka resistance. One precedent was in 2009, when Karayiannis and Ledbetter found that all plumcots were highly susceptible to PPV (Karayiannis and Ledbetter, 2009). Rubio et al. (2011b) obtained similar results, finding that the French selection 'J300' and the American 'Flavor Supreme' were susceptible to sharka. In a later work, Zhivondov (2012) obtained a P. domestica × P arm*eniaca* hybrid called 'Standesto' with tolerance to PPV. Other authors have reported the possibility of obtaining interspecific hybrids between *P. domestica* × *P, armeniaca* with PPV resistance through the hypersensitivity response (Neumüller et al., 2017), although these authors did not discuss plumcots. In any case, the present work seems to be the first deep approach to sharka resistance in plumcots.

The level of symptom expression shown by susceptible plumcots was similar to that observed in other works with plums and susceptible apricots (Martínez-Gómez and Dicenta, 2000; Rubio et al., 2011b).

The molecular characterization of resistance to PPV, using the markers PGS1.21 and ParPMC2 (Soriano et al., 2012; Polo-Oltra et al., 2020), has not been conclusive. However, the presence of the resistance allele could be the result of a decrease in susceptibility, with an important group of susceptible plumcots turning tolerant/resistant after the first phenotyping cycle. This behavior has already been reported by several authors (Karayiannis et al., 2008; Rubio et al., 2012) and has been linked to a mechanism of gene silencing of the virus.

5. Conclusions

Sharka disease is still a threat for most *Prunus* species, despite the fact that it was eliminated as a quarantine disease in the European Union. In our opinion, plumcots would be a good opportunity for markets. The high-quality fruits would satisfy consumers, and even more importantly, growers stand to benefit substantially from selected plumcots that are both self-fertile and sharka-resistant and can be used as future breeding lines. There are still challenges to solve in the inter-specific crossing, like androsterility or full resistance to PPV, as well as in determining the real characteristics of plumcots, thus avoiding the common mislabeling of 'plumcots' that in reality are just plum-type fruits.

CRediT authorship contribution statement

María Nicolás-Almansa: Conceptualization, Methodology, Writing – review & editing, Writing – original draft, Data curation, Visualization, Investigation, Supervision, Resources, Methodology. **David Ruiz:** Supervision, Conceptualization, Writing – review & editing. **Juan Alfonso Salazar:** Resources. **Alfonso Guevara:** Resources. **José Cos:** Writing – review & editing. **Pedro Martínez-Gómez:** Methodology, Writing – review & editing. **Manuel Rubio:** Conceptualization, Methodology, Writing – review & editing, Writing – original draft, Data curation, Visualization, Investigation, Supervision, Methodology.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Maria Nicolas Almansa reports financial support was provided by Spain Ministry of Science and Innovation.

Data availability

Data will be made available on request.

Acknowledgments and Funding

We want to acknowledge to the CEBAS-CSIC apricot breeding program led by Dr. Ruiz, Dr. Salazar and Dr. Egea, and the CEBAS-CSIC/IMIDA Japanese plum breeding program led by Dr. Ruiz and Dr. Guevara, for the availability of plant material for this work. María Nicolás-Almansa was supported by the Ministry of Economy, Industry and Competitiveness (MINECO, Spain) within a PhD programme grant (FPU16/03896).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2023.112131.

References

Abdallah, D., Baraket, G., Perez, V., BenMustapha, S., Salh i-Hannachi, A., Hormaza, J.I., 2019. Analysis of self-incompatibility and genetic diversity in diploid and hexaploid plum genotypes. Front. Plant Sci. 10, 896. https://doi.org/10.3389/fpls.2019.00896.

Ahmad, R., Potter, D., Southwick, S.M., 2004. Identification and characterization of plum and pluot cultivars by microsatellite markers. J. Hortic. Sci. Biotechnol. 79, 164–169. https://doi.org/10.1080/14620316.2004.11511743.

Bae, H., Yun, S.K., Jun, J.H., Yoon, I.K., Nam, E.Y., Kwon, J.H., 2014. Assessment of organic acid and sugar composition in apricot, plumcot, plum, and peach during fruit development. J. Appl. Bot. Food Qual. 87, 24–29. https://doi.org/10.5073/JABFQ.2014.087.004.

Beppu, K., Yamane, H., Yaegaki, H., Yamaguchi, M., Kataoka, I., Tao, R., 2002. Diversity of S-RNase genes and S-haplotypes in Japanese plum (Prunus salicina Lindl.). J. Hortic. Sci. Biotechnol. 77, 658–664. https://doi.org/10.1080/14620316.2002.11511553.

Boonprakob, U., Byrne, D.H., Graham, C.J., Okie, W.R., Beckman, T., Smith, B.R, 2001. Genetic relationships among cultivated diploid plums and their progenitors as determined by RAPD markers. J. Am. Soc. Hortic. Sci. 126, 451–461. https://doi.org/10.21273/JASHS.126.4.451.

Botstein, D., White, R.L., Skolnick, M., Davis, R.W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32, 314–331.

Brantley, W., 2004. About Pluots. Gastronomica 4, 84–89. https://doi.org/10.1525/gfc.2004.4.3.84.

Brown, G.S., Walker, T.D., 1990. Indicators of maturity in apricots using biplot multivariate analysis. J. Sci. Food Agric. 53, 321–331.

Burbank, L., 1914. Luther Burbank: His methods and discoveries and their practical application. Luther Burbank Press, New York, NY, USA. https://doi.org/10.5962/bhl.title.54007.

Byrne, D.H., Littleton, T.G., 1989. Interspecific hybrid verification of plum x apricot hybrids *via* isozyme analyses. HortScience 24, 132–134.

Cambra, M., Asensio, M., Gorris, M.T., Pérez, E., Camarasa, E., García, J.A., Moya, J.J., López-Abella, D., Vela, C., Sanz, A., 1994. Detection of plum pox potyvirus using monoclonal antibodies to structural and non-structural proteins. EPPO Bull. 24, 569–577. https://doi.org/10.1111/j.1365-2338.1994.tb01070.x.

Cipriani, G., Lot, G., Huang, W.G., Marrazzo, M.T., Peterlunger, E., Testolin, R., 1999. AC/GT and AG/CT microsatellite repeats in peach [Prunus persica (L) Batsch]: isolation, characterisation and cross-species amplification in Prunus. Theor. Appl. Genet. 99, 65–72. https://doi.org/10.1007/s001220051209.

Claverie, M., Bosselut, N., Lecouls, A.C., Voisin, R., Lafargue, B., Poizat, C., Kleinhentz, M., Laigret, F., Dirlewanger, E., Esmenjaud, D., 2004. Location of independent root-knot nematode resistance genes in plum and peach. Theor. Appl. Genet. 108, 765–773. https://doi.org/10.1007/s00122-003-1463-1.

Dirlewanger, E., Cosson, P., Tavaud, M., Aranzana, M.J., Poizat, C., Zanetto, A., Arús, P., Laigret, F., 2002. Development of microsatellite markers in peach [Prunus persica (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (Prunus avium L.). Theor. Appl. Genet. 105, 127–138. https://doi.org/10.1007/s00122-002-0867-7.

Doyle, J.J., Doyle, J.L., 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

FAO, 2022. Food and agriculture organization. Agricultural Statistics Database. FAO. https://www.fao.org/faostat/es/#home.

Faust, M., Surányi, D., Janick, J., 1998. Origin and dissemination of plums. Hortic. Rev. (Am. Soc. Hortic. Sci) 23, 179–231. https://doi.org/10.1002/9780470650752.ch4. John Wiley & Sons, Ltd.

García-Gómez, B., Razi, M., Salazar, J.A., Prudencio, A.S., Ruiz, D., Dondini, L., Martínez-Gómez, P., 2018. Comparative analysis of SSR markers developed in exon, intron, and intergenic regions and distributed in regions controlling fruit quality traits in prunus species: genetic diversity and association studies. Plant Mol. Biol. Rep. 36, 23–35. https://doi.org/10.1007/s11105-017-1058-7.

Gómez, E., Ledbetter, C., 1993. Transmission of biochemical flavor constituents from apricot and plum to their interspecific hybrid. Plant Breed. 111, 236–241. https://doi.org/10.1111/j.1439-0523.1993.tb00634.x.

Gómez, E., Ledbetter, C.A., 1997. Development of volatile compounds during fruit maturation: characterization of apricot and plum x apricot hybrids. J. Sci. Food Agric. 74, 541–546. https://doi.org/10.1002/(SICI)1097-0010(199708)74:4<541::AID−JSFA851>3.0.CO;2-D.

Gómez, E., Ledbetter, C.A., Hartsell, P.L., 1993. Volatile compounds in apricot, plum, and their interspecific hybrids. J. Agric. Food Chem. 41, 1669–1676. https://doi.org/10.1021/jf00034a029.

Guerra, M.E., López-Corrales, M., Wünsch, A., 2012. Improved S-genotyping and new incompatibility groups in Japanese plum. Euphytica 186, 445–452. https://doi.org/10.1007/s10681-012-0636-x.

Guerrero, B.I., Guerra, M.E., Herrera, S., Irisarri, P., Pina, A., Rodrigo, J., 2021. Genetic diversity and population structure of Japanese plum-type (Hybrids of p. salicina) accessions assessed by SSR markers. Agronomy 11, 1748. https://doi.org/10.3390/agronomy11091748.

Guerrero, B.I., Guerra, M.E., Rodrigo, J., 2022. Simple Sequence Repeat (SSR)-based genetic diversity in interspecific plumcot-type (Prunus salicina× Prunus armeniaca) hybrids. Plants 11, 1241. https://doi.org/10.3390/plants11091241.

Hakoda, N., Toyoda, R., Tabuchi, T., Ogiwara, I., Ishikawa, S., Shimura, I., 1998. Morphological characteristics of the interspecific hybrids between Japanese apricot (Prunus mume) and Plum (P. salicina). J. Jpn. Soc. Hortic. Sci. 67, 708–714.

Halász, J., Pedryc, A., Ercisli, S., Yilmaz, K.U., Hegedüs, A., 2010. S-genotyping supports the genetic relationships between Turkish and Hungarian apricot germplasm. J. Am. Soc. Hortic. Sci. 135, 410–417. https://doi.org/10.21273/JASHS.135.5.410.

Halász, J., Pedryc, A., Hegedus, A., 2007. Origin and dissemination of the pollen-part mutated SC haplotype which confers self-compatibility in apricot (Prunus armeniaca). New Phytol. 176, 792–803. https://doi.org/10.1111/j.1469-8137.2007.02220.x.

Hedrick, U.P., 1911. The Plums of New York. J.B. Lyon Company, State Printers, Albany, NY, USA.

Herrera, S., Lora, J., Hormaza, J.I., Herrero, M., Rodrigo, J., 2018. Optimizing production in the new generation of apricot cultivars: self-incompatibility, S-RNase allele identification, and incompatibility group assignment. Front. Plant Sci. 9, 1–12. https://doi.org/10.3389/fpls.2018.00527.

Howard, W.L., 1945. Luther Burbank's plant contributions. Calif. Agr. Exp. Sta. Bul. 691, 30–32.

Jun, J.H., Chung, K.H., 2007. Interspecific cross compatibility among plum, apricot and plumcot. Korean J. Hortic. Sci. Technol. 25, 217–222.

Jun, J.H., KwonJ, H., Chung, K.H, 2009. Morphological characteristics of interspecific hybrids between Japanese Plum (Prunus salicina Lindl.) cv. soldam and apricot (P. armeniaca L.) cv. harcot. Hortic. Sci. Technol. 27, 269–274.

Jun, J.H., Kwon, J.H., Chung, K.H., 2011. "Harmony" plumcot. J. Am. Pomol. Soc. 65, 47–51. https://doi.org/10.21273/HORTSCI10741-16.

Karayiannis, I., Ledbetter, C., 2009. Susceptibility of certain apricot and plumcot cultivars to plum pox virus infection. Acta Hortic. 825, 153–156. https://doi.org/10.17660/ActaHortic.2009.825.21.

Karayiannis, I., Thomidis, T., Tsaftaris, A., 2008. Inheritance of resistance to Plum pox virus in apricot (Prunus armeniaca L.). Tree Genet. Genomes 4, 143–148. https://doi.org/10.1007/s11295-007-0095-z.

Karp, D., 2015. Luther Burbank ' s Plums. HortScience 50, 189–194. https://doi.org/10.21273/HORTSCI.50.2.189.

Klabunde, G.H.F., Dalbó, M.A., Nodari, R.O., 2014. DNA fingerprinting of Japanese plum (Prunus salicina) cultivars based on microsatellite markers. Crop Breed. Appl. Biotechnol. 14, 139–145. https://doi.org/10.1590/1984-70332014v14n3a21.

Kwon, J.H., Choi, H.J., Nam, E.Y., Yun, S.K., Kim, S.J., Lee, J.S., 2020. Postharvest characteristics of plumcot cultivars bred in Republic of Korea. Acta Hortic. 1290, 191–196. https://doi.org/10.17660/ActaHortic.2020.1290.33.

Ledbetter, C.A., Peterson, S.J., Burgos, L., 1994. Variability of horticultural characteristics among plumcot progenies. J. Genet. Breed. 48, 117–124.

Liu, W., Chen, X., Liu, G., Liang, Q., He, T., Feng, J., 2007. Interspecific hybridization of Prunus persica with P. armeniaca and P. salicina using embryo rescue. Plant Cell Tissue Organ Cult. 88, 289–299. https://doi.org/10.1007/s11240-007-9201-z.

Martínez-Gómez, P., Dicenta, F., 2000. Evaluation of resistance of apricot cultivars to a Spanish isolate of plum pox potyvirus (PPV). Plant Breed. 119, 179–181. https://doi.org/10.1046/j.1439-0523.2000.00457.x.

Meier, U., Graf, H., Hack, H., Hess, M., Kennel, W., Klose, R., Mappes, D., Seipp, D., Stauss, R., Streif, J., Boom, T.V.D., 1994. Phanologische Entwicklungsstadien des Kernobstes (Malus domestica Borkh. und Pyrus communis L.). Nachrichtenbl Deut Pflanzenschutzd 46, 141–153.

Merkouropoulos, G., Ganopoulos, I., Tsaftaris, A., Papadopoulos, I., Drogoudi, P., 2017. Combination of high resolution melting (HRM) analysis and SSR molecular markers speeds up plum genotyping: case study genotyping the Greek plum GeneBank

collection. Plant Genet. Resour. Character. Util. 15, 366–375. https://doi.org/10.1017/S1479262116000022.
Mnejja, M., Garcia-Mas, J., Howad, W., Badenes, M.L., Arús, P., 2004. Simple-sequence repeat (SSR) markers of Japanese plum (Prunus salicina Lindl.) are highly polymorphic and transferable to peach and almond. Mol. Ecol. Notes 4, 163–166. https://doi.org/10.1111/j.1471-8286.2004.00603.x.
Morimoto, T., Kitamura, Y., Numaguchi, K., Akagi, T., Tao, R., 2019. Characterization of post-mating interspecific cross-compatibility in Prunus (Rosaceae). Sci. Hortic. 246, 693–699. https://doi.org/10.1016/j.scienta.2018.11.045.
Nam, E.Y., Jun, J.H., Chung, K.H., Kwon, J.H., Yun, S.K., Yun, I.K., Cho, K.H., 2016. Tiffany' red-fleshed plumcot. HortScience 51, 1304–1307. https://doi.org/10.21273/HORTSCI10741-16.
Neumüller, M., Dittrich, F., Hartmann, W., Hadersdorfer, J., Treutter, D., 2017. First report on the generation of Prunus domestica × P. armeniaca interspecific hybrids with hypersensitivity resistance to the Plum pox virus. Acta Hortic. 1172, 15–20. https://doi.org/10.17660/ActaHortic.2017.1172.3.
Niu, J.L., Liu, M.X., Peng, Q.M., 2015. Analysis of main index components and cluster of plumcot "Weihou" during different harvest periods. Xingjiang Agric. Sci. 52, 33–36.
Okie, W.R., 2006. Introgression of Prunus species in plum. New York Fruit. Q. 14, 29–37.
Okie, W.R., 2005. Spring Satin'Plumcot. J. Am. Pomol. Soc. 59, 119.
Okie, W.R., Hancock, J.F., 2008. Plums. In: Hancock, J.F. (Ed.), Temperate Fruit Crop Breeding. Springer, pp. 337–357. https://doi.org/10.1007/978-1-4020-6907-9_11.
Okie, W.R., Ramming, D.W., 1999. Plum breeding worldwide. Horttechnology 9, 162–176. https://doi.org/10.21273/HORTTECH.9.2.162.
Okie, W.R., Thompson, J.M., Reilly, C.G., Meredith, F.I., Robertson, J.A., Lyon, B.G., 1992. Segundo, Byrongold and Rubysweet plums and BY69- 1637P plumcot: fruits for the Southeastern United Sates. Fruit Var. J. 46, 102–107.
Okie, W.R., Weinberger, J.H., Janick, J., Moore, J.N., 1996. Plum. Fruit Breeding, Tree Trop. Fruits. John Wiley & Sons, Ltd, New York, pp. 559–609.
Peakall, R., Smouse, P.E., 2012. GenALEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28, 2537–2539.
Perez-Jimenez, M., Guevara-Gazquez, A., Carrillo-Navarro, A., Cos-Terrer, J., 2021. How carbon source and seedcoat influence the *in vitro* culture of peach (prunus persica l. batsch) immature seeds. HortScience 56, 136–137. https://doi.org/10.21273/HORTSCI15502-20.
Perez, S., Moore, J.N., 1985. Prezygotic endogenous barriers to interspecific hybridization in Prunus. J. Am. Soc. Hortic. Sci. 110, 267–273. https://doi.org/10.21273/JASHS.110.2.267.
Polo-Oltra, A., Romero, C., López, I., Badenes, M.L., Zuriaga, E., 2020. Cost-effective and time-efficient molecular assisted selection for PPV resistance in apricot based on ParPMC2 allele-specific PCR. Agronomy 10, 1292. https://doi.org/10.3390/agronomy10091292.
Qiao, Y.S., Fang, J.G., Cong, Y., Zhou, J., Zhang, Z., 2007. Analysis of genetic diversity of Japanese plum cultivars based on RAPD, ISSR and SSR markers. Acta Hortic. 763, 177–184. https://doi.org/10.17660/ActaHortic.2007.763.23.
Roeding G.C. 1908. New products of the trees: a treatise of Burbank's late introductions. Roeding, G.C. Fancher Creek Nurseries. Fresno, CA, USA.
Rubio, M., Dicenta, F., Martínez-Gómez, P., 2019. Plum Pox virus: detection and management. Integrated Management of Diseases and Insect Pests of Tree Fruit. Burleigh Dodds Science Publishing (ed.), pp. 179–198.
Rubio, M., Dicenta, F., Martínez-Gómez, P., Egea, J., Ruiz, D., 2011a. Evaluation of sharka resistance in new cultivars and selections of the apricot breeding programme at CEBAS-CSIC in Murcia (Spain). Acta Hortic. 899, 95–102. https://doi.org/10.17660/ActaHortic.2011.899.11.
Rubio, M., García-Ibarra, A., Dicenta, F., Martínez-Gómez, P., 2011b. Plum pox virus (sharka) sensitivity in Prunus salicina and Prunus cerasifera cultivars against a Dideron-type isolate. Plant Breed. 130, 283–286. https://doi.org/10.1111/j.1439-0523.2010.01813.x.
Rubio, M., Martínez-Gómez, P., García-Brunton, J., Pascal, T., García-Ibarra, A., Dicenta, F., 2012. Sensitivity of peach cultivars against a Dideron isolate of Plum pox virus. Sci. Hortic. 144, 81–86. https://doi.org/10.1016/j.scienta.2012.06.038.
Rubio, M., Martínez-Gómez, P., García, J.A., Dicenta, F., 2013. Interspecific transfer of resistance to Plum pox virus from almond to peach by grafting. Ann. Appl. Biol. 163, 466–474. https://doi.org/10.1111/aab.12069.
Rubio, M., Martínez-Gómez, P., Pinochet, J., Dicenta, F., 2005. Evaluation of resistance to sharka (Plum pox virus) of several Prunus rootstocks. Plant Breed. 124, 67–70. https://doi.org/10.1111/j.1439-0523.2004.01068.x.
Rubio, M., Ruiz, D., Egea, J., Martínez-Gómez, P., Dicenta, F., 2014. Opportunities of marker-assisted selection for Plum pox virus resistance in apricot breeding programs. Tree Genet. Genomes 10, 513–525. https://doi.org/10.1007/s11295-014-0700-x.
Ruiz, D., Cos, J., Egea, J., García, F., Nortes, M.D., Carrillo, A., Guevara, A., 2019. Progress in the Japanese plum (Prunus salicina L.) breeding program developed by CEBAS-CSIC and IMIDA in Murcia, Spain. Acta Hortic. 1260, 47–48. https://doi.org/10.17660/ActaHortic.2019.1260.9.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Sallom, A., Fatahi, R., Zamani, Z., Ebadi, A., 2021. Optimization *in vitro* conditions for plum × apricot embryo rescue and modeling some critical factors by using artificial neural networks technology. Sci. Hortic. 289, 110487 https://doi.org/10.1016/j.scienta.2021.110487.
Sanchez-Navarro, J.A., Aparicio, F., Herranz, M.C., Minafra, A., Myrta, A., Pallas, V., 2005. Simultaneous detection and identification of eight stone fruit viruses by one-step RT-PCR. Eur. J. Plant Pathol. 111, 77–84. https://doi.org/10.1007/s10658-004-1422-y.
Scorza, R., Sherman, W.B., Janick, J., Moore, J.N., 1996. Peaches. Fruit Breeding, Tree Trop. Fruits. John Wiley & Sons, New York, pp. 325–440.
Singh, D., Kumar, R., Selvan, T., 1997. Interspecific hybridization between plums and apricots to develop plumcots/cotplums. J. Tree Sci. 16, 96–100.
Sonneveld, T., Robbins, T.P., Tobutt, K.R., 2006. Improved discrimination of self-incompatibility S-RNase alleles in cherry and high throughput genotyping by automated sizing of first intron polymerase chain reaction products. Plant Breed. 125, 305–307. https://doi.org/10.1111/j.1439-0523.2006.01205.x.
Soriano, J.M., Domingo, M.L., Zuriaga, E., Romero, C., Zhebentyayeva, T., Abbott, A.G., Badenes, M.L., 2012. Identification of simple sequence repeat markers tightly linked to plum pox virus resistance in apricot. Mol. Breed. 30, 1017–1026. https://doi.org/10.1007/s11032-011-9685-4.
Sutula, C.L., Gillett, J.M., Morrissey, S.M., Ramsdell, D.C., 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70, 722–726. https://doi.org/10.1094/PD-70-722.
Szymajda, M., Napiórkowska, B., Korbin, M., Żurawicz, E., 2015. Studies on the interspecific crossing compatibility among three Prunus species and their hybrids. Hortic. Sci. 42, 70–82. https://doi.org/10.17221/273/2014-HORTSCI.
Szymajda, M., Studnicki, M., Kuras, A., Żurawicz, E., 2022. Cross-compatibility in interspecific hybridization between three Prunus species. S. Afr. J. Bot. 146, 624–633. https://doi.org/10.1016/j.sajb.2021.11.036.
Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.
Tao, R., Yamane, H., Sugiura, A., Murayama, H., Sassa, H., Mori, H., 1999. Molecular typing of S-alleles through identification, characterization and cDNA cloning for S-RNases in sweet cherry. J. Am. Soc. Hortic. Sci 124, 224–233. https://doi.org/10.21273/JASHS.124.3.224.
Tong, Z., Qu, S., Zhang, J., Wang, F., Tao, J., Gao, Z., Zhang, Z., 2012. A modified protocol for RNA extraction from different peach tissues suitable for gene isolation and real-time PCR analysis. Mol. Biotechnol. 50, 229–236. https://doi.org/10.1007/s12033-011-9433-3.
Topp, B.L., Russell, D.M., Neumüller, M., Dalbó, M.A., Liu, W., Badenes, M., Byrne, D., 2012. Plum. Fruit Breeding. Handbook of Plant Breeding. Springer, Boston, MA, pp. 571–621. https://doi.org/10.1007/978-1-4419-0763-9_15, 8.
Vilanova, S., Badenes, M.L., Burgos, L., Martínez-Calvo, J., Llácer, G., Romero, C., 2006. Self-compatibility of two apricot selections is associated with two pollen-part mutations of different nature. Plant Physiol. 142, 629–641. https://doi.org/10.1104/pp.106.083865.
Yaman, M., Uzun, A., 2020. Evaluation of superior hybrid individuals with intra and interspecific hybridization breeding in apricot. Int. J. Fruit Sci. 20, S2045–S2055. https://doi.org/10.1080/15538362.2020.1852151.
Yamane, H., Tao, R., Sugiura, A., Hauck, N.R., Iezzoni, A.F., 2001. Identification and characterization of S-RNnases in tetraploid sour cherry (Prunus cerasus). J. Am. Soc. Hortic. Sci. 126, 661–667. https://doi.org/10.21273/JASHS.126.6.661.
Yoshida, M., 1987. The origin of fruits, 2: Plums. Fruit Japan 42 (2), 49–53.
Yoshida, M., Kyotani, H., Yasuno, M., 1975. Studies on interspecific crossing in prunus spp.: I. cross compatibility. Jpn. J. Breed. 25, 17–23. https://doi.org/10.1270/jsbbs1951.25.17.
Zhivondov, A., 2012. Standesto", the first Bulgarian plumcot cultivar. Acta Hortic. 966, 219–222. https://doi.org/10.17660/ActaHortic.2012.966.34.
Zhivondov, A., Uzundzhalieva, K., 2012. Taxonomic classification of plum-apricot hybrids. Acta Hortic. 966, 211–218. https://doi.org/10.17660/ActaHortic.2012.966.33.
Zuriaga, E., Romero, C., Blanca, J.M., Badenes, M.L., 2018. Resistance to Plum Pox Virus (PPV) in apricot (Prunus armeniaca L.) is associated with down-regulation of two MATHd genes. BMC Plant Biol. 18, 1–13. https://doi.org/10.1186/s12870-018-1237-1.
Zuriaga, E., Soriano, J.M., Zhebentyayeva, T., Romero, C., Dardick, C., Cañizares, J., Badenes, M.L., 2013. Genomic analysis reveals MATH gene(s) as candidate(s) for Plum pox virus (PPV) resistance in apricot (Prunus armeniacaL.). Mol. Plant Pathol. 14, 663–677. https://doi.org/10.1111/mpp.12037.