

Sara Herrera Lagranja

Optimización del cultivo del albaricoquero. Necesidades de polinización, necesidades agroclimáticas y diversidad genética

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OPTIMIZACIÓN DEL CULTIVO DEL
ALBARICOQUERO. NECESIDADES DE
POLINIZACIÓN, NECESIDADES
AGROCLIMÁTICAS Y DIVERSIDAD GENÉTICA

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OPTIMIZACIÓN DEL CULTIVO DEL **ALBARICOQUERO**.

NECESIDADES DE POLINIZACIÓN,
NECESIDADES AGROCLIMÁTICAS,
Y DIVERSIDAD GENÉTICA

SARA HERRERA LAGRANJA



TESIS DOCTORAL 2022



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OPTIMIZACIÓN DEL CULTIVO DEL ALBARICOQUERO. NECESIDADES DE POLINIZACIÓN, NECESIDADES AGROCLIMÁTICAS Y DIVERSIDAD GENÉTICA.

Presentada por

Sara Herrera Lagranja

para optar al Grado de Doctor por la Universidad de Zaragoza

Programa de Doctorado en Ciencias Agrarias y del Medio Natural

Directores:

Javier Rodrigo García

Jorge Lora Cabrera

Septiembre 2022

D. JAVIER RODRIGO GARCÍA,
Investigador del Centro de Investigación y
Tecnología Agroalimentaria de Aragón, y



D. JORGE LORA CABRERA,
Científico titular del Consejo Superior de
Investigaciones Científicas en el Instituto de
Hortofruticultura Subtropical y Mediterránea
“La Mayora”,



CERTIFICAN

Que Dña. Sara Herrera Lagranja ha realizado bajo nuestra dirección, en el Centro de Investigación y Tecnología Agroalimentaria de Aragón, el trabajo que presenta en esta memoria con el título “Optimización del cultivo del albaricoquero. Necesidades de polinización, necesidades agroclimáticas y diversidad genética” y que reúne, a nuestro juicio, las condiciones requeridas para optar al Grado de Doctor, dentro del Programa de Doctorado en Ciencias Agrarias y del Medio Natural, por la Universidad de Zaragoza.

En Zaragoza, 14 de septiembre de 2022

Fdo: Javier Rodrigo García

Fdo: Jorge Lora Cabrera

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*Nessuna foglia è morta.
Prima è linfa per la terra
poi ritorna da farfalla.*

Ennio Cavalli

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RESUMEN

En los últimos años, se está produciendo una importante renovación varietal en el albaricoquero a nivel mundial, con un gran número de nuevas obtenciones. Los programas de mejora en todo el mundo tienen objetivos similares, entre ellos resistencia al virus de la sharka (Plum Pox Virus, PPV), el principal factor limitante para la producción del albaricoquero en Europa, así como autocompatibilidad, frutos de alta calidad y amplia adaptabilidad a las condiciones climáticas. Sin embargo, los genes de resistencia a PPV se encuentran principalmente en variedades de América del Norte que, además, se caracterizan por tener altas necesidades de frío, ser autoincompatibles y tener un período de maduración medio-tardío. El uso de estas variedades norteamericanas como parentales ha provocado variabilidad en las necesidades agroclimáticas y en el carácter de auto(in)compatibilidad de muchas de las nuevas variedades que, en muchos casos, se desconocen. En esta tesis, se han determinado las necesidades de polinización y agroclimáticas de un grupo de variedades de albaricoquero, incluyendo variedades tradicionales y nuevas variedades procedentes de programas de mejora de diversos países, y se ha evaluado el grado de diversidad genética del cultivo. La auto(in)compatibilidad se estableció mediante la observación del comportamiento de los tubos polínicos en flores autopolinizadas bajo el microscopio en 99 variedades, identificando 50 autocompatibles y 49 autoincompatibles. Para determinar las relaciones de incompatibilidad, se caracterizó el genotipo S en 170 variedades, de las que 87 presentaron el alelo S_c , relacionado con la autocompatibilidad. Cinco variedades autocompatibles no presentaron en su genotipo el alelo S_c . Las 49 accesiones autoincompatibles se asignaron a los correspondientes grupos de incompatibilidad, de los que se identificaron 9 nuevos grupos por primera vez [XVIII (S_1S_3), XIX (S_2S_3), XX (S_2S_9), XXI (S_3S_8), XXII (S_3S_9), XXIII (S_7S_9), XXIV (S_1S_6), XXV (S_1S_9) y XXVI (S_6S_8)]. Un solo alelo fue identificado en 29 variedades, lo que pudo deberse a una amplificación ineficiente por parte de la PCR, por lo que se desarrollaron dos nuevos pares de cebadores específicos para determinar los alelos S_1 y S_7 (Capítulos 1-4). Para proporcionar una visión general de la diversidad genética en el locus S en las variedades actualmente cultivadas, se realizó una compilación del genotipo S de 235 variedades, incluyendo los resultados obtenidos con resultados de trabajos previos. Los resultados revelaron que, a pesar del incremento de nuevas variedades autocompatibles en los últimos años, una proporción significativa de éstas son autoincompatibles y requieren de polinización cruzada para producir frutos (Capítulo 4). También se ha encontrado un biomarcador para determinar la salida de la endodormancia, la meiosis del polen. Para ello, el desarrollo del polen fue caracterizado histoquímicamente en 20 variedades durante 8 años, relacionando las etapas de desarrollo con las fases de reposo. La validez de su uso para la estimación de necesidades agroclimáticas se ha realizado mediante la comparación con tres metodologías previamente descritas, una metodología experimental y dos métodos estadísticos, en el estudio de las necesidades agroclimáticas de las 20 variedades (Capítulo 5). Finalmente, se han estudiado las relaciones de diversidad entre las principales variedades actualmente cultivadas usando 13 marcadores microsatélite para evaluar la erosión genética del cultivo. Las 202 accesiones se agruparon en función de su origen genealógico, diferenciándose claramente dos grupos: las variedades tradicionales y las variedades procedentes de programas de mejora. Las variedades comerciales se caracterizaron por presentar mayor diversidad que las variedades tradicionales. Sin embargo, se observó menor diversidad genética en las selecciones mejoradas más recientes, sugiriendo una pérdida de diversidad asociada con el uso reiterado de un grupo reducido de parentales (Capítulo 6). Los resultados de esta tesis revelan la importancia de estudiar la incompatibilidad, la adaptación ambiental y la diversidad genética para la optimización y desarrollo de los programas de mejora y el diseño y manejo de plantaciones del albaricoquero.

SUMMARY

In recent years, an important renewal of apricot cultivars is taking place worldwide, with the introduction of many new releases. Breeding programs have similar objectives, including the development of new cultivars resistant to the sharka virus (Plum Pox Virus, PPV), which is the main limiting factor in the apricot production in Europe, as well as self-compatibility, high fruit quality and climate adaptability. However, PPV-resistance genes are found primarily in North American cultivars that are also characterized by high chilling requirements, self-incompatibility, and medium-to-late ripening date. The use of these North American cultivars as parents in many breeding programs has caused variability in agroclimatic requirements and self-(in)compatibility of many of the new cultivars, which in many cases are unknown. In this thesis, the pollination and the agroclimatic requirements of a group of apricot cultivars, including traditional and newly-released cultivars from several breeding programs and countries, have been determined, and the current genetic diversity of the crop has been evaluated. Self-(in)compatibility was established by the observation of the pollen tube behavior in self-pollinated flowers under the microscope in 99 cultivars, identifying 50 cultivars as self-compatible and 49 as self-incompatible. To determine the incompatibility relationships, the *S*-genotype has been characterized in 170 cultivars, 87 of them presenting the *S_c*-allele that is related to self-compatibility. However, the *S_c*-allele was not present in the genotype of five self-compatible cultivars. The 49 self-incompatible accessions were assigned to the corresponding incompatibility groups, of which 9 new groups were identified for the first time [XVIII (*S₁S₃*), XIX (*S₂S₃*), XX (*S₂S₉*), XXI (*S₃S₈*), XXII (*S₃S₉*), XXIII (*S₇S₉*), XXIV (*S₁S₆*), XXV (*S₁S₉*) y XXVI (*S₆S₈*)]. A single allele was identified in 29 cultivars, which could be due to inefficient PCR amplification, so two new pairs of specific primers were developed for the identification of the *S₁* and *S₇*-alleles (Chapters 1-4). In addition, with the aim of providing an overview of the genetic diversity at the *S*-locus of currently grown apricot cultivars, a compilation of the *S*-genotype of 235 apricot cultivars was carried out, including the results obtained in this thesis and those from previous studies. The results revealed that despite the increase in new self-compatible cultivars in recent years, a significant proportion of new cultivars are self-incompatible and, therefore, require cross-pollination to produce fruit (Chapter 4). In addition, pollen meiosis has been determined as a biomarker to determine the end of endodormancy. For this purpose, pollen development was histochemically characterized in 20 cultivars over 8 years, and the developmental stages were related to dormancy phases. To validate its suitability for the estimation of agroclimatic requirements, the results were compared with the results of three previously described methodologies, an experimental methodology, and two statistical approaches (Chapter 5). On the other hand, the genetic relationships among most recent apricot releases was studied by using 13 microsatellite markers to evaluate the genetic erosion of the crop. Two main clusters according to the pedigree origin of the accessions were clearly differentiated in the phylogenetic analysis: landraces and releases from breeding programs. Bred cultivars showed higher diversity than landraces. However, a gradient of decreasing genetic diversity was observed in recent releases from breeding programs, suggesting a loss of diversity associated with the use of common parents in breeding programs (Chapter 6). The results of this thesis reveal the importance of studying incompatibility, environmental adaptation, and genetic diversity for the optimization and development of breeding programs and the design and management of apricot commercial orchards.

INTRODUCCIÓN GENERAL



INTRODUCCIÓN GENERAL

El albaricoquero

Origen y expansión del cultivo

El albaricoquero (*Prunus armeniaca* L.) es un frutal de hueso perteneciente al género *Prunus* de la subfamilia *Prunoideae*, que forma parte de la familia *Rosaceae* (APG IV, 2016). Dentro del género *Prunus*, la sección *Armeniaca* se presenta como un complejo de especies diploides ($2n = 16$) e interfértiles. Además de *P. armeniaca* L. (albaricoquero), en esta sección se encuentran especies como *P. ansu* Komar, *P. brigantiaca* Vill. (albaricoquero de los Alpes franceses), *P. dasycarpa* Ehrh. (albaricoquero negro), *P. holosericea* y *P. mume* Sieb. et Zucc. (albaricoquero tibetano o japonés) (Ledbetter, 2008). Estas especies, morfológica y ecológicamente variadas, son consideradas cercanas al albaricoquero, pero están escasamente cultivadas y su utilización en los programas de mejora es muy limitada. La mayoría de los albaricoqueros cultivados en la actualidad pertenecen a la especie *P. armeniaca* L. (Figura 1), que se cultiva en todo el mundo (Mehlenbacher et al., 1991; Faust et al., 1998).

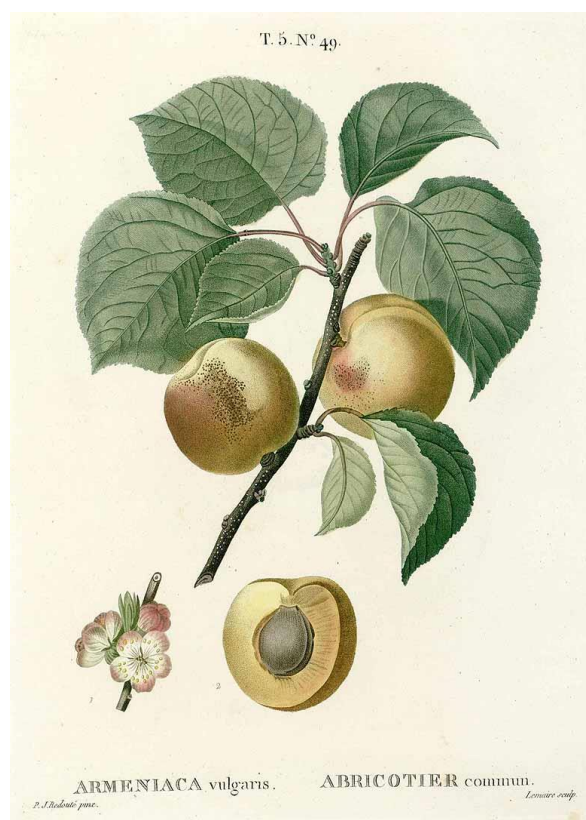


Figura 1. Dibujo de albaricoquero por P.J. Redouté (Duhamel du Monceau, 1812).

El albaricoquero se originó en Asia. El botánico ruso Vavilov estableció tres centros de origen (Vavilov, 1951). Un primer centro de origen en China Central y Occidental y Tíbet, un segundo en Asia Central [desde Tien-Shan hasta Cachemira, que incluye Afganistán, noroeste de India y Pakistán, Cachemira, Tayikistán, Uzbekistán, la provincia de Xinjing en China y el oeste de Tien-Shan, así como Turkmenistán que fue incluido posteriormente (Zhukovsky, 1971)], y un tercero en el Cercano Este (Irán, Cáucaso, Turquía), considerado como centro secundario (Figura 2). Sin embargo, estudios recientes usando marcadores moleculares SNPs (*'Single Nucleotide Polymorphisms'*) han determinado que tanto las variedades europeas como las chinas provienen de Asia Central, donde tuvieron lugar al menos dos eventos distintos de domesticación aproximadamente hace 2.000-3.000 años (Decroocq et al., 2016; Liu et al., 2019). Las variedades europeas y del área geográfica irano-caucásica son originarias de albaricoqueros silvestres del norte de Asia Central, mientras que las variedades chinas fueron domesticadas a partir de poblaciones silvestres del sur de Asia Central (Groppi et al., 2021). Adicionalmente, un estudio llevado a cabo con germoplasma de albaricoquero de todo el mundo reveló la aparición de un grupo distintivo en el Este de Asia que incluye a los albaricoqueros japoneses, lo que refuerza la hipótesis de que esta región es otro centro secundario de diversificación (Bourguiba et al., 2020).

Desde estos centros de origen, la especie se distribuyó al resto del mundo, probablemente a través de las rutas comerciales abiertas por Alejandro Magno en el siglo IV a.C. (Layne et al., 1996). Desde Asia oriental se introdujo en Japón hace unos 2.000 años (Bourguiba et al., 2020), y desde la región irano-caucásica se expandió hacia la región Mediterránea alrededor del siglo I a.C. (Zohary et al., 2012), dando origen a su nombre *P. armeniaca*, 'el que viene de la Gran Armenia' (Liu, 2019). Su diseminación en los países mediterráneos tuvo lugar siguiendo dos rutas: la primera al norte, por los Balcanes y al sur de Europa por Grecia e Italia, y la segunda a través del norte de África, que alcanzó el sur de España (Bourguiba et al., 2012). Sin embargo, un estudio posterior reveló que las variedades de Europa continental y de América del Norte, son originarias de albaricoqueros procedentes de Asia Central a partir de una tercera ruta de diseminación a través de los países de la Europa Continental, revelando tres grandes acervos genéticos (Bourguiba et al., 2020). Desde Europa continuó su dispersión hacia el Oeste y el hemisferio sur a partir del siglo XVI (Faust et al., 1998).

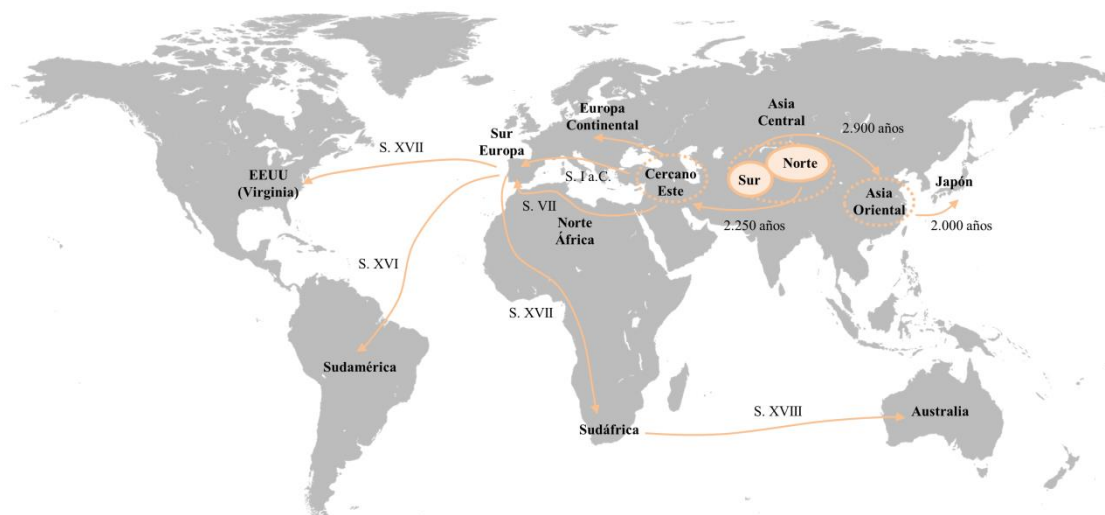


Figura 2. Esquema del origen y las rutas de domesticación mundial del albaricoquero cultivado.

Kostina (1969) propuso una clasificación de la especie en cuatro grupos eco-geográficos principales, subdivididos a su vez en grupos regionales, considerando el origen geográfico y las características morfológicas.

- Asia Central: es el grupo más antiguo y el de mayor diversidad en cuanto a sus caracteres vegetativos y fenológicos. Está caracterizado por árboles vigorosos y longevos con altas necesidades de frío y resistencia a las fluctuaciones de temperatura del final del invierno. Tienen una floración tardía y la mayoría son autoincompatibles. Los frutos tienen tamaño pequeño a medio y un alto contenido en azúcares, pero con la pulpa harinosa y sin aroma.
- Dzhungar-Zailij: este grupo incluye en su mayoría variedades autoincompatibles que se encuentran en el este de Kazajistán y oeste de China y se caracterizan por sus elevadas necesidades de frío, resistencia a las bajas temperaturas y producción de frutos pequeños.
- Irano-Caucásico: Los árboles de este grupo no son tan vigorosos ni longevos como los de Asia Central; tienen ramas más finas y hojas más anchas y brillantes. Incluye en su mayoría genotipos autoincompatibles con bajos requerimientos de frío. Las semillas son dulces y la pulpa de los frutos es blanca o poco coloreada.
- Europeo: es el grupo de origen más reciente y de menor diversidad. Incluye las variedades comerciales de América del Norte, Sudáfrica, Nueva Zelanda y

Australia. Los árboles de este grupo son poco vigorosos y precoces en floración, aunque las variedades del Norte de Europa están específicamente adaptadas a las heladas primaverales a través de su floración tardía (Faust et al., 1998; Zhebentyayeva et al., 2012). Son autocompatibles con frutos de menor grado de azúcar y acidez.

Posteriormente, esta clasificación se amplió a seis grupos eco-geográficos, (Layne et al., 1996). Sin embargo, los grupos de esta clasificación están menos definidos en la actualidad, debido la introducción de un gran número de nuevas variedades derivadas de cruces entre los genotipos de los diferentes grupos (Faust et al., 1998).

Importancia económica

El albaricoquero, con una producción mundial de 3,72 millones de toneladas, es el cuarto frutal de hueso en importancia económica a nivel mundial, por detrás del grupo melocotonero-nectarino, el ciruelo y el almendro (FAOSTAT, 2022). En los últimos años, la producción mundial se incrementó hasta un 83%, alcanzando en 2017 una producción de 4,79 millones de toneladas, debido principalmente al aumento de la superficie de cultivo en Asia y África (Zhebentyayeva et al., 2012). Turquía es el primer país productor (22,4 %; 833.398 t), seguido de Uzbekistán (14,2 %; 529.109 t), Irán (9 %; 334.408 t) y Argelia (5 %; 187.273 t) (Figura 3). En Europa, la producción aumentó a un ritmo menor, siendo Italia el quinto productor mundial (4,7 %; 173.380 t), seguido de España (séptimo) (3,5 %; 128.700 t) y Grecia (octavo) (3,4 %; 125.640 t), mientras que en América del Norte y Oceanía la producción ha disminuido (Zhebentyayeva et al., 2012). Cerca del 50% de la producción mundial se concentra en los países mediterráneos (FAOSTAT, 2022).

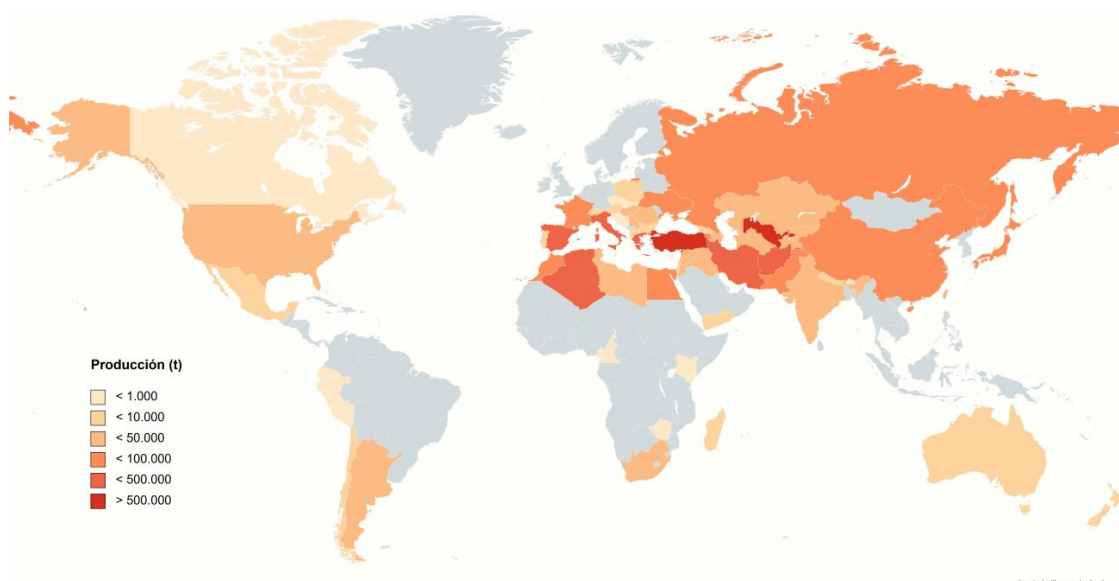


Figura 3. Distribución por países de la producción mundial (toneladas) de albaricoquero en el año 2020 (FAOSTAT, 2022).

En España, el albaricoquero es el quinto frutal de hueso en importancia con 127.231 t, por detrás del grupo melocotonero-nectarino (1.205.935 t), el almendro (371.460 t), el ciruelo (182.826 t) y el grupo cerezo-guindo (129.933 t) (MAPA, 2022). En 1991, llegó a alcanzar una producción de 210.900 t en una superficie de 23.900 ha, aunque su producción ha fluctuado en las últimas décadas, registrándose un importante descenso del 22 % en los últimos años (Figura 4). Actualmente, la superficie dedicada en España al cultivo del albaricoquero son unas 19.400 ha, localizándose principalmente en la cuenca mediterránea (Región de Murcia y Comunidad Valenciana, con 54.510 y 24.365 t, respectivamente) y Aragón, con 18.936 t (MAPA, 2022).



Figura 4. Producción y superficie cultivada de albaricoquero en España (1980-2021) (MAPA, 2022).

Biología floral¹

Diferenciación floral, floración y polinización

En las especies de *Prunus*, la inducción floral se produce durante el verano. Sin embargo, hasta finales de agosto no se diferencian los órganos florales, por lo que no se diferencian las yemas vegetativas y reproductivas. En otoño se produce la caída de hoja, a continuación la entrada en reposo en invierno y después la floración a finales de febrero y principios de marzo, antes de la aparición de nuevas hojas (Sedgley and Griffin, 1989). Cada yema fructífera puede presentar una flor, como en el albaricoquero (Julian et al., 2010), el almendro (*Prunus dulcis* Mill.) (Lamp et al., 2001) y el melocotonero (*Prunus persica* (L.) Batsch) (Luna et al., 1991), o varias flores, como ocurre en el cerezo (*Prunus avium* L.) (Fadón et al., 2015) y el ciruelo japonés (*Prunus salicina* Lindl.) (Guerra and Rodrigo, 2015). Las flores de *Prunus* son hermafroditas y están formadas por cuatro verticilos: carpelo, estambres, pétalos y sépalos, que se fusionan formando una copa en la base de la flor (Herrera et al., 2021b).

La floración del albaricoquero, como en otros frutales de hueso, está condicionada tanto por factores externos (condiciones ambientales, presencia de polinizadores) (Rodrigo and Herrero, 1996, 2002a) como por factores internos (estado nutricional de las yemas, factores genéticos) (Julian et al., 2010). Para que se produzca el cuajado de fruto, es necesaria una polinización adecuada de la flor donde insectos polinizadores como abejas o abejorros transporten los granos de polen hasta el estigma de la flor. La superficie del estigma es papilosa y húmeda cuando está receptivo, lo que proporciona un ambiente adecuado para la germinación de los granos de polen y su penetración en el tejido transmisor (Herrero, 1992; Lora et al., 2016). La germinación de los granos de polen tiene lugar en las siguientes 24 horas después de la polinización y el crecimiento del tubo polínico a lo largo del estilo tarda de 3 a 4 días hasta alcanzar el ovario (Rodrigo and Herrero, 2002b). En albaricoquero, el ovario contiene dos óvulos, pero solo uno de ellos es fecundado y desarrolla la semilla (Rodrigo and Herrero, 1998).

¹ Parte de la información incluida en este apartado se encuentra publicada en:

Herrera, S., Lora, J., Hormaza, J. I., and Rodrigo, J. (2021b). “Pollination Management in Stone Fruit Crops”, in: *Production Technology of Stone Fruits*, eds. M.M. Mir, M. Iqbal, S.A. Mir (Singapore: Springer), 75–102. doi:10.1007/978-981-15-8920-1_3 (Anexo I).

Incompatibilidad polen-pistilo

La incompatibilidad polen-pistilo es una barrera reproductiva en plantas con flores que inhibe la fecundación del óvulo por el polen propio o por polen de plantas filogenéticamente cercanas, lo que evita la endogamia y promueve el cruzamiento (de Nettancourt, 2001). La autoincompatibilidad es un mecanismo ampliamente expandido en el reino vegetal, regulado por diferentes mecanismos genéticos/moleculares (Igic et al., 2008). En Rosaceae, la autoincompatibilidad está determinada genéticamente mediante un sistema de autoincompatibilidad gametofítica (*Gametophytic Self-Incompatibility*, *GSI*), en el que el reconocimiento célula-célula ocurre en el estilo y está controlado por un locus llamado *S* (Kao and Mccubbin, 1996). El locus *S* codifica dos genes que determinan el genotipo del pistilo y del polen. En el pistilo, se expresa como una ribonucleasa, *S-RNasa*, que determina la especificidad del estilo (Tao et al., 1997). El determinante del polen es una proteína con una caja-F, denominada *SFB* (*S-haplotype-specific F-box*) (Ushijima et al., 2003). Las reacciones incompatibles se desencadenan cuando el alelo *S* del grano de polen (haploide) es el mismo que uno de los dos alelos expresados en el pistilo (diploide), por lo que el crecimiento del tubo polínico se detiene en el estilo, impidiendo así su llegada al ovario y la fecundación. Las *S-RNasas* funcionan dentro de los tubos polínicos como citotoxinas específicas inhibiendo su crecimiento al degradar el ARN del propio polen (McCubbin and Kao, 2000). Sin embargo, si los alelos *S* difieren entre sí, el tubo polínico es potencialmente capaz de alcanzar el ovario y fecundar al óvulo (Bedinger et al., 2017).

El gen de la *S-RNasa* está formado por cinco regiones conservadas (C1, C2, C3, C5 y la región específica de Rosaceae, RC4) y, además, incluye una región hipervariable, RHV (Ushijima et al., 1998). Adicionalmente, la estructura de la *S-RNasa* contiene dos intrones; el primer intrón se encuentra dentro de la unión entre el péptido señal y la proteína madura, y el segundo está situado dentro de la región hipervariable (RHV) (En capítulo 2 ver Figura 2). El tamaño de los intrones presenta una gran diversidad que varía en forma de haplotipo *S*, por lo que se ha usado para la identificación y clasificación de diferentes alelos (Romero et al., 2004; Vilanova et al., 2005). El gen *SFB* presenta un dominio de caja F, con dos regiones variables (V1, V2) y dos hipervariables (HV_a y HV_b). Además, contiene un intrón aguas arriba de la secuencia codificante. En albaricoquero, el alelo *S_c* se ha relacionado con la autocompatibilidad. El origen de este haplotipo es una mutación en la parte del polen en

el alelo *S₈* (Vilanova et al., 2006; Halász et al., 2007). La secuencia que codifica a la *RNasa* en este alelo está inalterada, pero una inserción de 358 pb en el gen *SFB₈* resulta en una proteína truncada que carece de los dos dominios esenciales 3'-hipervariables HVa y HVb, dando origen al alelo *SFB_c* (Vilanova et al., 2006).

*Estudio de la auto(in)compatibilidad*²

Tradicionalmente, en *Prunus*, la auto(in)compatibilidad y las relaciones de incompatibilidad entre variedades se han detectado mediante la evaluación de la fructificación en el campo tras polinizaciones controladas (Andres and Duran, 1998). Sin embargo, en estos ensayos la viabilidad del polen, la polinización y el desarrollo del fruto podrían verse fuertemente influenciadas por el medio ambiente, por lo que los resultados pueden no ser concluyentes. La evaluación al microscopio del crecimiento de los tubos polínicos en flores polinizadas en condiciones controladas también ha sido usada para evaluar la incompatibilidad (Rodrigo and Herrero, 1996; Burgos et al., 1997; Andres and Duran, 1998; Julian et al., 2010; Milatović et al., 2010, 2018; Herrera et al., 2020b), siendo una metodología más fiable que los ensayos de campo para determinar la auto(in)compatibilidad y las relaciones de incompatibilidad (Viti et al., 1997). Sin embargo, estos métodos requieren de árboles con flores, lo que significa al menos 3 años después de la obtención de las semillas.

Además de estos métodos de evaluación fenotípica, dos métodos moleculares/genéticos se han desarrollado para determinar las relaciones de incompatibilidad entre variedades. En un primer método, la detección de ribonucleasas estilares (*S-RNasas*), mediante su separación por electroforesis y posterior tinción, permite revelar las bandas de proteínas correspondientes a los alelos *S* (McClure et al., 1989; Burgos et al., 1998; Albuquerque et al., 2002). Un segundo método genético, basado en el genotipado de las variedades a través de la amplificación de los alelos *S* por PCR (siglas en inglés de ‘reacción en cadena de la polimerasa’), ha reemplazado

² Parte de la información incluida en este apartado se encuentra publicada en:

Herrera, S., Lora, J., Hormaza, J. I., and Rodrigo, J. (2020b). Necesidades de polinización en variedades de albaricoquero (Parte I): autoincompatibilidad. *Revista de Fruticultura* 77, 52–61 (Anexo II).

Herrera, S., Lora, J., Hormaza, J. I., and Rodrigo, J. (2020c). Necesidades de polinización en variedades de albaricoquero (Parte II): selección de variedades polinizantes. *Revista de Fruticultura* 78, 6–11 (Anexo III).

en los últimos años a las polinizaciones controladas, los ensayos in-vitro de crecimiento de tubo polínico y los ensayos enzimáticos utilizados para determinar el genotipo *S*. Esta metodología permite la identificación rápida de los genotipos *S* de variedades, con lo que se pueden establecer las relaciones de incompatibilidad. Además, tiene las ventajas adicionales de que no requiere árboles adultos ni depende de las condiciones ambientales (Yamane and Tao, 2009). En albaricoquero, se han identificado 33 alelos *S* de incompatibilidad que han permitido clasificar las variedades autoincompatibles en 26 grupos de incompatibilidad (I-XXXV) (Szabó and Nyéki, 1991; Egea and Burgos, 1996; Halász et al., 2010; Lachkar et al., 2013; Boubakri et al., 2021). Las variedades con los mismos alelos *S* son genéticamente incompatibles entre sí y están incluidas en el mismo grupo de incompatibilidad. Por otro lado, las variedades con al menos un alelo *S* diferente son compatibles entre sí y se asignan a diferentes grupos de incompatibilidad. Esto ha sido especialmente útil para los productores para seleccionar variedades polinizadoras eficientes. Las variedades clasificadas en el grupo 0 y las variedades autocompatibles pueden considerarse polinizadores universales. Para producir fruto, las variedades autoincompatibles deben ser cultivadas junto con variedades con las que sean intercompatibles, para que sus flores se puedan polinizar con polen compatible, y coincidentes en floración (Herrera et al., 2021b). Además, el genotipado *S* también se ha utilizado para diseñar cruces y seleccionar híbridos autocompatibles en programas de mejora donde, en muchos casos, todavía se utilizan parentales autoincompatibles.

Tradicionalmente, las necesidades de polinización no se han considerado un problema en albaricoquero, ya que la mayoría de las variedades europeas son autocompatibles (Hormaza et al., 2007). Sin embargo, la utilización de variedades autoincompatibles resistentes al virus de la sharka (Plum pox virus) procedentes de Norteamérica como parentales en los programas de mejora ha provocado la introducción de un gran número de variedades con necesidades de polinización desconocidas (Burgos et al., 1997; Herrera et al., 2020c).

El reposo invernal³

Importancia del reposo en la floración

La temperatura condiciona el ciclo anual de los frutales de clima templado. Para que tenga lugar la floración en primavera es necesario un periodo de reposo durante el invierno en el que los árboles necesitan acumular frío (Horvath et al., 2003). Este periodo, denominado reposo, letargo o latencia (*dormancy*), es un mecanismo de supervivencia para superar el frío invernal y está caracterizado por la supresión temporal del crecimiento visible de cualquier estructura que contenga un meristemo en la planta (Rohde and Bhalerao, 2007). Se han diferenciado tres etapas en este proceso: (1) endodormancia (*endodormancy*), regulada por factores fisiológicos internos de la estructura afectada, (2) paradormancia (*paradormancy*), regulada por factores fisiológicos externos a la estructura, y (3) ecodormancia (*ecodormancy*), regulada por factores ambientales (Lang et al., 1987). Tras la fase de endodormancia, los frutales necesitan acumular temperaturas cálidas durante la ecodormancia para que tenga lugar la floración (Fadón et al., 2020a). Tanto las necesidades de frío como las necesidades de calor son características de cada especie e incluso varían entre variedades dentro de la misma especie (Fadón et al., 2020b).

En los últimos años, el conocimiento de las necesidades de frío y calor de las variedades frutales ha cobrado mayor importancia debido al contexto del cambio climático. El aumento de temperaturas pronosticado para el futuro puede comprometer la viabilidad de algunas variedades de frutales y zonas productivas (Luedeling et al., 2011). Como consecuencia, la errática acumulación de frío invernal puede ocasionar problemas de producción, que se traducen en brotación irregular y dispersa, caída de yemas o malformaciones en las estructuras reproductivas (Viti et al., 2008; Campoy et al., 2011). Además, la disminución de frío invernal puede provocar falta de solapamiento de las épocas de floración en variedades con distintas necesidades agroclimáticas (Guerra et al., 2020).

³ Parte de la información incluida en este apartado se encuentra publicada en:

Fadón, E., **Herrera, S.**, Guerrero, B. I., Guerra, M. E., Rodrigo, J. (2020b). Chilling and Heat Requirements of Temperate Stone Fruits Trees (*Prunus* sp.). *Agronomy* 10, 409. <https://doi.org/10.3390/agronomy10030409> (Anexo IV).

Por ello, la caracterización de las necesidades agroclimáticas de variedades es cada vez más importante para el diseño y manejo de plantaciones, así como para determinar si puede adaptarse a una determinada zona de cultivo.

Establecimiento de la salida de la endodormancia

Para calcular las necesidades agroclimáticas es necesario determinar cuándo se produce el fin de la endodormancia, lo que permite cuantificar el frío acumulado hasta entonces (necesidades de frío) y el calor acumulado desde entonces hasta floración (necesidades de calor). Sin embargo, es difícil establecer el momento en que las yemas salen del estado de endodormancia y retoman su desarrollo cuando las condiciones ambientales lo permiten (Fadón and Rodrigo, 2018). Se han desarrollado diferentes métodos estadísticos y experimentales. Los modelos estadísticos determinan el fin de la endodormancia relacionando, mediante correlaciones o regresiones, las temperaturas invernales con las fechas de floración de varios años (Alonso et al., 2005; Luedeling et al., 2013). Por otro lado, en los modelos experimentales se monitoriza la brotación de las yemas en varetas recolectadas de forma periódica durante el reposo y mantenidas en condiciones controladas en cámara por un periodo determinado, considerando que las yemas han salido del reposo cuando se produce un aumento significativo de peso (Brown and Kotob, 1957) o un cambio de estado fenológico (Bennett, 1949). Los resultados obtenidos mediante las dos aproximaciones son útiles para la zona de estudio, pero no suelen ser extrapolables para otras zonas de cultivo de características climáticas diferentes (Fadón and Rodrigo, 2018). Esta situación ha provocado la búsqueda de indicadores biológicos que permitan determinar la salida de la endodormancia en condiciones reales de cultivo, aunque hasta la fecha no se ha encontrado ningún indicador fiable (Fadón et al., 2018). Estudios previos en albaricoquero han puesto en evidencia que la meiosis del polen es uno de los primeros procesos que tienen lugar una vez superado el periodo de reposo (Julian et al., 2011, 2014), por lo que sería interesante evaluar si el momento en que se desencadena la meiosis puede utilizarse como marcador del final de la endodormancia.

La cuantificación del frío y el calor

Se han desarrollado diferentes modelos para cuantificar la acumulación de frío invernal, siendo los más utilizados el “Modelo Weinberger” (horas frío) (Weinberger, 1950), “Modelo Utah” (unidades de frío) (Richardson et al., 1974) y “Modelo Dinámico” (porciones de frío) (Fishman et al., 1987). El modelo de Weinberger establece que las temperaturas entre 0 y 7,2°C son efectivas para la acumulación de frío y define que una hora frío es una hora en la cual la temperatura se encuentra entre ese rango (Weinberger, 1950). Posteriormente, surgieron otros modelos que contemplan rangos de temperatura con diferente eficiencia en la acumulación de frío. En el “Modelo Utah” se asigna a cada temperatura o intervalo de temperaturas un nivel de eficiencia para contribuir a la ruptura de la endodormancia. Solamente en el intervalo comprendido entre 2,5 y 9,1°C se produce la acumulación de unidades de frío (UF) completas. Las temperaturas entre 1,5 y 2,4°C, y entre 9,2 y 12,4°C, tienen una eficiencia del 50 % en la acumulación de UF. Las temperaturas inferiores a 1,4°C o superiores a 12,5°C no resultan efectivas para la ruptura del reposo, y las temperaturas superiores a 16 °C producen un efecto negativo (Richardson et al., 1974). Más reciente, el “Modelo Dinámico” establece la presencia de dos etapas en la ruptura del reposo, ya que considera que temperaturas cálidas pueden anular el efecto de temperaturas frías previas, y se expresa en porciones frío (PF) (Fishman et al., 1987). En función de la climatología, para una zona concreta es más fiable un modelo u otro (Dennis, 2003). El modelo de horas frío es el más utilizado por los agricultores debido a que es fácil de aplicar, pero tiene muchas limitaciones y no se ajusta bien en zonas templadas o cálidas (Ruiz et al., 2007, 2019), siendo más apropiado su uso en zonas más frías (Luedeling et al., 2013). Por otro lado, el “Modelo Utah” se adapta mejor en las áreas frías de las regiones templadas, mientras que el “Modelo Dinámico” es más indicado para zonas subtropicales (Dennis, 2003).

Una vez los árboles han acumulado el frío necesario para superar el periodo de endodormancia necesitan acumular cierta cantidad de calor, que varía en función tanto de la especie como de la variedad, para que tenga lugar la floración. El más utilizado para cuantificar el calor es el modelo “*Growing Degree Hours*” (GDH). Una GDH es 1 hora a una temperatura 1°C por encima de la temperatura base de 4,5°C, siendo 25°C la mayor temperatura efectiva, por encima de la cual el efecto es similar (Richardson et al., 1974).

Las variedades de albaricoquero que se cultivaban tradicionalmente en Europa tienen bajas necesidades de frío. Sin embargo, debido a la intensa renovación varietal producida en los últimos años y el uso de parentales con altas necesidades de frío, se desconocen las necesidades agroclimáticas de la mayoría de las variedades comerciales.

Caracterización varietal

Importancia de los recursos fitogenéticos locales

El germoplasma local y la variedades tradicionales actúan como fuentes naturales de rasgos de interés, ya que poseen características que las hacen muy útiles para los programas de mejora, como frutos con propiedades organolépticas interesantes y adaptabilidad a condiciones climáticas locales (Azeez et al., 2018). El albaricoquero, gracias a la amplia variabilidad de su germoplasma, ofrece claras oportunidades para el desarrollo de variedades que reúnen todas las características deseadas por productores, distribuidores y consumidores, y ayuden a paliar las problemáticas del cultivo (Bassi and Audergon, 2006). Sin embargo, la intensa renovación varietal en las últimas décadas ha provocado que las variedades tradicionales se estén sustituyendo por nuevas obtenciones, reduciéndose cada vez más su presencia en las plantaciones comerciales (Martín et al., 2010).

La conservación y el uso de recursos fitogenéticos debería ser una prioridad en la investigación agraria. A finales del siglo XIX se establecieron las primeras colecciones de germoplasma con el fin de conservar ese material vegetal. Los bancos de germoplasma suponen un sistema eficaz y económico para evitar la pérdida de variabilidad genética y permiten que sea más accesible para su uso en la mejora vegetal (Plucknett et al., 1983). Los análisis de diversidad genética en colecciones de germoplasma pueden determinar una clasificación adecuada de las accesiones y suministrar información sobre su origen y proximidad genética, así como facilitar el establecimiento de colecciones nucleares que contengan individuos bien caracterizados y representativos de la diversidad original (Campoy et al., 2016; Bourguiba et al., 2020).

La caracterización de la variabilidad genética

Tradicionalmente, los estudios de diversidad genética se han llevado a cabo mediante marcadores morfológicos y agronómicos. Sin embargo, estos métodos son lentos y costosos, por lo que se realizaban pocos estudios y no eran muy precisos. Además, es común observar incongruencias entre los análisis basados en datos morfológicos y los basados en datos moleculares (Wiens, 2004). Posteriormente, se desarrollaron los marcadores moleculares, que permiten detectar polimorfismos en las secuencias de ADN. Los más conocidos son RFLPs (*‘Restriction Fragment Length Polymorphism’*), RAPDs (*‘Random Amplified Polymorphic DNA’*), AFLPs (*‘Amplified Fragment Length Polymorphism’*), SSRs (*‘Simple Sequence Repeats’*), ISSRs (*‘Inter-Simple Sequence Repeats’*), CAPS (*‘Cleaved Amplified Polymorphic Sequence’*) y SNPs (*‘Single Nucleotide Polymorphism’*) (Martínez-Gómez et al., 2005).

Los microsatélites (SSRs) son secuencias cortas de ADN (de 1 a 10 pares de bases) que se repiten en tándem en el genoma. Su utilización para estudios de diversidad genética ha mostrado varias ventajas respecto a otros marcadores moleculares. Los SSRs no son codificantes, por lo que las mutaciones se transmiten; además son abundantes, están uniformemente distribuidos por el genoma, son altamente polimórficos, presentan herencia mendeliana simple y son fáciles de identificar, medir y analizar (Carneiro Vieira et al., 2016). Otra de sus ventajas es que son transferibles entre grupos relacionados, lo que permite hacer estudios comparativos entre especies o géneros (Dirlewanger et al., 2002; Guerrero et al., 2021).

En los últimos 20 años, los marcadores microsatélite SSRs han sido ampliamente utilizados para caracterizar accesiones de numerosas especies de Rosáceas, como cerezo (Dirlewanger et al., 2002; Wünnch and Hormaza, 2002; Liu et al., 2018), ciruelo europeo (Urrestarazu et al., 2018), ciruelo japonés (Guerrero et al., 2021), manzano (Pina et al., 2014) y melocotonero (Dirlewanger et al., 2002). En albaricoquero, se han utilizado para caracterizar variedades comerciales tradicionales (Hormaza, 2002) y locales de distintos países como España (Martínez-Mora et al., 2009; Martín et al., 2010), Irán (Raji et al., 2014), Túnez (Bourguiba et al., 2013; Batnini et al., 2016) y Turquía (Murathan et al., 2017).

Evaluación de la diversidad y la estructura poblacional

Los datos obtenidos con marcadores microsatélites y otros marcadores moleculares permiten evaluar la similitud y obtener distancias entre individuos para realizar estudios de variabilidad genética e identificar estructuras o subgrupos dentro de una población. El estudio de la diversidad genética puede ser analizado por un método específico o por una combinación de métodos (Mohammadi and Prasanna, 2003). Los principales parámetros de diversidad genética que se evalúan son: i) el polimorfismo (P), que indica el número de locus variables en una población cuando se detecta más de un alelo, ii) la riqueza alélica (A_r), que cuantifica el número medio de alelos (N_a) que presenta un locus en una población, y iii) la frecuencia alélica, que refleja la proporción de un alelo específico de un determinado locus en un grupo de genotipos o en la población, información que es útil para determinar la presencia de alelos privados (P_a). La frecuencia alélica es una medida que permite evaluar la evolución en una población, ya que representa la transmisión de los genes. Si el valor de las frecuencias alélicas de una generación a la siguiente cambia, indica que la población está experimentando cambio evolutivo. Si, por el contrario, las frecuencias alélicas permanecen constantes quiere decir que no ha ocurrido evolución (Vellend and Geber, 2005).

Adicionalmente, la heterocigosidad es otra medida que representa la variabilidad genética en una población. La heterocigosidad observada (H_o) representa la relación entre el número de individuos heterocigotos observados para un locus y el número total de individuos analizados para ese determinado locus. La heterocigosidad esperada o diversidad genética (H_e) representa la probabilidad de que dos alelos elegidos al azar en un individuo para un determinado locus sean diferentes entre sí (Hughes et al., 2008). Por ello, la identificación de desviaciones entre las heterocigosidades (esperada y observada) se ha utilizado como un indicador de la dinámica de una población (Nei, 1973). Para estimar la pérdida de heterocigosis conforme a la ley del equilibrio de Hardy-Weinberg (H-W), se utilizan los índices Estadísticos F de Wright, considerando tres niveles jerárquicos en una población (organismos individuales, subpoblaciones, y la población total) (Wright, 1951). El equilibrio H-W plantea que, en una población en la que el cruzamiento es aleatorio, la composición genética permanece en equilibrio mientras no haya mutaciones, migraciones o selección natural. Esta teoría funciona como hipótesis nula y se utiliza como referencia para evaluar la magnitud del cambio evolutivo en las poblaciones analizadas comparando las frecuencias genotípicas

estimadas en las poblaciones naturales que estudiamos con las que esperaríamos encontrar según el equilibrio H-W. El parámetro F_{IS} (coeficiente de endogamia) estima la reducción en la heterocigosidad de un individuo debido al cruzamiento no aleatorio dentro de su subpoblación y el parámetro F_{ST} (coeficiente de diferenciación genética o índice de fijación) mide el grado de diferenciación genética entre poblaciones. Por otro lado, la estructuración o distribución de la diversidad genética dentro y entre grupos de poblaciones se puede evaluar mediante un Análisis de Varianza Molecular (AMOVA), aplicándose también a diferentes niveles jerárquicos (Excoffier et al., 1992).

Tras el análisis de diversidad genética, el estudio de la estructura poblacional de la especie contribuye a mejorar el conocimiento de las dinámicas temporales y espaciales, así como de los factores evolutivos que explican la variabilidad genética (Liu et al., 2019). Los análisis de agrupamiento permiten clasificar individuos o entidades en función de sus características, de forma que los individuos con descriptores similares se agrupan juntos (Van de Peer and Salemi, 2009). Además, permiten revelar patrones de diversidad e identificar duplicados (Urrestarazu et al., 2018). Los principales procedimientos utilizados en estos análisis son i) los métodos basados en las distancias genéticas, como UPGMA (*Unweighted Pair Group Method with Arithmetic Average*) (Sneath and Sokal, 1973) y *Neighbour-Joining* (Saitou and Nei, 1987), ii) los métodos basados en caracteres, como máxima parsimonia o la inferencia bayesiana (Speed et al., 2006), y iii) los métodos basados en análisis multivariados, como el Análisis Discriminatorio de Componentes Principales (DAPC) (Jombart et al., 2010).

Los estudios de diversidad realizados en albaricoquero se han centrado en el estudio de la historia evolutiva del cultivo, así como en la caracterización de variedades locales en diferentes países. Sin embargo, la intensa renovación varietal llevada a cabo en los últimos años ha provocado que se desconozcan las relaciones de diversidad de la mayoría de las nuevas variedades desarrolladas en los programas de mejora.

Renovación varietal y mejora genética ⁴

Problemática del cultivo

A pesar de la importancia del cultivo del albaricoquero, tanto a nivel mundial como nacional presenta una serie de deficiencias y problemas que han sido la causa de su evolución decreciente durante los últimos años en muchas regiones del mundo. La aparición y diseminación del virus de la sharka (Plum pox virus) es uno de los mayores factores limitantes del cultivo del albaricoquero (García and Cambra, 2007). El virus se transmite por insectos y causa graves afecciones, principalmente en hojas (decoloración, manchas o anillos cloróticos, amarilleamiento de nervios, o incluso deformación) y frutos (anillos con depresiones y deformaciones con gomosis), lo que impide su comercialización. La principal estrategia para combatir esta enfermedad es el uso de material vegetal resistente que sustituya las variedades afectadas. Sin embargo, las variedades de origen europeo han demostrado susceptibilidad a la sharka (Martínez-Gómez et al., 2000; García and Cambra, 2007), por lo que ha sido necesaria una renovación varietal cuya adaptación ambiental también necesita ser estudiada.

El albaricoquero es una de las especies frutales más restringidas geográficamente para su cultivo debido a sus fuertes condicionantes climáticos (Guerriero and Bartolini, 1991). Es un cultivo de clima templado y, aunque es posible encontrar albaricoqueros en hábitats muy diversos (Mehlenbacher et al., 1991), su cultivo comercial está restringido a determinadas áreas geográficas con altitudes inferiores a 600 m (Got, 1963). La baja capacidad de adaptación de algunas variedades podría estar relacionada con la necesidad de cubrir adecuadamente las necesidades de frío. Además, el albaricoquero es un cultivo que presenta cuajados erráticos caracterizados por bajos cuajados y grandes oscilaciones de cosecha. Las variedades cultivadas tradicionalmente en Europa eran variedades autocompatibles, pero el número de variedades autoincompatibles ha aumentado en los últimos años (Herrera et al., 2021c), por lo que la incompatibilidad polen-pistilo podría ser una de las causas más frecuentes de falta de cuajado.

⁴ Parte de la información incluida en este apartado se encuentra publicada en:

Herrera, S., Lora, J., Hormaza, J. I., and Rodrigo, J. (2021c). Albaricoquero: renovación varietal y necesidades de polinización. *Agricultura* 1047, 52-56 (Anexo V).

Por otro lado, en cuanto a calidad de fruto, han aparecido nuevas exigencias en los mercados (Gatti et al., 2009). Las características exigidas por los consumidores han evolucionado hacia la demanda de frutos de piel y pulpa naranja con amplia chapa roja en la epidermis y elevada calidad gustativa.

Situación actual del albaricoquero

Los productores y los consumidores exigen una necesidad continua de desarrollo de nuevas variedades para dar una respuesta a las necesidades de producción del cultivo (Krška, 2018). En los últimos años, se han desarrollado programas de mejora en todo el mundo tanto de financiación pública como privados, que han lanzado al mercado variedades de albaricoquero con características mejoradas (Zhebentyayeva et al., 2012). Los principales objetivos de los programas de mejora del albaricoquero son resistencia a estreses bióticos (como la enfermedad de la sharka, podredumbre parda causada por *Monilinia* spp., enfermedades bacterianas causadas por *Pseudomonas* spp. y *Xanthomonas arboricola* pv. *pruni* (Smith)), adaptabilidad al entorno (requerimientos de temperatura, déficit hídrico), autocompatibilidad, extensión de la temporada de cosecha, calidad de la fruta para consumo en fresco y para procesamiento, productividad, y vigor y estructura adecuados de los árboles (Bassi and Audergon, 2006). Como consecuencia de esta situación, se está produciendo una intensa renovación varietal que ha ocasionado la introducción en el mercado de 322 nuevas variedades en Europa durante el periodo 1995-2020 (CPVO, 2022).

Sin embargo, el rápido lanzamiento de nuevas variedades ha provocado que se carezca de información agronómica de la mayoría de ellas. Por ello, es importante conocer sus necesidades de polinización, para la elección de variedades polinizadoras, así como sus necesidades agroclimáticas, para determinar su adaptación a cada zona de cultivo. También es importante determinar el grado de diversidad actual de la especie, incluyendo las nuevas variedades, y compararlo con la diversidad anterior para evitar la pérdida de germoplasma local de interés.

OBJETIVOS

El objetivo principal de la tesis doctoral es determinar las necesidades de polinización y las necesidades agroclimáticas de diferentes variedades de albaricoquero, así como evaluar el grado de diversidad genética del cultivo en la actualidad. El trabajo se ha estructurado en tres objetivos específicos:

Objetivo 1. Determinación de las necesidades de polinización

Determinación de la auto(in)compatibilidad de variedades de albaricoquero mediante la observación al microscopio de fluorescencia del crecimiento de tubos polínicos en flores autopolinizadas, establecimiento de las relaciones de incompatibilidad entre variedades mediante la caracterización del genotipo S por PCR y estudio de la diversidad genética actual del locus S (Capítulos 1-4).

Objetivo 2. Determinación de las necesidades agroclimáticas

Evaluación de la meiosis del polen como un marcador biológico para la determinación del fin de la endodormancia en albaricoquero y validación del método mediante la comparación con otros métodos previamente desarrollados para la estimación de las necesidades agroclimáticas en variedades comerciales (Capítulo 5).

Objetivo 3. Diversidad genética

Análisis de la diversidad genética, relaciones filogenéticas y estructura poblacional en variedades tradicionales de albaricoquero y nuevas obtenciones comerciales procedentes de diferentes programas de mejora de diversos países mediante marcadores moleculares tipo SSR (Capítulo 6).

CAPÍTULO 1. Determination of Self- and Inter-(in)compatibility Relationships in Apricot Combining Hand-Pollination, Microscopy and Genetic Analyses

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CAPÍTULO 1

Determination of Self- and Inter-(in)compatibility Relationships in Apricot Combining Hand-Pollination, Microscopy and Genetic Analyses

Determinación de las relaciones de auto(in)compatibilidad e inter(in)compatibilidad en albaricoquero combinando polinizaciones controladas, microscopía y análisis genéticos

RESUMEN

La autoincompatibilidad en Rosaceae está determinada por un Sistema Gametofítico de Autoincompatibilidad (GSI) que está controlado principalmente por el locus multialélico *S*. En albaricoquero, la determinación de las relaciones de auto(in)compatibilidad e inter(in)compatibilidad es cada vez más importante, ya que el desarrollo de un gran número de nuevas obtenciones ha resultado en el aumento de variedades con necesidades de polinización desconocidas. En este trabajo, se describe una metodología que combina la determinación de la auto(in)compatibilidad mediante polinizaciones controladas en laboratorio y microscopía con la identificación del genotipo *S* por análisis PCR. Para la determinación de la auto(in)compatibilidad, se recolectaron en campo flores en estado de botón globoso de cada variedad, se polinizaron manualmente en el laboratorio, se fijaron y se tiñeron con azul de anilina para observar el comportamiento de los tubos polínicos bajo el microscopio de fluorescencia. Para el establecimiento de relaciones de incompatibilidad entre variedades, se extrajo ADN de cada variedad a partir de hojas jóvenes y se identificaron alelos *S* por PCR. Esta metodología permite establecer grupos de incompatibilidad y dilucidar relaciones de incompatibilidad entre variedades, lo que proporciona información valiosa para elegir variedades polinizadoras adecuadas en el diseño de nuevas plantaciones y para seleccionar los parentales apropiados en programas de mejora.

Determination of Self- and Inter-(in)compatibility Relationships in Apricot Combining Hand-Pollination, Microscopy and Genetic Analyses

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Abstract

Self-incompatibility in Rosaceae is determined by a Gametophytic Self-Incompatibility System (GSI) that is mainly controlled by the multiallelic locus *S*. In apricot, the determination of self- and inter-(in)compatibility relationships is increasingly important, since the release of an important number of new cultivars has resulted in the increase of cultivars with unknown pollination requirements. Here, we describe a methodology that combines the determination of self-(in)compatibility by hand-pollinations and microscopy with the identification of the *S*-genotype by PCR analysis. For self-(in)compatibility determination, flowers at balloon stage from each cultivar were collected in the field, hand-pollinated in the laboratory, fixed, and stained with aniline blue for the observation of pollen tube behavior under the fluorescence microscopy. For the establishment of incompatibility relationships between cultivars, DNA from each cultivar was extracted from young leaves and *S*-alleles were identified by PCR. This approach allows establishing incompatibility groups and elucidate incompatibility relationships between cultivars, which provides a valuable information to choose suitable pollinizers in the design of new orchards and to select appropriate parents in breeding programs.

Introduction

Self-incompatibility is a strategy of flowering plants to prevent self-pollination and promote outcrossing¹. In Rosaceae, this mechanism is determined by a Gametophytic Self-Incompatibility System (GSI) that is mainly controlled by the multiallelic locus *S*². In the style, the *RNase* gene encodes the *S*-stylar determinant, a *RNase*³, while a F-box protein,

which determines the *S*-pollen determinant, is codified by the *SFB* gene⁴. The self-incompatibility interaction takes place

through the inhibition of pollen tube growth along the style preventing the fertilization of the ovule^{5, 6}.

In apricot, a varietal renewal has taken place worldwide in the last two decades^{7, 8}. This introduction of an important number of new cultivars, from different public and private breeding programs, has resulted in the increase of apricot cultivars with unknown pollination requirements⁸.

Different methodologies have been used to determine pollination requirements in apricot. In the field, self-(in)compatibility may be established by controlled pollinations in caged trees or in emasculated flowers and subsequently recording the percentage of fruit set^{9, 10, 11, 12}. In addition, controlled pollinations have been carried out in the laboratory by semi-in vivo culture of flowers and analysis of the pollen tube behavior under fluorescence microscopy^{8, 13, 14, 15, 16, 17}. Recently, molecular techniques, such as PCR analysis and sequencing, have allowed the characterization of incompatibility relationships based on the study of the *RNase* and *SFB* genes^{18, 19}. In apricot, thirty-three S-alleles have been reported (*S*₁ to *S*₂₀, *S*₂₂ to *S*₃₀, *S*₅₂, *S*₅₃, *S*_V, *S*_X), including one allele related with self-compatibility (*S*_C)^{12, 18, 20, 21, 22, 23, 24}. Up to now, 26 incompatibility groups have been established in this species according to the S-genotype^{8, 9, 17, 25, 26, 27}. Cultivars with the same S-alleles are inter-incompatible, whereas cultivars with at least one different S-allele and, consequently, allocated in different incompatible groups, are inter-compatible.

To define the pollination requirements of apricot cultivars, we describe a methodology that combines the determination of self-(in)compatibility by fluorescence microscopy with the identification of the S-genotype by PCR analysis in apricot cultivars. This approach allows establishing incompatibility

groups and elucidate incompatibility relationships between cultivars.

Protocol

1. Self-(in)compatibility determination

1. Sample the flowers in the field. It is necessary to collect the flowers at balloon stage (**Figure 1A**), corresponding to stage 58 on the BBCH scale for apricot²⁸, to avoid unwanted previous pollination.
2. **Self- and cross-pollinations in the laboratory**
 1. Remove the anthers of the flowers at balloon stage and place them on a piece of paper to dry at laboratory temperature.
 2. After 24 h, sieve the pollen grains by using a fine mesh (0.26 mm) (**Figure 1B**).
 3. Emasculate a group of 30 flowers at the same balloon stage for each self-pollination and cross-pollination and place the pistils on florist foam in water at laboratory temperature (**Figure 1C**).
 4. Hand pollinate the pistils with the help of a paintbrush with pollen from flowers of the same cultivar 24 h after emasculation. In addition, pollinate another set of pistils of each cultivar with pollen from flowers of a compatible pollinizer as control (**Figure 1D**).
 5. After 72 h, fix the pistils in a fixative solution of ethanol/ acetic acid (3:1) for at least 24 h at 4 °C²⁹. Then discard the fixative and add 75% ethanol ensuring that the samples are completely submerged in the solution. Samples can be conserved in this solution at 4 °C until use^{8, 17, 30, 31, 32}.

3. Evaluating pollen viability through in vitro pollen germination

1. To prepare the germination medium, weight 25 g of sucrose, 0.075 g of boric acid (H_3BO_3) and 0.075 g of calcium nitrate ($Ca(NO_3)_2$)³³.
2. Add the components of the medium in 250 mL of distilled water and dissolve completely.
3. Solidify the medium adding 2 g of agarose and mix by swirling.
4. Check the pH of the medium using a pH meter and adjust the value to 7.0 with NaOH or HCl solution.
5. Autoclave the mixture to sterilize the medium.
6. After autoclaving, cool down the medium and distribute it into Petri dishes in a sterile laminar flow hood.
7. Scatter the pollen grains of the same cultivars used for the controlled pollinations in the solidified pollen germination medium and observe them under the microscope after 24 h⁶.

NOTE: To sterilize the laminar flow hood, clean the surface with 70% ethanol and switch on the UV lamp during 10 min.

8. Store the Petri dishes in a refrigerator at 4 °C until use.

4. Microscopy observations

1. Wash the pistils three times for 1 h with distilled water and leave them in 5% sodium sulphite at 4 °C. After 24 h, autoclave them at 1 kg/cm² during 10 min in sodium sulphite to soften the tissues³⁴.
2. Place the autoclaved pistils over a glass slide and, with the help of a scalpel, remove the trichomes around the ovary to get a better visualization of the

pollen tubes. Then, squash the pistils with a cover glass.

3. Prepare 0.1% (v/v) aniline blue stain: mix 0.1 mL of aniline blue in 100 mL of 0.1 N potassium phosphate tribasic (K_3PO_4). Apply a drop of aniline blue over the preparations to stain callose depositions during pollen tube growth.
4. Observe the pollen tubes along the style by a microscope with UV epifluorescence using 340-380 bandpass and 425 longpass filters.

2. DNA extraction

1. Sample 2-3 leaves in the field in spring. It is recommended to sample the leaves at young stages since DNA obtained is of higher quality and lower levels of phenolic compounds compared to old leaves.
2. Extract Genomic DNA following the steps described in a commercially available kit (see **Table of Materials**).
3. Analyze the quantity and quality of DNA concentrations using UV-vis spectrophotometer (260 nm).

3. S-allele identification

1. Setting up of the PCR Reactions

1. Prepare a 50 ng/μL dilution in distilled water of each DNA extraction sample.
2. Thaw out the PCR reagents slowly and keep them on ice. Leave the DNA polymerase in the freezer until needed.
3. Prepare the amplification reactions using the different combinations of primers. Create the PCR reaction mix by combining the components in **Table 1**. Vortex the PCR reaction mix well and distribute the volume

indicated for the different combinations of primers to each well of the PCR plate. Then, add 1 μL of the DNA dilution in each well.

4. Place the PCR plate in the thermocycler and run the corresponding PCR program shown in **Table 1**.

2. Analyze the amplified fragments. There are mainly two different ways to analyze the PCR amplified fragments: capillary electrophoresis (CE) with fluorescent-labelled primers or as visualize amplicons of agarose gel electrophoresis with not-labelled primers.

1. Capillary Electrophoresis

1. To prepare the loading buffer, mix 35 μL of deionized formamide with 0.45 μL of labeled sizing standard. Vortex the reagent to mix well, and then dispense 35.5 μL into the well of the reader plate.
2. Add 1 μL of the PCR product into the well. In addition, add a drop of mineral oil to prevent water evaporation.
3. Prepare the separation plate adding separation buffer.
4. Use the commercial software included with the gene analyzer (see **Table of Materials**). Create a new sample plate and save the sample names for all wells on the plate.
5. Select the method of analysis. In this case, denature the samples at 90 °C for 120 s, inject at 2.0 kV for 30 s, and separate at 6.0 kV for 35 min.
6. Insert the two plates into the gene analyzer. Fill the capillary array with distilled water.
7. Load the patented linear polyacrylamide (LPA) gel. Finally, click **Run**.

2. Gel Electrophoresis

1. Prepare a 1% agarose gel adding 1.5 g of molecular biology grade agarose in 150 mL of 1x TAE (Tris-acetate-EDTA) electrophoresis running buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA at pH 8.0). Dissolve the agarose by microwave heating for 2-3 min.
2. To visualize the DNA, add 4 μL of a nucleic acid stain (see Table of Materials) and mix gently.
3. Add a gel comb, with sufficient wells for ladders, controls and samples, into a gel tray. Then, pour slowly the mix into the middle of the gel tray and avoid bubbles.
4. Let the gel cool down for 30-45 min at room temperature until the gel has completely solidified. Introduce the gel in the electrophoresis chamber, remove the gel comb and fill the chamber with enough 1x TAE buffer to cover the gel.
NOTE: Check the placement of the gel. The wells should be placed close to the negative pole since negatively charged DNA migrates towards the cathode.
5. Add 5 μL of loading buffer (0.1% (v/v) bromophenol blue) to the PCR products and mix well.
6. To estimate the size of the bands, load 5 μL of DNA molecular weight ladder (see **Table of Materials**).
7. Load the samples into the additional wells of the gel.
8. Once all the samples and the DNA molecular weight ladder are loaded, run the gel at 90 V for

1-1.5 h, until the blue dye line is approximately at 75% the length of the gel.

9. Visualize the bands in a transilluminator for nucleic acids.

Representative Results

Pollination studies in apricot require the use of flowers at the late balloon stage one day before anthesis (**Figure 1A**). This stage is considered the most favorable for both pollen and pistil collection, since floral structures are nearly mature, but anther dehiscence has not yet occurred. This prevents the interference of undesired pollen, not only of pollen from the same flower but also from other flowers, since the closed petals impede the arrival of insects carrying external pollen. The pollen grains are easily sieved through a fine mesh (**Figure 1B**) from dehiscent anthers previously placed on a piece of paper for 24 h at room temperature or with slight extra heat. Likewise, pistils are obtained from flowers at balloon stage after the removing of petals, sepals and stamens with the help of tweezers or fingernails (**Figure 1C**). Pistils can be self- and cross-pollinated with a fine brush (**Figure 1D**).

The hermaphroditic flowers of apricot have five dark red sepals, five white petals (**Figure 1A**), a single pistil (**Figure 2A**) and 25-30 stamens. The pistil has three main structures: stigma, style and ovary. The ovary has two ovules, and the fertilization of at least one of them is required for fruit setting. During pollination, insects, mainly bees, transfer pollen grains to the stigma (**Figure 1A**), where they germinate (**Figure 2B**) within 24 h following pollination. A pollen tube is produced from each germinating pollen grain, which grows through the pistil structures to reach the ovary after 3-4 days and fertilize one of the two ovules after around 7 days. In self-incompatible cultivars in which the *S* allele of the pollen grain is the same as one of the two of the pistils, pollen tube stops growing at

the upper style, preventing fertilization (**Figure 2C**). However, the pollen tubes from a compatible cultivar, with a different *S* allele, can grow through the style (**Figure 2D**), reach the ovary (**Figure 2E**) and fertilize one of the two ovules.

The analysis of in vitro pollen germination showed good pollen viability in all the cultivars analyzed here, since most pollen tubes were longer than the length of the pollen grain after 24 h in the culture medium. Germinated pollen grains were observed at the stigma surface (**Figure 2B**) in pistils from all pollinations, indicating adequate pollination (**Figure 3**).

To determine the self-(in)compatibility for each cultivar, pollen tube behavior in self- and cross-pollinations done in laboratory-controlled conditions was observed under fluorescence microscopy. Pollen tube growth was recorded along the style in all the pistils examined. Cultivars were considered as self-incompatible when pollen tube growth was arrested along the style in most self-pollinated pistils (**Figure 2C**, **Figure 3**) and self-compatible when at least one pollen tube reached the base of the style in most of the pistils examined (**Figure 2E**, **Figure 3**).

The study of the *S*-locus by PCR analysis allowed characterizing the *S*-genotype of each cultivar. Firstly, the *S*-alleles were identified by the amplification of the first *S-RNase* intron using the primers SRC-F/SRC-R (**Table 2**). The size of the amplified fragments was analyzed by capillary electrophoresis (**Figure 4A**) and was used to classify the genotypes analyzed in their corresponding incompatibility group (I.G.) (**Table 3**).

Some pairs of alleles, such as *S*₁ and *S*₇ or *S*₆ and *S*₉, showed similar fragment sizes for the first intron. Thus, the differentiation of these alleles was done by amplifying a region of the second intron of the *RNase* with the primers Pru-C2/

PruC4R, SHLM1/SHLM2 and SHLM3/SHLM4 (**Table 2**). The PruC2/PruC4R primer combination was used to distinguish between S_6 and S_9 . For S_6 , a fragment of 1300 bp was amplified whereas a fragment of around 700 bp was observed for the S_9 allele (**Figure 4B**, **Table 3**). The specific primers SHLM1/SHLM2 and SHLM3/SHLM4 amplified a fragment of approximately 650 bp in the S_1 allele and 413 bp in the S_7 allele (**Figure 4C**, **Table 3**).

The primers AprFBC8-(F/R) that amplify the V2 and HVb variable regions of the *SFB* gene were used to distinguish S_C and S_8 alleles since both alleles show identical *RNase* sequence. The S_8 allele showed a PCR-fragment

of approximately 150 bp whereas a 500 bp fragment corresponded to the S_C allele (**Figure 4D**, **Table 3**). Once the S -genotype was determined for all the cultivars, self-incompatible cultivars were assigned to their corresponding incompatibility groups based on their S -alleles (**Table 3**).

This approach requires determining the self-(in)compatibility of each cultivar by controlled self- and cross-pollinations in the laboratory (**Figure 5A**) concomitantly with the characterization of the S -genotype by genetic analysis (**Figure 5B**). As a result, the pollination requirements of each cultivar and the incompatibility relationships among apricot cultivars can be determined.

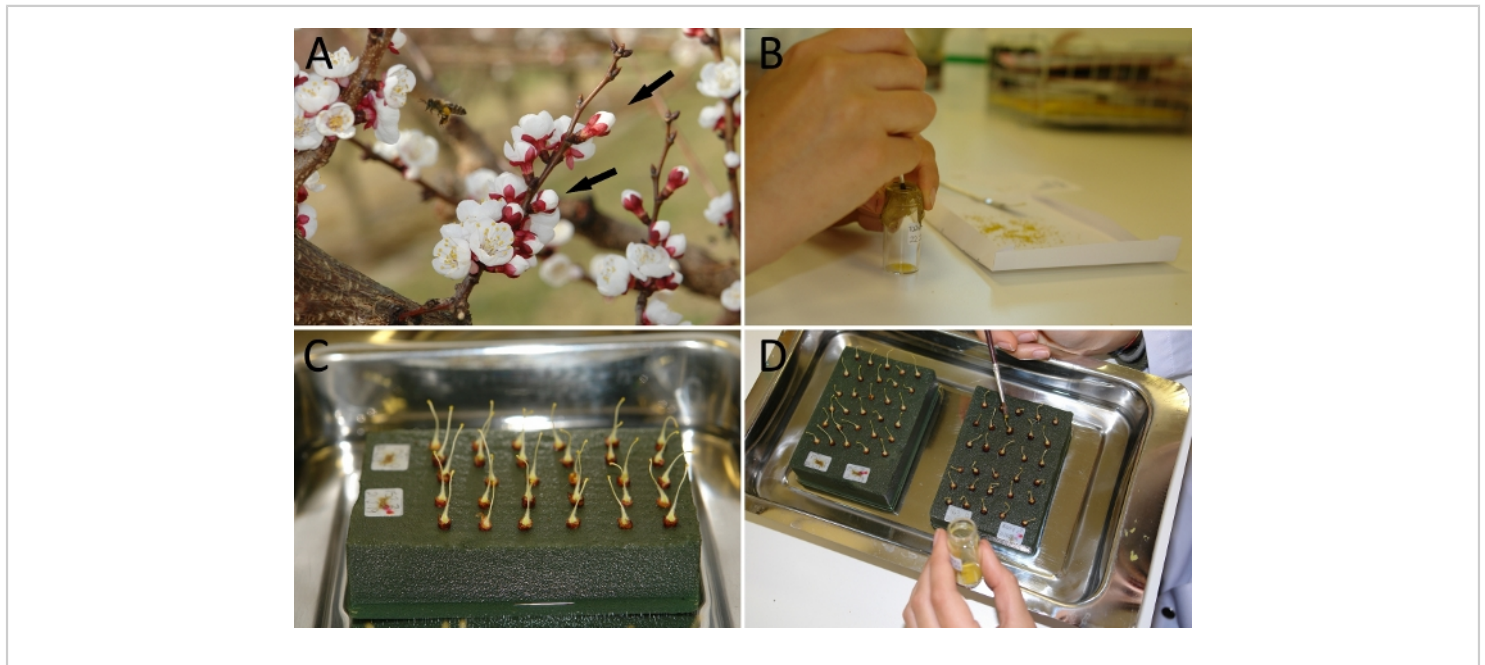


Figure 1. Experimental set up for the determination of self-(in)compatibility in apricot.

(A) Flowers at balloon stage (black arrows) in the field. (B) Sieve of pollen grains using a fine mesh. (C) Pistils placed on florist foam in water. (D) Hand-pollination of the pistils with the help of a paintbrush. [Please click here to view a larger version of this figure.](#)



Figure 2. Diagrammatic representation of gametophytic incompatibility relationships in apricot flowers.

(A) In Gametophytic Self-Incompatibility (GSI), both compatible and incompatible pollen grains germinate on the stigma. The pollen grain carries one of two S-alleles of the original genotype, in this case either S_1 or S_2 . If the S-allele of the pollen grain matches one of the two S-alleles of the pistil, in this case S_1S_3 , pollen tube growth is inhibited in the upper one-third of the style. (B) Germination of pollen grains on the stigma surface. (C) Pollen tube arrested in the style indicating an incompatible behavior. (D) Pollen tubes growing along the style. (E) Pollen tubes at the base of the style indicating a compatible behavior. Scale bars, 100 μ m. [Please click here to view a larger version of this figure.](#)



Figure 3. Representative results of pollen germination and pollen tube growth through the style for self-compatible and self-incompatible cultivars after self-pollinations.

Percentage of pistils with pollen grains germinating at the stigma surface, with pollen tubes in halfway the style, at the base of the style, and reaching the ovule. [Please click here to view a larger version of this figure.](#)

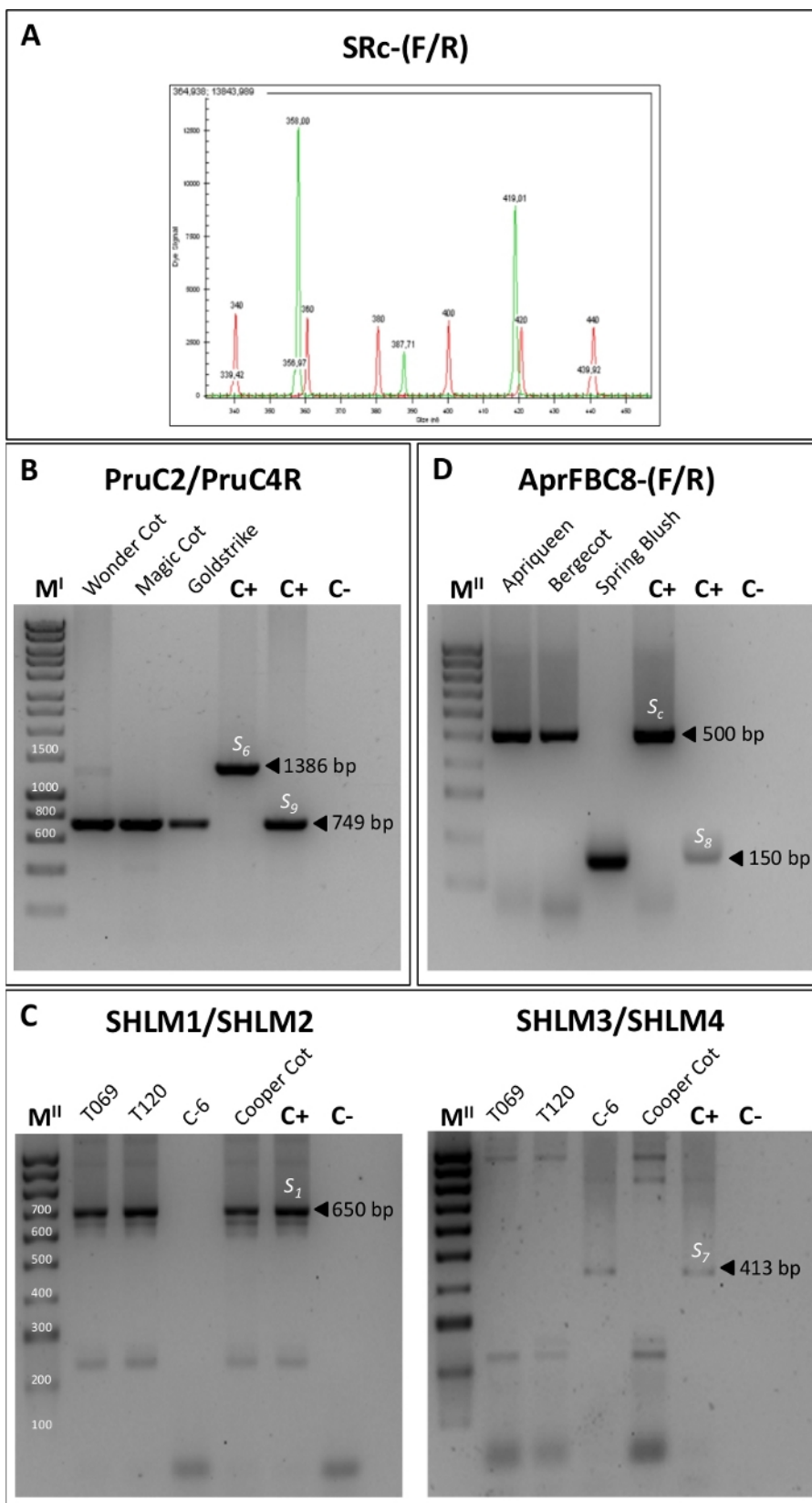


Figure 4. PCR fragment amplification using five primer pair combinations for the identification of S-alleles.

(A) Gene analyzer output for the SRc-(F/R) primers showing the size of the two amplified fragments of the *RNase* first intron region corresponding to the S-alleles. (B) PCR amplification using the primers PruC2/PruC4R for the identification of the S_6 and S_9 alleles. (C) PCR products obtained using the specific primers SHLM1 and SHLM2 for the differentiation of the S_1 allele and SHLM3 and SHLM4 to distinguish the S_7 allele. (D) PCR amplification with the AprFBC8-(F/R) primers for identifying S_C and S_8 alleles. M^I : 1 kb DNA Ladder. M^{II} : 100 bp DNA Ladder. [Please click here to view a larger version of this figure.](#)

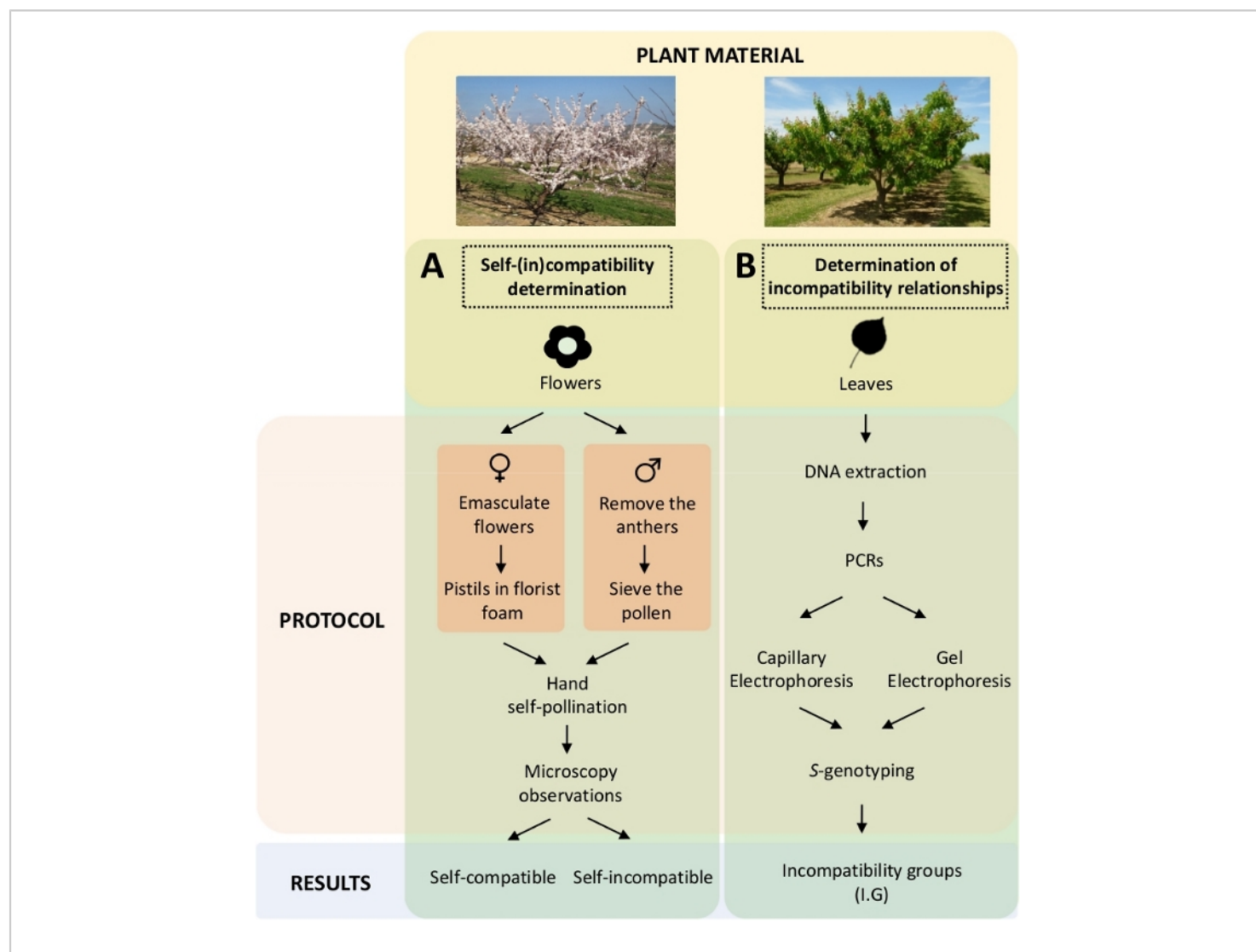


Figure 5. Scheme of the experimental design to elucidate the self- and inter-(in) compatibility relationships in apricot cultivars.

(A) Workflow of self-(in)compatibility determination by controlled pollinations in the laboratory. (B) Workflow of the S-allele identification by molecular approaches. [Please click here to view a larger version of this figure.](#)

PCR Master Mix				Thermocycler conditions			
Components	Final Concentration	15 µL reaction		Cycle Step	Temperature	Time	Cycles
10x NH ₄ Reaction Buffer	10x	1.5 µL		Initial denaturation	94 °C	3 min	1
50 mM MgCl ₂ Solution	25 mM	1.2 µL		Denaturing	94 °C	1 min	35
100 mM dNTP	2.5 mM	0.6 µL		Annealing	55 °C	1 min	
Primer SRc-F	10 µM	0.6 µL		Extension	72 °C	3 min	
Primer SRc-R	10 µM	0.6 µL		Final Extension	72 °C	5 min	1
500 U Taq DNA Polymerase	0.5 U	0.2 µL			4 °C	hold	
H ₂ O		8.3 µL					
Components	Final Concentration	25 µL reaction	Cycle Step	Temperature	Time	Cycles	
10x PCR buffer	10x	2.5 µL	Initial denaturation	94 °C	2 min	1	
5x Q-solution	5x	5 µL	Denaturing	94 °C	10 s	10	
100 mM dNTP	2.5 mM	0.5 µL	Annealing	55 °C	2 min		
Primer PruC2	10 µM	0.2 µL	Extension	68 °C	2 min		
Primer C4R	10 µM	0.2 µL	Denaturing	94 °C	10 s	25	
250 U Taq DNA Polymerase	10 U	0.13 µL	Annealing	58 °C	2 min		

H ₂ O		15.5 µL
Components	Final Concentration	25 µL reaction
10x PCR buffer	10x	2.5 µL
5x Q-solution	5x	5 µL
100 mM dNTP	2.5 mM	0.5 µL
Primer SHLM1	10 µM	0.2 µL
Primer SHLM2	10 µM	0.2 µL
250 U Taq DNA Polymerase	10 U	0.13 µL
H ₂ O		15.5 µL
Components	Final Concentration	20 µL reaction
5x PCR Buffer	5x	4 µL
dNTP	2.5 mM	1.6 µL
Primer SHLM3	10 µM	1 µL
Primer SHLM4	10 µM	1 µL

Extension*	68 °C	2 min	
Final Extension	72 °C	5 min	1
	4 °C	hold	
* with 10 s added each cycle to the 68 %C extension step.			
Cycle Step	Temperature	Time	Cycles
Initial denaturation	94 °C	2 min	1
Denaturing	94 °C	30 s	35
Annealing	62 °C	1.5 min	
Extension	72 °C	2 min	
Final Extension	72 °C	5 min	1
	4 °C	hold	
Cycle Step	Temperature	Time	Cycles
Initial denaturation	98 °C	30 s	1
Denaturing	98 °C	10 s	35
Annealing	51 °C	30 s	
Extension	72 °C	1 min	

100 U DNA Polymerase	5 U	0.2 μ L
H ₂ O		12.4 μ L
Components	Final Concentration	25 μL reaction
10x PCR buffer	10x	2.5 μ L
100 mM dNTP	2.5 mM	2 μ L
Primer FBC8-F	10 μ M	1 μ L
Primer FBC8-R	10 μ M	1 μ L
250 U Taq DNA Polymerase	10 U	0.125 μ L
H ₂ O		17.4 μ L

Final Extension	72 °C	5 min	1
	4 °C	hold	
Cycle Step	Temperature	Time	Cycles
Initial denaturation	94 °C	2 min	1
Denaturing	94 °C	30 s	35
Annealing	55 °C	1.5 min	
Extension	72 °C	2 min	
Final Extension	72 °C	5 min	1
	4 °C	hold	

Table 1. Reaction and cycling conditions for different primer combinations used in this protocol.

Primers	Sequence	Reference
SRc-F	5'-CTCGCTTTCCTTGTCTTGC-3'	18
SRc-R	5'-GGCCATTGTTGCACCCCTTG-3'	18
Pru-C2	5'- CTTTGGCCAAGTAATTATTCAAACC-3'	35
Pru-C4R	5'-GGATGTGGTACGATTGAAGCG-3'	35
SHLM1-F	5'-GGTGGAGGTGATAAGGTAGCC-3'	17
SHLM2-R	5'-GGCTGCATAAGGAAGCTGTAGG-3'	17
SHLM3-F	5'-TATATCTTACTCTTTGGC-3'	17
SHLM4-R	5'-CACTATGATAATGTGTATG-3'	17
AprFBC8-F	5'-CATGGAAAAAGCTGACTTATGG-3'	26
AprFBC8-R	5'- GCCTCTAATGTCATCTACTCTTAG-3'	26

Table 2. Primers used in this protocol, sequence and reference for S-genotype characterization in *Prunus armeniaca*.

Cultivar	SRc-(F/R) (bp)	PruC2/PruC4R (bp)	SHLM1/SHLM2 (bp)	SHLM3/SHLM4 (bp)	AprFBC8-(F/R) (bp)	S-Genotype	Incompatibility group (I.G)
Wonder Cot ⁸	420, 420	749, 1386				S ₆ S ₉	VIII
Magic Cot ⁸	334, 420	749				S ₂ S ₉	XX
Goldstrike ⁸	334, 420	749				S ₂ S ₉	-
T069 ¹⁷	334, 408		650			S ₁ S ₂	I
T120 ¹⁷	334, 408		650			S ₁ S ₂	-
C-6	334, 408			413		S ₂ S ₇	IV
Cooper Cot ⁸	274, 408		650			S ₁ S ₃	XVIII
Apriqueen	358, 358				500	S _c S _c	-
Bergecot ⁸	334, 358				500	S ₂ S _c	-
Spring Blush ⁸	274, 358				150	S ₃ S ₈	XXI

Table 3. S-genotyping of apricot cultivars with five primer pairs used in this protocol and incompatibility group assignment. The different polymerase chain reaction product sizes of S-alleles amplified using SRc-(F/R), PruC2/PruC4R, SHLM1/ SHLM2, SHLM3/SHLM4, and AprFBC8-(F/R) primers are shown in the table.

Discussion

Traditionally, most commercial apricot European cultivars were self-compatible³⁶. Nevertheless, the use of North American self-incompatible cultivars as parents in breeding programs in the last decades has resulted in the release of an increasing number of new self-incompatible cultivars with unknown pollination requirements^{7, 8, 37}. Thus, the determination of self- and inter-(in)compatibility relationships in apricot cultivars is increasingly important. This is accentuated in those areas where winter chilling is decreasing, since high year to year variations in the time of flowering are preventing the coincidence in flowering of cultivars and their pollenizers in many cases, especially in

cultivars with high chilling requirements³⁸. The methodology described herein, combining hand-pollination, microscopy and genetic analyses has been very useful to determine the self(in)compatibility of each cultivar and to establish its potential pollinizer cultivars.

Pollination requirements can be determined through field-control experiments in orchard conditions^{11, 39}. However, the exposition to external factors including meteorological adverse conditions can cause pollination failure¹⁰, which may result in erroneous diagnoses of self-incompatibility. The methodology described herein allows to evaluate self-(in)compatibility more accurately by microscopy observations of hand-pollinated flowers in laboratory-controlled conditions,

avoiding environmental influence. Moreover, this approach allows analyzing a higher number of cultivars per year, since only a small number of flowers is required instead of several adult trees for each cultivar that are required in field experiments⁴⁰.

Incompatibility relationships can be established combining hand-pollinations and microscopy¹⁴. However, pollinations can only be performed for a short period during the flowering season in spring, and adult trees near the laboratory are needed, since the lifespan of the flowers collected is very short. Thus, the number of incompatibility relationships that can be analyzed by controlled hand-pollinations in each season is very low. The characterization of the genes encoded by the *S*-locus has enabled the development of PCR-based methods for *S*-allele genotyping^{18, 41}. This approach accelerates *S*-allele identification since it does not require flowers, and the experiments can be carried out with any vegetative tissue⁴². This extends the period during which plant material, usually young leaves, can be collected⁴³. Furthermore, the leaves can be lyophilized or frozen, so that the analysis can be done at any time of the year, unlike pollinations that can only be done on fresh flowers during the flowering season⁴⁴. An additional benefit is that leaves can be collected from young trees even before entering flowering age, facilitating the collection of samples and the early obtaining of results⁴⁵.

The genetic analysis allows a better differentiation of self-incompatibility alleles since it provides precise results of amplified fragment sizes^{21, 46}. To date, thirty-three *S*-alleles have been identified in apricot^{12, 18, 20, 21, 22, 23, 24}, which has allowed to establish 36 incompatibility groups based on *S*-genotype^{8, 9, 17, 25, 26, 27}. On the other hand, a drawback of this methodology is that different alleles in the

same range size or mutations can be erroneously identified as the same allele. Thus, *S_C* and *S₈* alleles are identical for the *RNase* sequence but a 358-bp insertion is found in the *SFB* gene of *S_C*¹⁹. Likewise, the first intron region of the alleles *S₁* and *S₇* are identical and are indistinguishable using the primers *SRc-F/SRc-R*. In addition, several homologies, such as *S₆* and *S₅₂*⁸ or *S₂₀* and *S₅₅*, and *S₇*, *S₁₃* (EF062341) and *S₄₆*¹⁷, have been found because some of these alleles have been partially sequenced or by failures during PCR amplification and, consequently, further work is needed to distinguish them correctly.

PCR analysis and *S-RNase* sequencing are adequate for establishing incompatibility relationships through the identification of *S*-alleles and the allocation of cultivars in their corresponding Incompatibility Group^{8, 17, 26, 27}. However, this methodology has the limitation of preventing the determination of the self-(in)compatibility for particular apricot cultivars. Self-compatibility (SC) has been associated to particular *S*-alleles in other *Prunus* species⁴⁷, as almond (*S_f*)^{48, 49} or sweet cherry (*S₄*)^{50, 51}. However, in apricot, the *S_C* allele, which has been associated to SC²¹, can be erroneously identified as *S₈*, a self-incompatible allele^{19, 22}, and possible mutations not linked to the *S* locus, as the *M*-locus^{12, 52}, conferring SC have been identified. Recently, the *M*-locus has been genotyped using SSR markers¹². Therefore, the genetic identification of SC for apricot genotypes needs further research and, in order to avoid mistakes due to factors not linked to the *S* locus, in this work the characterization of self-(in)compatibility has been determined also by phenotyping the behavior of the pollen tubes through the pistil of self-pollinated flowers.

The methodology described herein combining the determination of self-(in)compatibility by hand-pollinations

in laboratory conditions with the subsequent observation of the behavior of pollen tubes in the pistil of controlled self-pollinations under the fluorescence microscopy and the identification of the S-genotype by PCR analysis allows establishing the pollination requirements of apricot cultivars. This provides a valuable information for growers and breeders, since it allows establishing the incompatibility relationships between cultivars to choose suitable pollinizers in the design of new orchards as well as to select appropriate parents to design new crosses in apricot breeding programs.

Disclosures

The authors have nothing to disclose.

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CAPÍTULO 2. Optimizing Production in the New Generation of Apricot Cultivars: Self-incompatibility, *S-RNase* Allele Identification, and Incompatibility Group Assignment

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CAPÍTULO 2

Optimizing Production in the New Generation of Apricot Cultivars: Self-incompatibility, *S-RNase* Allele Identification, and Incompatibility Group Assignment

Optimización de la producción en la nueva generación de variedades de albaricoquero: auto(in)compatibilidad, identificación de alelos *S-RNasa* y clasificación en grupos de incompatibilidad

RESUMEN

El albaricoquero (*Prunus armeniaca* L.) es una especie de las Rosáceas que se originó en Asia Central, desde donde se introdujo en Europa a través de Armenia. El desarrollo de un número cada vez mayor de nuevas variedades procedentes de diferentes programas de mejora está provocando una importante renovación de material vegetal a nivel mundial. Aunque la mayoría de las variedades tradicionales de albaricoquero en Europa son autocompatibles, el uso de variedades autoincompatibles como genotipos parentales para la mejora genética está conduciendo a la introducción de una serie de nuevas variedades autoincompatibles. Como consecuencia, existe una creciente necesidad de cultivar esas nuevas variedades con variedades intercompatibles que actúen de polinizantes para asegurar la producción de frutos en las plantaciones comerciales. Sin embargo, se desconocen las necesidades de polinización de muchas de estas nuevas variedades. En este trabajo, hemos analizado las necesidades de polinización de un grupo de 92 variedades de albaricoquero, incluyendo variedades tradicionales y nuevas variedades comerciales de diferentes programas de mejora de diversos países. La auto(in)compatibilidad se estableció mediante la observación del comportamiento de los tubos polínicos en flores autopolinizadas bajo el microscopio. Las relaciones de incompatibilidad entre variedades se establecieron mediante la identificación de los alelos *S* mediante análisis PCR. En este trabajo, se ha reportado por primera vez la auto(in)compatibilidad de 68 variedades y el genotipo *S-RNasa* de 74 variedades. Aproximadamente la mitad de las variedades (47) se comportaron como autocompatibles y las otras 45 como autoincompatibles. La identificación de los alelos *S* en las variedades autoincompatibles permitió clasificarlas en 11 grupos de incompatibilidad, seis de ellos descritos aquí por primera vez. La determinación de las necesidades de polinización y las relaciones de incompatibilidad entre variedades es muy valiosa para la selección adecuada de variedades albaricoquero en plantaciones comerciales y de genotipos parentales en los programas de mejora. La metodología descrita puede ser transferida a otros cultivos leñosos perennes con problemas similares.



Optimizing Production in the New Generation of Apricot Cultivars: Self-incompatibility, S-RNase Allele Identification, and Incompatibility Group Assignment

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Apricot (*Prunus armeniaca* L.) is a species of the Rosaceae that was originated in Central Asia, from where it entered Europe through Armenia. The release of an increasing number of new cultivars from different breeding programs is resulting in an important renewal of plant material worldwide. Although most traditional apricot cultivars in Europe are self-compatible, the use of self-incompatible cultivars as parental genotypes for breeding purposes is leading to the introduction of a number of new cultivars that behave as self-incompatible. As a consequence, there is an increasing need to interplant those new cultivars with cross-compatible cultivars to ensure fruit set in commercial orchards. However, the pollination requirements of many of these new cultivars are unknown. In this work, we analyze the pollination requirements of a group of 92 apricot cultivars, including traditional and newly-released cultivars from different breeding programs and countries. Self-compatibility was established by the observation of pollen tube behavior in self-pollinated flowers under the microscope. Incompatibility relationships between cultivars were established by the identification of S-alleles by PCR analysis. The self-(in)compatibility of 68 cultivars and the S-RNase genotype of 74 cultivars are reported herein for the first time. Approximately half of the cultivars (47) behaved as self-compatible and the other 45 as self-incompatible. Identification of S-alleles in self-incompatible cultivars allowed allocating them in 11 incompatibility groups, six of them reported here for the first time. The determination of pollination requirements and the incompatibility relationships between cultivars is highly valuable for the appropriate selection of apricot cultivars in commercial orchards and of parental genotypes in breeding programs. The approach described can be transferred to other woody perennial crops with similar problems.

Keywords: *Prunus armeniaca*, self-incompatibility, S-alleles, S-genotype, ovary, pollen tube, pollination, style

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is considered as one of the most delicious temperate fruits (Faust et al., 1998). Apricot is a species of the Rosaceae, one of the most economically important plant families in temperate regions worldwide (Dirlewanger et al., 2004). Although the Latin name of apricot (*armeniaca*) and later its scientific name (*P. armeniaca*) could wrongly suggest an origin from Armenia, apricot was indeed originated in Central Asia, where the first orchards of apricot were described 406–250 BC (Janick, 2005), whereas Armenia was the route by which apricot first entered Europe. Apricot was already mentioned as *Mela armeniaca* by Roman authors around 50 A.D., which could indicate its introduction in the Roman empire during the first century (Faust et al., 1998). The English name apricot (apricock in the old spelling) derives from the Arabic and Greek term *al-praeocx* that means “early fruit” (Faust et al., 1998; Janick, 2005). Traditionally, apricot cultivars have been classified in six main groups depending on the geographical origin: Dzhungar-Zailij, East Chinese, European Iranian-Caucasian, Middle-Asian, and North Chinese (Layne et al., 1996).

Apricot cultivars from the Central Asian, the Dzhungar-Zailij, and the Iranian-Caucasian groups are mostly self-incompatible. However, cultivars from the European group, which is the least variable and the most recent, are mainly self-compatible and include most of the commercial cultivars (Mehlenbacher et al., 1991; Hormaza et al., 2007). In the Rosaceae, the incompatibility mechanism to reduce self-fertilization and promote outcrossing is based on cell-cell recognition that is determined genetically by a gametophytic self-incompatibility System (GSI). This mechanism acts through the inhibition of pollen tube growth in the style (de Nettancourt, 2001) and is controlled by a multiallelic locus named *S*, encoding two linked genes that determine the pistil and pollen genotypes (Charlesworth et al., 2005). A ribonuclease (*S*-RNase), which is a glycoprotein secreted in the style mucilage, determines the allele specificity of the style (Tao et al., 1997) whereas an F-box protein (SFB) specifically expressed in pollen determines pollen allele specificity (Ushijima et al., 2003).

The introduction of an increasing number of new apricot cultivars from different breeding programs is resulting in an important renewal of plant material worldwide (Zhebentyayeva et al., 2012). Thus, the initial classification of six ecogeographical groups is becoming increasingly complex, since many of the new cultivars are derived from crosses between genotypes of different ecogeographical groups (Faust et al., 1998; Halász et al., 2007). Moreover, although most traditional apricot cultivars in Europe are self-compatible (Burgos et al., 1997), the use of self-incompatible cultivars developed in North America as parental genotypes in several breeding programs, with the objective of incorporating resistance to sharka (Hormaza et al., 2007; Zhebentyayeva et al., 2012), is leading to the introduction of new self-incompatible cultivars. As a consequence, there is an increasing need to interplant those new cultivars with cross-compatible cultivars to ensure fruit set in commercial orchards. However, the pollination requirements of many of these new cultivars are unknown.

The pollination requirements of a cultivar can be established by carrying out controlled pollinations in the field and recording the percentage of fruit set. Final fruit set in apricot is usually established during the first 4 weeks following pollination (Rodrigo et al., 2009; Julian et al., 2010). However, incompatibility can be determined more accurately under a fluorescence microscope by the observation of pollen tube growth through the style in self- and cross-pollinated flowers in squash preparations of pistils after staining with aniline blue (Burgos et al., 1993; Rodrigo and Herrero, 1996, 2002; Julian et al., 2010). In self-incompatible genotypes and incompatible crosses, pollen tube growth is arrested in the style and, therefore, fertilization of the ovules is prevented since no pollen tubes reach the ovary. However, in self-compatible genotypes and compatible crosses, pollen tubes can grow along the style and reach the ovary, where fertilization of some of the two ovules can take place. This histochemical approach allows the identification of pollination failure from diverse environmental factors that can affect fruit set under field conditions (Guerra and Rodrigo, 2015).

In addition, advances in the study of the molecular determinants of self-incompatibility have allowed developing tools to analyze the allelic composition of the self-incompatibility locus. Thus, the identification of the *S*-RNase gene in apricot (Romero et al., 2004; Sutherland et al., 2004) allowed developing an *S*-allele genotyping PCR strategy, similar to those developed for cherry or almond (Sutherland et al., 2004). To date, 33 *S*-alleles (*S*₁ to *S*₂₀, *S*₂₂ to *S*₃₀, *S*₅₂, *S*₅₃, *S*_v, and *S*_x), including one allele for self-compatibility (*S*_c), have been identified in apricot (Halász et al., 2005; Vilanova et al., 2005; Zhang et al., 2008; Muñoz-Sanz et al., 2017; Murathan et al., 2017), although additional alleles have been included in the NCBI database and not yet published. These studies allowed the determination of different apricot *S*-genotypes from different countries (Halász et al., 2010; Kodad et al., 2013a,b; Muñoz-Sanz et al., 2017) that are included in, up to now, 17 incompatibility groups (Szabó and Nyéki, 1991; Egea and Burgos, 1996; Halász et al., 2010; Lachkar et al., 2013).

Due to the increasing release of a high number of apricot cultivars in the last years with unknown self-incompatibility genotypes, in this work we analyze the pollination requirements of a group of 92 apricot cultivars, including traditional and new cultivars released from different breeding programs. Self-compatibility was established by the observation of pollen tube behavior under the microscope following self-pollination. Incompatibility relationships between cultivars were established by the identification of *S*-alleles by PCR analysis. The results obtained allowed assigning each cultivar to its corresponding incompatibility group.

MATERIALS AND METHODS

Plant Material

Leaf and flower samples from 92 apricot cultivars, including traditional cultivars from different origins and new cultivars from different breeding programs (Table 1), were collected from diverse collections for pollination experiments and *S*-RNase genotyping.

TABLE 1 | Country of origin, number of pistils examined, percentage of pistils with pollen tubes halfway the style, at the base of the style, and reaching the ovule, percentage of style traveled by the longest pollen tube, mean number of pollen tubes at the base of the style, and self-incompatibility (SI) or self-compatibility (SC) of 92 apricot cultivars analyzed in this work.

Cultivar	Country of origin	Number of pistils examined	Pistils (%) with pollen tubes			Percentage of style traveled by the longest pollen tube	Mean number of pollen tubes at the base of the style	SI/SC
			Halfway the style	At the base of the style	Reaching the ovule			
AC1	USA	13	100	0	0	82	0	SI
ASF0401	France	17	94	0	0	65	0	SI
ASF0402	France	24	100	0	0	65	0	SI
Avirine (Bergarouge)	France	13	100	0	0	62	0	SI
CA-26 (Almater)	Spain	20	100	5	5	70	0	SI
Colorado	Spain	30	90	0	0	64	0	SI
Cooper Cot	USA	10	100	0	0	65	0	SI
Durobar (Almadulce)	Spain	23	100	0	0	67	0	SI
Farely	France	10	100	0	0	63	0	SI
Feria Cot	France	10	100	0	0	78	0	SI
Flash Cot	USA	10	70	0	0	54	0	SI
Flodea	Spain	11	100	0	0	71	0	SI
Goldbar	USA	20	100	0	0	62	0	SI
Goldrich	USA	72	94	3	3	69	0	SI
Goldstrike 01 ^a	USA	40	100	0	0	71	0	SI
Goldstrike 02 ^a	USA	20	100	0	0	72	0	SI
Harcot	Canada	44	95	0	0	62	0	SI
Hargrand	Canada	49	100	14	14	77	0	SI
Henderson	USA	47	91	15	9	75	0	SI
Holly Cot	France	20	100	0	0	61	0	SI
JNP	Spain	20	100	5	5	75	0	SI
Lilly Cot	USA	47	96	2	0	67	0	SI
Magic Cot	USA	30	100	0	0	65	0	SI
Maya Cot	France	10	100	0	0	66	0	SI
Medaga	France	10	100	0	0	71	0	SI
Megatea	Spain	10	100	0	0	62	0	SI
Moniqui	Spain	18	100	6	0	79	0	SI
Monster Cot	USA	10	100	0	0	70	0	SI
Muñoz	Spain	21	100	0	0	72	0	SI
Orangered	USA	10	90	0	0	64	0	SI
Pandora	Greece	23	100	4	0	75	0	SI
Peñaflor 01 ^a	Spain	29	100	7	7	71	0	SI
Perle Cot	USA	28	93	4	0	72	0	SI
Pinkcot	France	34	97	9	0	83	0	SI
Priabel	France	10	90	10	0	81	0	SI
Robada	USA	25	96	0	0	63	0	SI
Spring Blush	France	40	83	3	3	55	0	SI
Stark Early Orange	USA	51	98	33	16	87	0	SI
Stella	USA	13	100	23	15	85	0	SI
Sun Glo	USA	64	100	2	0	71	0	SI
Sunny Cot	USA	10	100	0	0	65	0	SI
Sweet Cot	USA	20	95	0	0	66	0	SI
Vanilla Cot	USA	20	100	0	0	79	0	SI
Veecot	Canada	29	100	3	3	74	0	SI
Wonder Cot	USA	37	100	0	0	69	0	SI

(Continued)

TABLE 1 | Continued

Cultivar	Country of origin	Number of pistils examined	Pistils (%) with pollen tubes			Percentage of style traveled by the longest pollen tube	Mean number of pollen tubes at the base of the style	SI/SC
			Halfway the style	At the base of the style	Reaching the ovule			
AC2	USA	10	100	100	100	100	2.2	SC
Aprix 20	Spain	15	100	73	53	100	1.4	SC
Aprix 33	Spain	10	100	100	100	100	1.1	SC
Aprix 9	Spain	14	100	86	64	100	2.1	SC
ASF0404 (Apriqueen)	France	22	100	91	91	100	3	SC
Berdejo	Spain	10	100	100	100	100	1.9	SC
Bergecot	France	20	100	95	95	100	2.5	SC
Canino	Spain	29	100	100	83	100	2.0	SC
Charisma	South Africa	23	100	100	100	100	3	SC
Corbato	Spain	60	100	98	93	100	3.2	SC
Delice Cot	France	15	100	87	53	100	1.1	SC
Faralia	France	11	100	100	100	100	2	SC
Farbaly	France	22	100	86	77	100	2.0	SC
Farbela	France	6	100	100	100	100	1.8	SC
Farclo	France	9	100	100	89	100	1.4	SC
Fardao	France	9	100	100	100	100	4.1	SC
Farfia	France	10	100	100	100	100	3	SC
Farhial	France	10	100	100	100	100	3	SC
Farius	France	12	100	100	100	100	2	SC
Farlis	France	22	100	100	100	100	2	SC
Fartoli	France	10	100	100	100	100	3	SC
Flopria	Spain	10	100	100	100	100	2	SC
Golden Sweet	USA	21	100	95	95	100	2	SC
Lady Cot	France	26	100	77	77	100	2.3	SC
Lorna	USA	17	100	100	100	100	3	SC
Luizet	France	10	100	90	80	100	1	SC
Medflo	France	8	100	100	100	100	1.9	SC
Mediabel	France	12	100	100	100	100	1.2	SC
Mediva	France	9	100	89	89	100	2.3	SC
Mirlo Anaranjado	Spain	10	100	100	100	100	2.1	SC
Mirlo Blanco	Spain	10	100	100	100	100	2	SC
Mitger	Spain	50	100	100	100	100	2.4	SC
Palsteyn	South Africa	30	100	100	100	100	3	SC
Paviot	France	12	100	91	91	100	1.2	SC
Peñaflor 02 ^a	Spain	6	100	83.3	66.6	100	1.4	SC
Pepito del Rubio	Spain	12	100	100	90	100	2.2	SC
Playa Cot	France	10	100	100	70	100	1.7	SC
Pricia	France	9	100	100	100	100	2	SC
Primidi	France	9	89	78	78	100	2	SC
Rouge Cot	France	10	100	90	70	100	1.55	SC
Rubista	France	19	95	89	89	100	1.7	SC
Sandy Cot	France	10	100	100	100	100	2.3	SC
Soledane	France	21	100	100	100	100	3	SC
Swired	Switzerland	9	100	100	90	100	1.8	SC
Tadeo	Spain	36	100	97	97	100	2.4	SC
Tom Cot	USA	10	100	100	100	100	3	SC
Victor 1		14	100	93	93	100	2.1	SC

^aDiverse origin.

Pollination Experiments

To explore self-(in)compatibility, self-pollinations of the 92 apricot cultivars were carried out in the laboratory. Pollen tube growth was observed in self-pollinated flowers under the microscope (Table 1). As control, a group of flowers of each cultivar were cross-pollinated with pollen from “Canino” or “Katy,” which are considered as universal pollinizers for apricot (Zuriaga et al., 2013).

Pollen was extracted from flowers collected at the balloon stage. For this purpose, the anthers were removed and dried at laboratory temperature during 24 h. After that, pollen grains were sieved by using a fine mesh (0.26 mm) and used immediately or frozen at -20°C until further use. Pistils were obtained from flowers collected 1 day before anthesis, at balloon stage. After the removing of petals, sepals and stamens, the pistils were maintained on wet florist foam at laboratory temperature (Rodrigo and Herrero, 1996). For each self- and cross-pollination, a group of 20–25 flowers were hand pollinated with the help of a paintbrush 24 h after emasculation. After 72 h, they were fixed in ethanol (95%)/acetic acid (3:1, v/v) during 24 h, and conserved at 4°C in 75% ethanol (Williams et al., 1999). When observations of pollen tube growth were not clear, each cross was repeated every year during the flowering period up to 4 years. In order to evaluate pollen viability, after hand pollination, pollen from each cultivar was scattered on a solidified pollen germination medium (Hormaza et al., 1996). After 24 h, preparations were observed under the microscope. Pollen grains were considered viable when the length of the growing pollen tubes was higher than the pollen grain diameter.

For histochemical preparations, the pistils were washed three times for 1 h with distilled water and left in 5% sodium sulphite at 4°C for 24 h. Then, to soften the tissues, they were autoclaved at 1 kg/cm^2 during 10 min in sodium sulphite (Jefferies and Belcher, 1974). To stain callose, the softened pistils, were stained with 0.1% (v/v) aniline blue in 0.1 N K_3PO_4 (Linskens and Esser, 1957). The observation of pollen tube behavior along the style was performed by a Leica DM2500 microscope (Cambridge, UK) with UV epifluorescence using 340–380 bandpass and 425 longpass filters. The percentage of style traveled by the longest pollen tube and the mean number of pollen tubes at the base of the style were recorded on at least 10 pistils in each cross. Cultivars were considered as self-incompatible when pollen tube growth was arrested in the style in most pistils from self-pollinated flowers, and as self-compatible when more than half of the pistils displayed at least one pollen tube reaching the base of the style.

DNA Extraction

For the identification of *S*-alleles, young leaves were collected in spring. Genomic DNA from 92 cultivars (Table 2) was isolated following the protocol described by Hormaza (2002) and using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). NanoDropTM ND-1000 spectrophotometer (Bio-Science, Budapest, Hungary) was used to measure DNA concentrations to analyze the quantity and quality of DNA.

TABLE 2 | Incompatibility group (I.G.) and *S*-*RNase* genotype of 92 apricot cultivars analyzed in this study and 30 additional cultivars analyzed in previous studies.

I.G.	<i>S</i> - <i>RNase</i> genotype	Cultivars analyzed in this study	Cultivars analyzed in previous studies	References
I	S_1S_2	AC1 ^x	Hargrand	2, 5, 14
			Katy	13, 14
			Goldrich	1, 2, 3, 5, 6, 10, 13, 14
			Castleton	14
			Farmingdale	9
			Giovanniello	9
			Lambertín-1	2, 14
II	S_8S_9	Pinkcot ^x Perle Cot ^x	Ceglédi óriás	5, 8
			Cologlu	12
			Kadioglu	12
			Ligeti óriás	5, 8
			Seftalioglu	12
			Szegedi M.	14
III	S_2S_6	ASF0401 ^x Avirine (Bergarouge) ^x Moniqui	Iri Bitirgen	2, 6, 14 12
V	S_2S_8	Holly Cot ^x Sweet Cot ^x	Alyanak	12
			Ziraat Okulu	12
VIII	S_6S_9	Orangered ^w ASF0402 ^x Wonder Cot ^x Stark Early Orange ^w Feria Cot ^x Sunny Cot ^x JNP ^x	Cataloglu	14 12
			Ozal	12
			Soganci	12
XVIII ^z	S_1S_3	Cooper Cot ^x	Perfection	1
XIX ^z	S_2S_3	Mayacot ^x Sun Glo		2, 3, 4, 6
XX ^z	S_2S_9	Magic Cot ^x Goldstrike 02 ^{v,x}	Hasanbey	12
XXI ^z	S_3S_8	Spring Blush ^x Lilly Cot ^x	Kayseri P.A	12

(Continued)

TABLE 2 | Continued

I.G.	S-RNase genotype	Cultivars analyzed in this study	Cultivars analyzed in previous studies	References
XXII ^z	S ₃ S ₉	Durobar (Almadulce) ^x Henderson ^w Flodea ^x	Akcadag Günay	14 12
XXIII ^z	S ₇ S ₉	Goldbar ^x	Kurukabuk	12
	S ₂	Pandora ^x Veecot Muñoz ^x Peñaflor 01 ^{v, x} Hardgrand Goldrich	Búlida Lorna ^y Perla	9 9 9
	S ₃	Colorado ^y	Ninfa	9
	S ₄	Harcot		
	S ₆	Stella		
	S ₈	Vanilla Cot ^x Robada ^x	Katy Krimskyi Medunec	7 5
	S ₉	Flash Cot ^x Goldstrike 01 ^{v, x} CA-26 (Almater) ^x Farely ^x Medaga ^x Megatea ^x Monster Cot ^x Priabel ^x		
Self-compatible cultivars	S ₂ S _c	Berdejo ^x Canino Paviot ^x Pepito del Rubio Peñaflor 02 ^{v, x} Bergecot ^x Mediva ^x Primidi ^x Sandy Cot ^x		3, 10, 13 2, 3
	S ₃ S _c	Pricia ^x Rubista ^x		
	S ₆ S _c	Aprix 20 ^x Aprix 9 ^x Faralia ^x Farlis ^x Medflo ^x Mediabel ^x		

(Continued)

TABLE 2 | Continued

I.G.	S-RNase genotype	Cultivars analyzed in this study	Cultivars analyzed in previous studies	References
	S ₇ S _c	Charisma ^x		
	S ₉ S _c	AC2 ^x Flopria ^x Tom Cot ^x		
	S _c	Soledane ^x ASF0404 (Apriqueen) ^x Mirlo Blanco Mitger Tadeo Corbato ^y Aprix 33 ^x Delice Cot ^x Farbaly ^x Farbela ^x Farclo ^x Fardao ^x Farfia ^x Farhial ^x Farius ^x Fartoli ^x Lady Cot ^x Mirlo Anaranjado Luizet ^x Playa Cot ^x Swired ^x Rouge Cot ^x	11	
	S ₁ S ₂	Lorna ^{x, y} Palsteyn ^{x, y}		
	S ₂ S ₉	Victor 1 ^x		
	S ₃	Golden Sweet ^x		

(1) Egea and Burgos (1996); (2) Burgos et al. (1998); (3) Albuquerque et al. (2002); (4) Sutherland et al. (2004); (5) Halász et al. (2005); (6) Vilanova et al. (2005); (7) Chen et al. (2006); (8) Halász et al. (2007); (9) Donoso et al. (2009); (10) Raz et al. (2009); (11) Egea et al. (2010); (12) Halász et al. (2010); (13) Zuriaga et al. (2013); (14) Muñoz-Sanz et al. (2017).

^vDiverse origin.

^wS₉ and S₁₇ have been considered the same allele.

^xS-RNase genotypes first reported in this study.

^yCultivars in which S-RNase genotype reported herein differs from that reported in other studies.

^zIncompatibility groups first reported in this study.

S-RNase Allele Identification by PCR Analysis

Amplification reactions for the first intron region of the S-RNase gene were carried out with the combination of the fluorescently labeled forward primer SRc-F (5'-CTCGCTTTCCTTGTTCTTGC-3') with the reverse primer SRc-R (5'-GGCCATTGTTGCACAAATTG-3'; Romero et al., 2004; Vilanova et al., 2005). PCR amplifications were carried out in 15 µl reaction volumes, containing 10x NH₄ Reaction Buffer, 25 mM Cl₂Mg, 2.5 mM of each dNTP, 10 µM of each primer, 100

ng of genomic DNA and 0.5 U of BioTaq™ DNA polymerase (Bioline, London, UK). The temperature profile used had an initial step of 3 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C and 3 min at 72°C, and a final step of 5 min at 72°C.

The sizes of the products obtained by PCR were analyzed in a CEQ™ 8000 capillary electrophoresis DNA analysis system (Beckman Coulter, Fullerton, CA, USA) and compared and classified according to Vilanova et al. (2005) and Kodad et al. (2013b). Primers Pru-C2 (5'-CTTTGGCCAAGTAATTATTCAAACC-3') and Pru-C4R (5'-GGATGTGGTACGATTGAAGCG-3') were used for the amplification of the second intron region as recommended by Vilanova et al. (2005), but with the addition of 10 cycles and using 55°C of annealing temperature as indicated by Sonneveld et al. (2003). Amplified fragments of the second intron were separated on 1% (w/v) agarose gels and DNA bands were visualized using the nucleic acid stain SYBR Green (Thermo).

Sequencing of Genomic PCR Products

Two PCR fragments of 420 and 430 bp obtained by the automatic sequencer were isolated using the NucleoSpin Gel and PCR Clean-up (Macherey-Nagel). Cloning was performed using CloneJET PCR Cloning Kit (Thermo) and by electroporation in *E. coli* Single-Use JM109 Competent Cells (Promega). The search for similarities in the sequences of the NCBI database was performed with BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>, version 2.2.10). The 420 bp fragment resulted in a fragment of 414 bp after one sequencing reaction whereas the initial 430 bp fragment resulted in a fragment of 421 bp after two sequencing reactions.

RESULTS

Pollination Experiments

Self-compatibility of 92 apricot cultivars was established by the observation of pollen tube behavior in pistils under the microscope after self-pollinations (Table 1). Germinated pollen grains were observed in the stigma (Figure 1A) in all the pollinations performed. The establishment of self-incompatibility or self-compatibility could be carried out for all the cultivars. Approximately half of the cultivars (47) behaved as self-compatible, displaying most pistils with pollen tubes growing along the style (Figure 1B) and at least one pollen tube reaching the base of the style (Figure 1C). On the other hand, in 45 cultivars pollen tubes arrested their growth in the style (Figure 1D) and no pollen tubes reached the base of the style in most of the pistils. Consequently, these cultivars were considered as self-incompatible. As expected, in all cross-pollinations pistils displayed pollen tubes at the base of the style. Between one and four pollen tubes at the base of the style were observed in self-compatible cultivars.

S-RNase Allele PCR Analysis

To confirm the results obtained in the pollination experiments, PCR analyses using specific primers from conserved regions

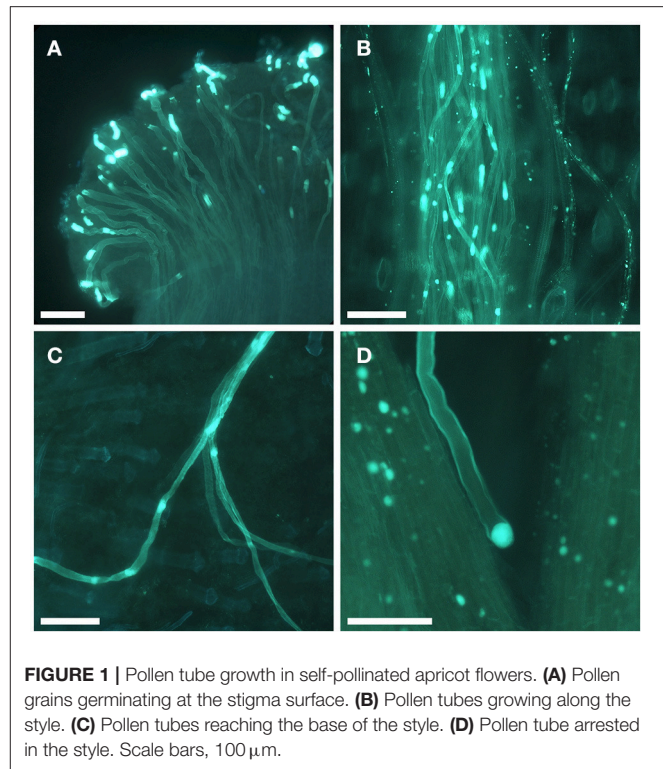


FIGURE 1 | Pollen tube growth in self-pollinated apricot flowers. **(A)** Pollen grains germinating at the stigma surface. **(B)** Pollen tubes growing along the style. **(C)** Pollen tubes reaching the base of the style. **(D)** Pollen tube arrested in the style. Scale bars, 100 μm.

of the apricot *S-RNase* locus were used to identify the *S-RNase* alleles of 92 apricot cultivars (Table 2). The information reported herein has been compiled with the *S-RNase* genotype of 30 additional cultivars previously determined showing the compatibility relationships among all the cultivars whose *S-RNase* genotype is known (Table 2). Cultivars have been allocated according their *S-RNase* alleles in 11 incompatibility groups, six of them reported here for the first time. Some cultivars with previously reported *S*-genotypes were initially used to confirm the size of *S-RNase* alleles previously identified using the primer pairs SRC-F and SRC-R (Vilanova et al., 2005) that amplify the first intron of the apricot *S-RNase* and allowed identifying the *S-RNase* alleles of the rest of the cultivars analyzed (Figure 2).

Two alleles, *S*₁ and *S*₇, could not be distinguished with the primers SRC-F/SRC-R, since these alleles showed similar fragment sizes in the first intron (Table 3). Thus, the PruC2/PruC4R primer combination designed from *P. avium* *S-RNase*-cDNA sequences (Tao et al., 1999) was additionally used to amplify the second intron. The alleles *S*₆ and *S*₈ had also similar fragment sizes in the first intron, and, in this case, the self-compatibility or self-incompatibility observed in the pollination experiments was used to distinguish between both alleles in each cultivar. A fragment of 420 or 430 bp was detected in some of the cultivars. These band sizes are close to the *S*₆ allele, which has been reported as 424 bp (Kodad et al., 2013b) or 423 bp (Halász et al., 2010). To elucidate if the 430 bp and 420 bp bands obtained by an automatic sequencer correspond to new or pre-existing alleles, both fragments were cloned and sequenced, resulting in fragments of 421 and 414 bp, respectively.

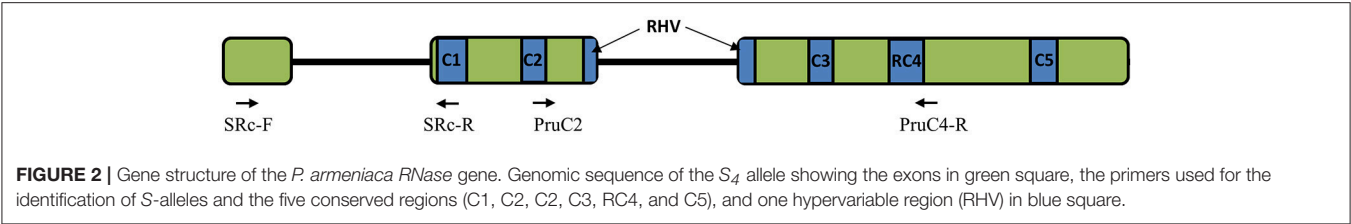


TABLE 3 | *S*-alleles identified or/and sequenced in *Prunus armeniaca*.

Alleles	Gen bank accession	Sequence	Fragment 1st intron	Fragment 2nd intron	References
<i>S</i> _c	EF491872/DQ386735	Partial CDS, 1st intron	355 ^{a,e}	2800 ^c	Halász et al., 2007
<i>S</i> ₁	AY587561	CDS, 1st and 2nd intron	408 ^{a,e}	2260 ^{b,e}	Romero et al., 2004
<i>S</i> ₂	AY587562	CDS, 1st and 2nd intron	334 ^{a,e}	990 ^b	Romero et al., 2004
<i>S</i> ₃			274 ^{a,e}	~450 ^{b,e}	Vilanova et al., 2005
<i>S</i> ₄	AY587564	CDS, 1st and 2nd intron	249 ^{a,e}	247 ^{b,e}	Romero et al., 2004
<i>S</i> ₅			375 ^a	1400 ^b	Vilanova et al., 2005
<i>S</i> ₆ / <i>S</i> ₅₂	KF951503 (<i>S</i> ₅₂)	CDS, 1st and 2nd intron	421 ^{a,e}	1386 ^{b,e}	Unpublished
<i>S</i> ₇			408 ^{a,e}	900 ^{b,e}	Vilanova et al., 2005
<i>S</i> ₈	AY884212	Partial CDS, 2nd Intron	355 ^{a,e}	691 ^b	Feng et al., 2006; Halász et al., 2007
<i>S</i> ₉	AY864826	Partial CDS	414 ^{a,e}	749 ^{b,e}	Feng et al., 2006
<i>S</i> ₁₀	AY846872	Partial CDS, 2nd Intron		583 ^d	Feng et al., 2006
<i>S</i> ₁₁	DQ868316	Partial CDS, 2nd Intron		464 ^d	Zhang et al., 2008
<i>S</i> ₁₂	DQ870628	Partial CDS, 2nd Intron		360 ^d	Zhang et al., 2008
<i>S</i> ₁₃	DQ870629	Partial CDS, 2nd Intron		401 ^d	Zhang et al., 2008
<i>S</i> ₁₄	DQ870630	Partial CDS, 2nd Intron		492 ^d	Zhang et al., 2008
<i>S</i> ₁₅	DQ870631	Partial CDS, 2nd Intron		469 ^d	Zhang et al., 2008
<i>S</i> ₁₆	DQ870632	Partial CDS, 2nd Intron		481 ^d	Zhang et al., 2008
<i>S</i> ₁₇	DQ870633	Partial CDS, 2nd Intron		487 ^d	Zhang et al., 2008
<i>S</i> ₁₈	DQ870634	Partial CDS, 2nd Intron		1337 ^d	Zhang et al., 2008
<i>S</i> ₁₉	EF133689	Partial CDS, 2nd Intron		546 ^d	Zhang et al., 2008
<i>S</i> ₂₀	EF160078	Partial CDS, 2nd Intron		1934 ^d	Zhang et al., 2008
<i>S</i> ₂₂	HM053569	Partial CDS, 2nd Intron		550 ^d	Unpublished
<i>S</i> ₂₃	EU037262	Partial CDS, 2nd Intron		505 ^d	Wu et al., 2009
<i>S</i> ₂₄	EU037263	Partial CDS, 2nd Intron		168 ^d	Wu et al., 2009
<i>S</i> ₂₅	EU037264	Partial CDS, 2nd Intron		583 ^d	Wu et al., 2009
<i>S</i> ₂₆	EU037265	Partial CDS, 2nd Intron		289 ^d	Wu et al., 2009
<i>S</i> ₂₇	EU836683	Partial CDS, 2nd Intron		230 ^d	Wu et al., 2009
<i>S</i> ₂₈	EU836684	Partial CDS, 2nd Intron		948 ^d	Wu et al., 2009
<i>S</i> ₂₉	EF185300	Partial CDS, 2nd Intron		285 ^d	Wu et al., 2009
<i>S</i> ₃₀	EF185301	Partial CDS, 2nd Intron		956 ^d	Wu et al., 2009

^aAmplified using SRc-(F/R).
^bAmplified using Pru-C2 and Pru-C4R.
^cAmplified using Pru-C2 and Pru-C3R.
^dAmplified using other primers.
^eOur results.

The 421 bp fragment showed a 99% identity with the *S*₅₂ present in the NCBI database. This allele was initially included in the NCBI database and unpublished but, recently, it has been reported in some Turkish apricot cultivars (Murathan et al., 2017). Since the *S*₆ allele had not been previously sequenced, the *S*₅₂ allele could indeed correspond to the *S*₆ allele. The *S*₆ allele could also be identified with the primers Pru-C2/Pru-C4, showing a PCR-fragment of around 1400 bp (1386 bp) that

included the second intron; a 1386 bp fragment was also amplified in the *S*₅₂ allele with the same primer combination strongly suggesting that *S*₆ and *S*₅₂ could be the same allele. Thus, in this work, the 421 bp fragment was assigned to the *S*₆ allele.

The sequence of the 414 bp fragment showed high sequence similarity to *S*-alleles from other *Prunus* species, but not to any *S*-allele of *Prunus armeniaca* present in NCBI databases. The second intron of this allele was amplified with the primers

Pru-C2/Pru-C4, showing a PCR-fragment of around 700 bp. Its cloning, sequencing and alignment revealed a 99% identity with the *S₉* allele [AY853594 (Feng et al., 2006)] and, consequently, the 414 bp fragment was assigned to the *S₉* allele.

The 45 self-incompatible cultivars were grouped in incompatibility groups according to their *S* genotypes following the numbering proposed by Halász et al. (2010) and Lachkar et al. (2013). While 26 of the cultivars analyzed were assigned to 11 different incompatibility groups, 19 cultivars were not assigned since only one *S-RNase* allele was detected (Table 2). *S₂* was the most frequent allele, appearing in 22 cultivars, followed by *S_c* (21), *S₉* (19), *S₆* (16), *S₃* (10), *S₈* (6), and *S₁* (4), while *S₇* was the least frequent allele found in only two cultivars.

DISCUSSION

Self-pollination of the 92 apricot cultivars analyzed in this work and observation of pollen tubes under the microscope showed that 47 behaved as self-compatible and 45 as self-incompatible. The self-(in)compatibility of 68 cultivars is reported herein for the first time. The results in the remaining 23 cultivars have been compared with previous reports in which self-(in)compatibility was determined by the evaluation of the percentage of fruit set after self-pollinations in the field (Egea and Burgos, 1996; Rodrigo and Herrero, 1996; Burgos et al., 1997; Egea et al., 2010; Muñoz-Sanz et al., 2017) or by the observation of pollen tube growth in pistils after self- and cross-pollinations (Egea and Burgos, 1996; Rodrigo and Herrero, 1996; Egea et al., 2010; Milatovic et al., 2013a,b). Thus, results herein agree with previous reports for “Canino,” “Corbato,” “Luizet,” “Mirlo Blanco,” “Mirlo Anaranjado,” “Mitger,” “Palsteyn,” “Paviot,” “Pepito del Rubio,” “Tadeo,” and “Tom Cot” as self-compatible, and also for “Bergarouge,” “Goldrich,” “Goldstrike,” “Harcot,” “Hargrand,” “Moniqui,” “Orangered,” “Pinkcot,” “Robada,” “Stark Early Orange,” “Stella,” “Sun Glo,” and “Veecot” as self-incompatible.

Approximately half of the cultivars analyzed (49%) were self-incompatible, a very high percentage compared to the situation some years ago when most European cultivars were self-compatible (Mehlenbacher et al., 1991), including the most traditional cultivars (Burgos et al., 1997). The other half (51%) were self-compatible. Due to this dramatic increase in the number of self-incompatible apricot cultivars, knowing their pollination requirements is necessary in order to choose compatible pollinizers in designing new commercial orchards as well as in selecting parental genotypes in apricot breeding programs.

The first case of cross-incompatibility of apricot cultivars in the European group was reported in the early 1990s in the Spanish cultivar Moniqui (Egea et al., 1991). The first incompatibility group in apricot, which consisted of three North American cultivars, was established several years later based on microscopic observations (Egea and Burgos, 1996), and, later, a more extensive study with 123 apricot cultivars, reported self-incompatibility in 42 cultivars (Burgos et al.,

1997). Afterward the *S-RNase* proteins of seven *S*-alleles (*S₁*–*S₇*) and one allele associated with self-compatibility (*S_c*) were identified; the proteins were separated by non-equilibrium pH gradient electrofocusing (NEpHGE) in a gel that was later stained for ribonuclease activity (Albuquerque et al., 2002). The identification of the gene involved in GSI allowed *S*-allele identification by PCR analysis (Romero et al., 2004). In this first study, three *S*-alleles were sequenced (*S₁*, *S₂*, and *S₄*) (Romero et al., 2004). In a later study, *S*-allele genotyping using the *SRc-F* and *SRc-R* primers, which have also been used in our study and amplify the first intron, identified four self-incompatibility alleles (*S₃*, *S₅*, *S₆*, and *S₇*) and one allele for self-compatibility (*S_c*) (Vilanova et al., 2005). Nine additional *S*-alleles (*S₈*–*S₁₆*) were identified by Halász et al. (2005) in 23 apricot accessions, mostly from Hungary. These last 13 alleles (*S₃*, *S₅*–*S₁₆*) were only identified by PCR analysis. From then on, several studies have identified additional *S*-alleles by sequencing [*S₉* and *S₁₀* (Feng et al., 2006); *S₁₁*–*S₂₀* (Zhang et al., 2008); *S₂₃*–*S₃₀* (Wu et al., 2009)], but some of them have only been included in the NCBI database and not yet published. Some unpublished alleles such as *S₅₂* (Murathan et al., 2017)/*S₆* (our results) and *S₂₂* (Muñoz-Sanz et al., 2017), have already been associated to several cultivars. Most of these studies have been performed in apricot cultivars from different origins like China, Turkey, Hungary, or Spain and, in some cases, the use of different primers or just the inaccuracy of band identification in a gel or even analyzed by an automatic fragment analyzing system can result in misidentification and the appearance of numerous homologies (Muñoz-Sanz et al., 2017). For example, differences in reported fragment size can be found in the *S₂* [334 bp herein; 327 bp in Vilanova et al. (2005); 332 bp in Kodad et al. (2013b)] or *S_c* [358 bp herein; 353 bp in Vilanova et al. (2005); 355 bp in Kodad et al. (2013b)] alleles in addition to the *S₆* allele mentioned above. Moreover, many of the *S*-alleles, such as *S₁₀*–*S₃₀* alleles, in which the first intron is still unknown, have only been partially sequenced.

From the 92 cultivars analyzed herein, 74 have been reported for the first time. Two alleles could be identified in most cultivars. However, a single allele was identified in 19 self-incompatible cultivars that may be due to inefficient PCR amplification of the *S-RNase* allele, in which the PCR primers may have a preferential amplification of the detected allele or caused by mismatching of PCR primers. Alternatively, the similar size of two alleles that show overlapping PCR fragments could make their identification difficult. Thus, the identification of additional *S-RNase* alleles in these genotypes requires more work focused on characterizing the *S*-locus. In 22 self-compatible cultivars, the identification of a unique allele could also be due to homozygosity of the self-compatible allele (*S_c*). Higher *S*-allele frequencies in the cultivars analyzed correspond to alleles *S₂*, *S_c*, and *S₉* (19–22%). The allele *S_c* is associated with self-compatibility, and the alleles *S₂* and *S₉* are present in different cultivars resistant to sharka from USA (“Goldrich,” “Henderson,” “Orangered,” and “Sun Glo”) and Canada (“Hargrand” and “Veecot”), which have been used as parental genotypes in different breeding programs (Hormaza et al., 2007; Zhebentyayeva et al., 2012). On the other hand, the allele *S₇* is the least frequent and is present in only two cultivars: “Charisma” from South Africa, and “Goldbar” from USA.

Results herein, while confirmed the *S-RNase* genotype of 16 cultivars reported in previous studies, showed differences in the *S-RNase* genotype of “Corbato,” “Colorado,” “Lorna,” and “Palsteyn” reported previously (Burgos et al., 1998; Alburquerque et al., 2002; Vilanova et al., 2005; Donoso et al., 2009; Raz et al., 2009; Muñoz-Sanz et al., 2017). To clarify their *S*-genotype, it would be necessary to identify their *S*-alleles in additional samples of the same cultivars. In this sense, sequencing can reveal the differences between a band fragment of an initial size, such as in our case, in which the initial fragment of 420 and 430 bp resulted in a *S*-allele of 414 bp (*S*₉) and 421 bp (*S*₆) respectively, after sequencing. Moreover, the sequence of the *S*₆ (fragment of 421 bp) could reveal its similarity to *S*₅₂ that has been recently assigned to Turkish apricot cultivars (Murathan et al., 2017).

The self-incompatibility identification has allowed allocating the cultivars to their corresponding incompatibility groups. This information, compiled with those reported in previous studies, has allowed describing six new incompatibility groups (XVIII–XXIII). Thus, self-incompatible cultivars within the same incompatibility group have the same *S*-genotype and are genetically incompatible with each other. On the other hand, cultivars with at least one different *S*-allele are placed in different incompatibility groups and are inter-compatible.

Although self-incompatibility was observed in nearly half of the cultivars analyzed herein, self-compatibility was still found in a good number of apricot cultivars. Self-compatibility has been related to particular *S*-alleles in different self-incompatible *Prunus* species, as almond (Fernández i Martí et al., 2014; Company et al., 2015), Japanese apricot (Ushijima et al., 2003), peach (Tao et al., 2006; Hanada et al., 2014), sour cherry (*Prunus cerasus* L.) (Yamane et al., 2003), and sweet cherry (Wunsch and Hormaza, 2004; Marchese et al., 2007; Cachi and Wünsch, 2014). The breakdown of the incompatibility system has been reported in these *Prunus* species affecting the function of *S*-locus in the stylar *S*-determinant (*S-RNase*) and in the pollen *S*-determinant (*F*-box protein, *SFB*). It has also been related to mutations outside the *S*-locus (Hegedus et al., 2012; Company et al., 2015). In apricot, self-compatibility has been related with the insertion of a 358-bp fragment in the *S*-haplotype-specific *F*-box (*SFB*) gene. This insertion has been reported in self-compatible Spanish (Vilanova et al., 2006) and Hungarian (Halász et al., 2007) cultivars and, since they only differ in two nucleotides in the intron region, a common origin for them has been suggested (Halász et al., 2007). Moreover, a detailed study on the *S*₈ allele, a very common allele in Hungarian cultivars, suggests that *S*₈ could derive from the *S*₈ allele (Halász et al., 2007). Thus, the insertion of 358-bp in the *SFB* gene is only present in the *S*₈ haplotype and both alleles can only be distinguished by primers designed based on the *SFB* sequence such as the degenerate primers AprSFB-F1/R (Halász et al., 2007) or, as in our case, by pollination experiments.

The microscopy observations herein revealed self-compatibility in “Lorna” and “Palsteyn,” cultivars with the *S*₁*S*₂ genotype, “Victor 1” with the genotype *S*₂*S*₉, and “Golden Sweet” with the allele *S*₃, but the *S*₆ allele was not reported. A different mutation that is not linked to the *S*-locus has also

been related to self-compatibility with a loss of pollen *S*-activity (Vilanova et al., 2006) and it has recently been associated with the *M*-locus (Zuriaga et al., 2013; Muñoz-Sanz et al., 2017). Due to its limited distribution, it has been suggested that the *M*-locus would be in a very early stage of dispersion (Muñoz-Sanz et al., 2017). Interestingly, the *S*₆ allele is generally found in cultivars with the *m*-haplotype suggesting that this could be the result of a relaxed constrain for self-compatible selection (Muñoz-Sanz et al., 2017). A similar *S*-genotype is also found in self-compatible “Katy,” in which the compatibility was associated to the *M*-locus (Zuriaga et al., 2013). A further analysis could reveal if self-compatibility in “Lorna,” “Palsteyn,” “Victor 1,” and “Golden Sweet” is also related to the *M*-locus.

Studies on *S*-allele identification can also provide information on the genetic diversity of the species. Thus, Chinese cultivars are mostly self-incompatible with a higher number of *S*-alleles (Zhang et al., 2008) compared with the limited number of *S*-alleles found in Western countries (Halász et al., 2007). A study on genetic diversity using AFLP markers showed a decreasing genetic diversity of apricot cultivars from the former USSR to Southern Europe (Hagen et al., 2002; Halász et al., 2007), which is coherent with the Asian origin of the species. However, although most traditional European cultivars are self-compatible (Burgos et al., 1997), due to breeding and crosses with Asian cultivars, the number of self-incompatibility cultivars is recently increasing in Western countries (Muñoz-Sanz et al., 2017; Murathan et al., 2017) and our study confirms this trend. Moreover, our results also show six new incompatibility groups in addition to the initial 17 incompatibility groups described so far.

Thus, the results obtained in this work using pollen tube growth observation in self-pollinated pistils, has allowed establishing the self-compatibility or self-incompatibility of 92 cultivars, including traditional and the main current cultivars as well as new cultivars from different breeding programs. *S-RNase* allele identification has allowed the allocation of a number of cultivars to their corresponding incompatibility groups, determining the incompatibility relationships between cultivars. The combination of these two complementary approaches results in valuable information for the appropriate selection of cultivars in commercial orchards and for the selection of parental genotypes in apricot breeding programs and a similar approach could be used in other woody perennial crops.

AUTHOR CONTRIBUTIONS

JR, JH, and MH: conceived the study; SH, JL, JR, JH, and MH: designed the experiments and wrote the paper; SH and JL performed the microscope observations, the PCR analysis, and analyzed the data; SH and JL contributed equally to this work.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CAPÍTULO 3. Identification of Self-Incompatibility Alleles by Specific PCR Analysis and *S-RNase* Sequencing in Apricot

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CAPÍTULO 3

Identification of Self-Incompatibility Alleles by Specific PCR Analysis and *S-RNase* Sequencing in Apricot

Identificación de los alelos de auto(in)compatibilidad mediante análisis por PCR específica y secuenciación de *S-RNasa* en albaricoquero

RESUMEN

La auto(in)compatibilidad (AI) es uno de los mecanismos más eficientes para promover el cruzamiento en plantas. Sin embargo, la AI podría ser un problema para la producción frutal. Un ejemplo es el albaricoquero (*Prunus armeniaca*), en el que, como en otras especies de Rosaceae, la AI está determinada por un Sistema Gametofítico de Autoincompatibilidad (GSI). Las relaciones de incompatibilidad entre variedades pueden ser establecidas mediante el genotipado de alelos *S* por PCR. Hasta hace poco, la mayoría de las variedades tradicionales europeas de albaricoquero eran autocompatibles, pero varios programas de mejora han introducido un creciente número de nuevas variedades cuyas necesidades de polinización se desconocen. Para llenar este vacío, se han identificado los alelos *S* de 44 genotipos de albaricoquero, de los cuales 43 son descritos en este trabajo por primera vez. La identificación del alelo *S_c* en 15 genotipos indica que estas variedades son autocompatibles. En cinco genotipos, el análisis PCR no permitió distinguir entre los alelos *S_c* y *S_g*, por lo que la auto(in)compatibilidad se estableció mediante la observación del crecimiento de los tubos polínicos en flores autopolinizadas. Los genotipos autoincompatibles se asignaron a sus correspondientes grupos de incompatibilidad. El conocimiento de las relaciones de incompatibilidad entre variedades de albaricoquero puede ser una herramienta muy valiosa para el desarrollo de futuros programas de mejora mediante la selección de los parentales apropiados y para el diseño eficiente de plantaciones mediante la introducción de variedades autocompatibles e intercompatibles.



Article

Identification of Self-Incompatibility Alleles by Specific PCR Analysis and *S-RNase* Sequencing in Apricot

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Abstract: Self-incompatibility (SI) is one of the most efficient mechanisms to promote out-crossing in plants. However, SI could be a problem for fruit production. An example is apricot (*Prunus armeniaca*), in which, as in other species of the Rosaceae, SI is determined by an S-RNase-based-Gametophytic Self-Incompatibility (GSI) system. Incompatibility relationships between cultivars can be established by an S-allele genotyping PCR strategy. Until recently, most of the traditional European apricot cultivars were self-compatible but several breeding programs have introduced an increasing number of new cultivars whose pollination requirements are unknown. To fill this gap, we have identified the S-allele of 44 apricot genotypes, of which 43 are reported here for the first time. The identification of S_c in 15 genotypes suggests that those cultivars are self-compatible. In five genotypes, self-(in)compatibility was established by the observation of pollen tube growth in self-pollinated flowers, since PCR analysis could not allowed distinguishing between the S_c and S_8 alleles. Self-incompatible genotypes were assigned to their corresponding self-incompatibility groups. The knowledge of incompatibility relationships between apricot cultivars can be a highly valuable tool for the development of future breeding programs by selecting the appropriate parents and for efficient orchard design by planting self-compatible and inter-compatible cultivars.

Keywords: apricot; Gametophytic Self-Incompatibility; pollen TUBE; pollination; *Prunus armeniaca*; S-alleles; S-genotype

1. Introduction

Different physical and genetic strategies have been developed by plants to prevent self-pollination and promote out-crossing, and thus, ensure genetic variability [1]. A physical barrier can be found in bisexual flowers by different temporal maturation of the male and female parts (dichogamy) or by a spatial separation of the female and male parts (herkogamy) [2]. The genetic barrier, self-incompatibility (SI), is considered to be one of the most efficient mechanisms to promote out-crossing [3]. SI is a widespread mechanism that has been reported in nearly one-half of angiosperm species [4]. However, SI has only been characterized in a few families and the underlying molecular and genetic factors that are involved in SI have only been described in detail in the Sporophytic Self-Incompatibility System (SSI) and in the Gametophytic Self-Incompatibility System (GSI) [5]. In those systems, self-incompatibility is genetically controlled by a multiallelic locus named S [6]. Following the discovery of sporophytic and gametophytic incompatibility [6], an array of other incompatibility

systems, such as late-acting self-incompatibility [7] or cryptic self-incompatibility [8], have been reported in different families, although the cells and the mechanisms that are involved have not been identified. Interestingly, cryptic self-incompatibility has been found in herkogamous species in which the growth of cross-pollen tubes is faster than that of self-pollen tubes [7], a pollen tube behavior that, to the best of our knowledge, has not been studied in GSI and should also be taken into account. However, this scenario has not been described in Rosaceae and it seems unlikely to occur in this family [9].

SSI has been mainly characterized in the Brassicaceae family [10]. In SSI, recognition factors are coded by the sporophyte and deposited extra-cellularly on the pollen grain wall. Pollen germination is arrested at the stigma surface when the *S*-allele of the haploid pollen matches either of the *S*-alleles of the diploid pistil [11]. GSI is the most abundant SI in angiosperms and it has been characterized in 18 families, including the Rosaceae [7]. GSI is controlled by a multiallelic locus *S*, which encodes the stilar and pollen *S*-determinants, and cell-cell recognition generally takes place in the style during pollen tube growth [12]. The stilar *S*-determinant, a ribonuclease (*S*-RNase), is a glycoprotein secreted into the style mucilage [13] that is composed of five highly conserved regions (C1–C5). Additionally, a single hypervariable region (RHV) is found in the *S*-RNase of Rosaceae, while two hypervariable regions (HVa and HVb) are observed in the *S*-RNase of Solanaceae and Plantaginaceae [14]. The pollen *S*-determinant is an F-box protein that contains two variable (V1 and V2) and two hypervariable (HVa and HVb) regions, and it is coded by pollen-specific F-box genes (*SFB*) [15].

Rosaceae constitutes the third most economically important family in temperate regions of the world, including several important crops, such as pear (*Pyrus communis*), apple (*Malus domestica*), sweet cherry (*Prunus avium*), almond (*Prunus dulcis*), and apricot (*Prunus armeniaca*) [16]. Most European common apricot cultivars have been traditionally considered as self-compatible [17]. However, with the purpose of introducing a source of resistance to sharka, some incompatible cultivars that were developed in North America have been used as parents in several breeding programs [18,19]. Sharka is the most important virus disease affecting *Prunus* in Europe and it has caused important economic damages in the last decades, becoming a limiting factor for apricot production in some areas. The release of these new breeding lines has resulted in the introduction of an increasing number of new apricot cultivars with unknown incompatibility information.

Self and intercompatibility relationships have been traditionally determined by performing field-controlled pollinations. The (in)compatibility is established by recording the percentage of fruit set four weeks after self- and cross-pollinations [20,21]. However, the efficiency of this method is conditioned by uncontrolled environmental factors [22]. Incompatibility can also be evaluated by observing pollen tube growth through the style in hand-pollinated flowers under the microscope. The stigma of apricot is papillate and wet, and the style shows a compact transmitting tissue enveloped by vascular bundles. Pollen grains germinate on the moist surface of the stigma within one day after pollination. The pollen tube penetrates into the stigma between the papillae, reaches the transmitting tissue and grows along the style in the following days. Pollen tube growth along the style takes 3–4 days [22]. However, pollen tube behavior can also be affected by environmental factors, mainly temperature. Thus, warm temperatures prior to flower opening result in lower fruit set, related to an asynchrony between the reproductive organs and the showy part of the flower [22]. Moreover, high temperatures can accelerate pollen tube growth kinetics [23]. Thus, in order to minimize environmental effects, the (in)compatibility phenotype can be evaluated in semi-in vivo culture of flowers in laboratory-controlled self- and cross-pollinations, followed by the subsequent observation of pollen tube behavior through the style under a fluorescence microscopy. This approach has been successfully used to determine self-(in)compatibility and incompatibility relationships between cultivar, in apricot [24] and other *Prunus* species [25].

Moreover, since the first isolation of a partial cDNA encoding an *S*-locus specific glycoprotein from *Brassica oleracea* [26], the development of molecular techniques based on the sequence of the *S*-locus has allowed for the identification of self-(in)compatibility and the incompatibility relationships

among cultivars in numerous species. In *Prunus*, the sequence of the *S-RNase* revealed two introns with considerable length polymorphism that have been used to distinguish among *S*-haplotypes [27–31]. Thirty-three alleles have been reported so far in apricot (S_1 to S_{20} , S_{22} to S_{30} , S_{52} , S_{53} , S_v , S_x), including one allele for self-compatibility (S_c) [32–36]. Additional alleles have been included in the NCBI database but not yet published showing misidentification and the appearance of numerous homologies [24,35]. *S*-genotyping has allowed for classifying the cultivars in their corresponding incompatibility group according to their compatibility relationships. Self-incompatibility is considered when the genotype of pollen matches with one of the *S*-alleles of the pistil. Thus, those self-incompatible cultivars with the same *S*-alleles are inter-incompatible and are allocated in the same incompatibility group, whereas cultivars from different groups with at least one different *S*-allele are inter-compatible [35]. In apricot, 23 incompatibility groups have been established [24,37–40], which provides useful information to apricot growers and breeders.

To fill the lack of knowledge on self-(in)compatibility and incompatibility relationships in the increasing number of new apricot cultivars, in this work we identified the *S*-genotype of 44 apricot cultivars and selections by PCR analysis, and classified them according to their *S-RNase* alleles in nine incompatibility groups. Of those, the *S*-genotype of 43 is reported here for the first time. Additionally, in those cases in which the *S*-genotype could not be identified by molecular approaches, self-incompatibility was established by the observation of pollen tube behavior under the microscope following self-pollination. Our results elucidated incompatibility relationships among a high number of apricot cultivars with previous unknown pollination requirements providing valuable information to design new crosses in apricot breeding programs and selecting self-compatible and inter-compatible varieties in new apricot orchards.

2. Results

Self-compatibility of 44 cultivars and selections (Table 1) was evaluated by *S-RNase* allele identification using PCR analysis, amplifying the conserved regions of the apricot *S-RNase* locus. We first identified the *S-RNase* alleles using the primers SRC-F/SRC-R for the amplification of the first intron (Tables 1 and 2, Figure 1). Because some *S-RNase* alleles, such as S_1 and S_7 or S_6 and S_9 showed amplified fragments of similar size of the first intron in 27 genotypes, we also used the primers Pru-C2 and PruC4R that amplify the second *S-RNase* intron. These additional primers enabled identifying the *S*-allele of 13 of those 27 genotypes. The primers Pru-C2 and PruC4R also amplified a fragment of 2 kb in the genotype ‘T007’. This fragment size in the second intron has not been previously reported in other apricot cultivars. Thus, we further analyzed this PCR fragment by cloning and sequencing (Figure S1). We obtained a sequence of 2002 bp that showed a 99% of identity with the S_{20} allele (EF160078, [34]) and the unpublished S_{55} allele (KT223014). Thus, we identified one of the alleles of the *S*-genotype of ‘T007’ as S_{20} .

The combination of the primers Pru-C2 and PruC4R was inefficient in 15 genotypes with no amplification fragments obtained. Therefore, new specific primers were designed to amplify the second intron of the S_1 and S_7 alleles (Table 2, Figure 1). The primers SHLM1 and SHLM2 amplified a fragment of 650 bp from the S_1 allele (Figures 1 and 2). However, while the sequence of S_1 has been previously reported [41] (AY587561), the S_7 allele has only been identified by PCR analysis and no sequence has been yet included in the NCBI database. Thus, using the primers Pru-C2 and PruC4R, we cloned and sequenced the fragment of 900 bp that was obtained from the cultivar ‘Charisma’ (S_c/S_7 , [24]), which corresponds to the S_7 allele [33]. Surprisingly, the cloned sequence resulted in a fragment of 915 bp (Figure S2) that showed a 99% identity with the partial coding sequence (790 bp, EF062341, unpublished), of the S_{13} allele and with a fragment of 830 bp that corresponds to the unpublished S_{46} allele (HQ342876). The S_{13} allele (DQ870629) has also been identified by Zhang et al. [34], but the sequence of the gen bank accessions EF062341 and DQ870629 are different, and, consequently, both sequences have been considered as two different *S*-alleles. Our results suggest that S_7 , S_{13} (EF062341), and S_{46} could indeed be the same allele. Based on the sequence of 915 bp,

we designed the primers SHLM3 and SHLM4 that amplified a fragment of 413 bp. The primers Pru-C2/PruC4R, SHLM1/SHLM2, and SHLM3/SHLM4 enabled the identification of the *S* genotype of 27 additional cultivars and selections (Table 1).

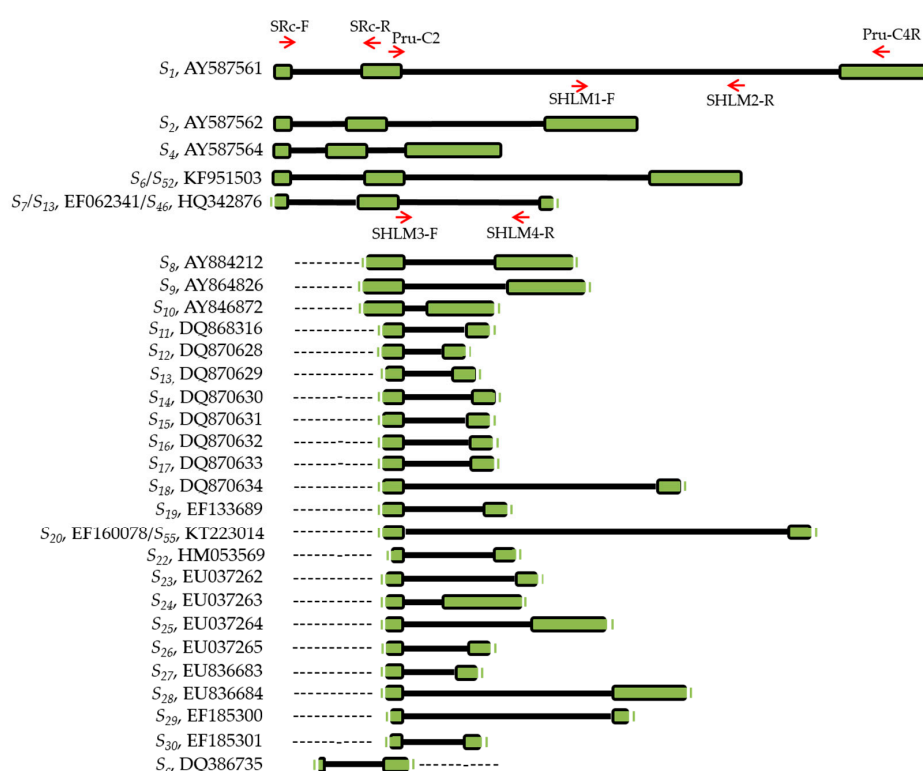
Table 1. Incompatibility groups and *S-RNase* genotype of the 44 apricot cultivars and selections analyzed in this study.

Incompatibility Group	<i>S-RNase</i> Genotype	Apricot Genotypes Analyzed in this Study
I	S_1S_2	T069 T120 T139A
II	S_8S_9	C007 ¹
V	S_2S_8	C012 ¹
VIII	S_6S_9	Cheyenne T001
XVIII	S_1S_3	A150
XXII	S_3S_9	A153 Kosmos
XXIV ²	S_1S_6	Primaya
XXV ²	S_1S_9	A106
XXVI ²	S_6S_8	C009 ¹
Self-compatible cultivars	S_2S_c	Kalao
		Regibus
	S_3S_c	C014 ¹
		Rambo
	S_4S_c	T002
	S_7S_c	Beliana
	S_9S_c	C003 ¹
		Lido
	S_c	Dorada
		Memphis
		Milord
		Murciana
		Oscar
		Sherpa
		T003
	S_1	A154
		T004
		T005
		T109
		T124
		T139B
		T140
	S_2	Cyrano
		T098
	S_3	Mikado
		T006
	S_7	T116
	S_9	A151
		A152
		A155
	S_{20}	T007

¹ S_c/S_8 allele identified using fluorescence microscopy; ² Incompatibility groups first reported in this study.

Table 2. Primers used in this study for the identification of *S*-alleles in *Prunus armeniaca*.

Primers	Amplified Region	Sequence (5' → 3')	Reference
SRc-F	<i>S</i> -RNase 1st intron	CTCGCTTTCCTTGTCTTGC	[41]
SRc-R	<i>S</i> -RNase 1st intron	GGCCATTGTTGCACCCCTTG	[41]
Pru-C2	<i>S</i> -RNase 2nd intron	CTTTGGCCAAGTAATTATTCAAACC	[27]
Pru-C4R	<i>S</i> -RNase 2nd intron	GGATGTGGTACGATTGAAGCG	[27]
AprFBC8-F	<i>SFB</i>	CATGGAAAAAGCTGACTTATGG	[39]
AprFBC8-R	<i>SFB</i>	GCCTCTAATGTCATCTACTCTTAG	[39]
SHLM1-F ¹	<i>S</i> ₁ -RNase 2nd intron	GGTGGAGGTGATAAGGTAGCC	
SHLM2-R ¹	<i>S</i> ₁ -RNase 2nd intron	GGCTGCATAAGGAAGCTGTAGG	
SHLM3-F ¹	<i>S</i> ₇ -RNase 2nd intron	TATATCTTACTCTTTGGC	
SHLM4-R ¹	<i>S</i> ₇ -RNase 2nd intron	CACTATGATAATGTGTATG	

¹ Specific primers designed in this study.**Figure 1.** Gene structure of the *S*-alleles sequenced, showing the exons in green square and including the primers used in this study for the alleles *S*₁ and *S*₇/*S*₁₃/*S*₄₆. Unknown sequences are represented by dotted lines. The first column shows *S*-genotype and gen bank accession. *S*₁ [41], *S*₂ [41], *S*₃ [41], *S*₄ [41], *S*₆/*S*₅₂, *S*₇/*S*₁₃ and *S*₄₆ [Unpublished], *S*₈ [42,43], *S*₉ [42], *S*₁₀ [42], *S*₁₁ [34], *S*₁₂ [34], *S*₁₃ [34], *S*₁₄ [34], *S*₁₅ [34], *S*₁₆ [34], *S*₁₇ [34], *S*₁₈ [34], *S*₁₉ [34], *S*₂₀ [34]/*S*₅₅ [Unpublished], *S*₂₂ [44], *S*₂₃ [44], *S*₂₄ [44], *S*₂₅ [44], *S*₂₆ [44], *S*₂₇ [44], *S*₂₈ [44], *S*₂₉ [44], *S*₃₀ [44], *S*_c [43].

Due to the identical sequence of the *RNase* gene in the *S*_c and *S*₈ haplotypes [43], the pairs of primers SRc-F/SRc-R and Pru-C2/PruC4R could not be used for distinguishing between those two alleles. Therefore, the *S*_c and *S*₈ alleles can only be identified using the primers AprFBC8-F and AprFBC8-R that amplify a fragment of approximately 500 bp in the *S*_c allele and 150 bp in the *S*₈ allele from the V2 and HVb variable regions of the *SFB* gene [39]. Thus, the *S*-genotype of 13 cultivars was identified using the primers AprFBC8-F and AprFBC8-R (Table 2). Moreover, self-(in)compatibility was also evaluated by direct observation of pollen tube growth in the pistil after self-pollination in five selections (“C003”, “C007”, “C009”, “C012”, and “C014”), in which the primers

AprFBC8-F/AprFBC8-R were not able to differentiate between the S_c and S_8 alleles. Pollen germination was observed on the stigma in all cultivars. Pollen tube growth was arrested in the upper half of the style, showing a terminal callose plug and end thickening (Figure 3A) in three genotypes (“C007”, “C009”, and “C012”) that were considered as self-incompatible. Self-compatibility was considered in two genotypes (“C003” and “C014”), in which the pollen tubes grew along the style (Figure 3B) and at least one of them reached the base of the style in most of the pistils that were analyzed (Figure 3C). As expected, the pollen used was viable and all cross-pollinated pistils displayed pollen tubes at the base of the style (Table 3).

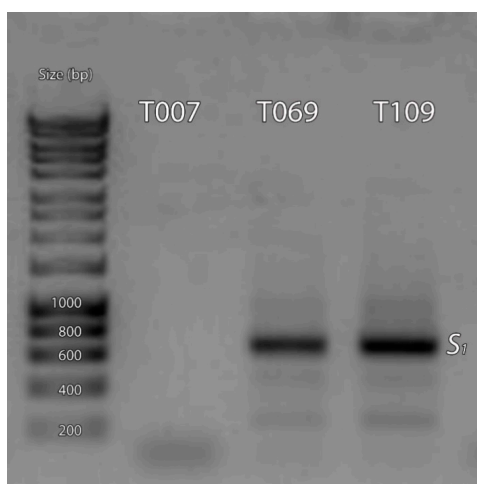


Figure 2. PCR amplification of the *RNase* second intron region using the specific primers SHLM1 and SHLM2 for the identification of the S_1 allele in T007 (S_{20}), T069 (S_1S_2), and T109 (S_1).

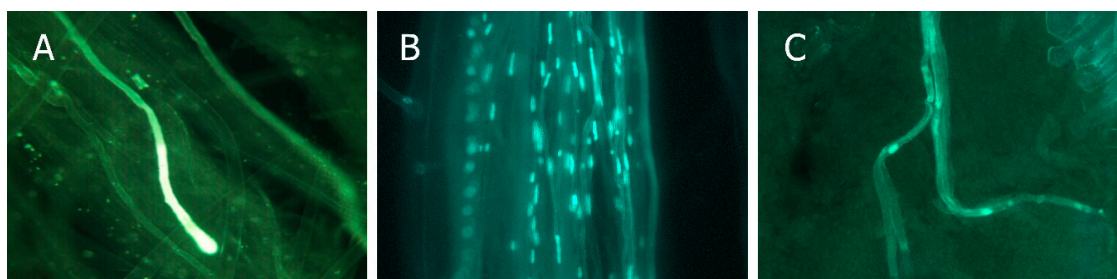


Figure 3. Pollen tube growth in apricot pistils. (A) Pollen tube arrested in the upper half of the style in a self-incompatible cultivar; (B) Pollen tubes growing along the style; and, (C) Pollen tubes at the base of the style in a compatible cross. Scale bars, 100 μ m.

Table 3. Pollen tube behavior in the pistils of five apricot selections after self- and cross-pollinations.

Cultivar	Number of Pistils Examined	Pistils (%) with Pollen Tubes			Percentage of Style Travelled by the Longest Pollen Tube	Mean Number of Pollen Tubes at the Base of the Style	Compatible (C) or Incompatible (I) Behavior
		in the Middle of the Style	at the Base of the Style	Reaching the Ovule			
Self-pollination							
C003	10	100	90	80	100	2	C
C014	18	100	94	83	100	2.1	C
C007	8	75	0	0	57	0	I
C009	10	60	0	0	56	0	I
C012	9	100	0	0	66	0	I
Cross-pollination							
C003 × Katy	18	100	78	78	100	1	C
C014 × Katy	5	100	100	100	100	3.4	C
C007 × Katy	5	100	80	80	100	1	C
C009 × Katy	5	100	80	80	100	1.6	C
C012 × Katy	4	100	100	100	100	1.5	C

3. Discussion

The S-allele composition of 44 apricot genotypes, including new cultivars and advanced selections of three breeding programs, of which 43 have been reported here for the first time, has been identified. S-genotyping by PCR analysis allowed for the identification of the two S-alleles in 21 genotypes. Approximately half of the genotypes analyzed (55%) were self-compatible, including commercial cultivars, such as “Murciana” or “Dorada” [45,46]. The remaining genotypes, mostly advanced breeding selections, were considered as self-incompatible. This S-allele identification can speed up breeding programs by selecting self-compatible genotypes at the seedling stage or genotypes with the desired S-allele composition. The results confirm the tendency of the increase in the number of self-incompatibility genotypes in apricot that is in contrast with the previous scenario, when most European cultivars were self-compatible [47].

According to their S-allele composition, 13 self-incompatible cultivars have been allocated in nine previously determined incompatibility groups [24,37–40]. “Primaya” (S_1S_6), “A106” (S_1S_9), and “C009” (S_6S_8) have been assigned to XXIV–XXVI groups, three new incompatibility groups that have been reported herein for the first time. By contrast, 15 cultivars had the S_c allele and they were characterized as self-compatible, and, consequently, can be considered as universal pollen donors. Results herein confirm the S-alleles of ‘Beliana’ (S_cS_7) [48].

The PCR fragment of the first intron of S_8 was identical to S_c . Moreover, both S-RNases showed equal isoelectric points [43], suggesting that the S-RNase of S_8 and S_c alleles are identical. Thus, it is likely that S_c could derive from the S_8 allele [43]. In this case, as it has also been reported in other *Prunus* species [25,49], the breakdown of the incompatibility system can be related to a mutation outside of the S-locus [50]. Indeed, an insertion of 358 bp in the *SFB* gene causes a loss of the incompatibility [50] that was also observed in the *SFB_c* gene but not in the sequence of *SFB₈* [43]. That is why primers have been designed based on the *SFB* sequence for the identification of S_c and S_8 , such as the primer pair AprFBC8-R/F. Thus, using these primers, we could distinguish between S_c and S_8 in 13 apricot genotypes. However, S_c and S_8 could not be distinguished in five genotypes. Although the PCR-based method has been a preferred technique for S-allele identification, mismatching of PCR primers can result in no amplification. This might be the case in these five selections, in which an amplified fragment was not obtained using the primer pair AprFBC8-R/F. As an alternative, we performed controlled pollinations that were carried out in the laboratory and pollen tube growth was observed under the microscopy to determine self-(in)compatibility. The results showed that two genotypes behaved as self-compatible and they were assigned with allele S_c , whereas the other three were self-incompatible and were assigned with allele S_8 .

The inaccuracy of the PCR-based method could also be reflected in the amplification of just one allele. Indeed, 23 of the 44 genotypes that were analyzed in this work show only one S-allele after PCR amplification. This could be due either to homozygosity in the case of the S_c allele or with amplification problems in the other genotypes. Thus, the S-genotype of “Dorada”, “Memphis”, “Milord”, “Murciana”, “Oscar”, “Sherpa”, and “T003” is likely to be S_cS_c . Further sequencing work is under way to identify the other S allele in the remaining 16 genotypes. S-allele analysis by band identification in a gel can also provide inaccurate identification. It also should be taken into account that the S-RNase sequence has only been revealed in some S-alleles, and, in most of the cases, such as S_9 , S_{15} , or S_{27} , it has been only partially sequenced, and/or not including introns that are essential for fragment sizing in S-allele identification. The use of an automatic fragment analyzing system can also report small differences in fragment size; for example, in S_2 allele (327 bp [33]; 334 bp [24]; 332 bp [51]) or S_c (353 bp [33]; 358 bp [24]; 355 bp [51]). Taken together, all this has led to numerous homologies [2,4,35]. That is the case of S_6 and S_{52} [24] or S_{20} and S_{55} , and S_7 , S_{13} (EF062341) and S_{46} shown in this study.

There is a need to standardize the criteria for the S-allele identification in different laboratories, such as the use of the same primer pairs and the complete sequencing of the S-alleles, including introns. This will facilitate S-allele identification avoiding confusion and it will provide valuable information

for apricot growers and breeders. Results herein allow for establishing incompatibility relationships among apricot cultivars with previous unknown pollination requirements, allowing the selection of the appropriate parents in the design of new crosses in apricot breeding programs and self-compatible and inter-compatible cultivars in new commercial orchards. Moreover, the approach that is followed in this work can be of interest to other fruit crops in the Rosaceae with similar problems to those faced by apricot.

4. Materials and Methods

4.1. Plant Material

Plant material was collected from different apricot germplasm collections and orchards of Aragon, Cataluña, and Extremadura (Spain). Young leaves from 16 new apricot cultivars and 28 advanced selections from different breeding programs were used in this study for PCR analysis (Table 1). Moreover, flowers were collected from five of the selections (“C003”, “C007”, “C009”, “C012”, and “C014”) for pollination experiments (Table 3).

4.2. DNA Extraction

Young leaves from the 44 cultivars and selections were collected for the identification of self-incompatibility alleles. Genomic DNA was extracted following the protocol described by Hormaza [52] and using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The DNA quantification was performed by using a NanoDrop™ ND-1000 spectrophotometer (Bio-Science, Budapest, Hungary).

4.3. *S*-RNase Allele Identification by PCR Analysis

Self-incompatibility was established by *S*-allele identification through PCR amplification of *RNase* and *SFB* regions. Fluorescently labelled forward primer SRc-F with the reverse primer SRc-R [33,41] were used to amplified the first intron region of the *S-RNase* gene. PCR amplifications were carried out in 15 µL reaction volumes, containing 10× NH₄ Reaction Buffer, 25 mM Cl₂Mg, 2.5 mM of each dNTP, 10 µM of each primer, 100 ng of genomic DNA, and 0.5 U of BioTaq™ DNA polymerase (Bioline, London, UK). The temperature profile used had an initial step of 3 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 3 min at 72 °C, and a final step of 5 min at 72 °C. *S_c* and *S₈* alleles had an identical fragment length of 355 bp from the first intron, amplified using the primers SRc-(F/R) [42,43]. Thus, the *SFB* specific primers, AprFBC8-F and AprFBC8-R, were used to distinguish between both alleles. The identification was carried out according to the PCR conditions of Halasz et al. [39]. The sizes of the products that were obtained by PCR were analyzed in a CEQ™ 8000 capillary electrophoresis DNA analysis system (Beckman Coulter, Fullerton, CA, USA) and compared and classified according to Vilanova et al. [33] and Kodad et al. [51]. For the amplification of the *RNase* second intron region, the primers Pru-C2 and Pru-C4R were used, as recommended by Vilanova et al. [33], but with the addition of 10 cycles and using 55 °C of annealing temperature, as indicated by Sonneveld et al. [53]. The amplified fragments were separated on 1% (*w/v*) agarose gels and DNA bands were visualized using the nucleic acid stain SYBR Green (Thermo Scientific, St Leon-Rot, Germany).

Since the PruC2/PruC4R primer combination was inefficient to distinguish *S₁* and *S₇* alleles, new specific primers were designed from the second intron, SHLM1 and SHLM2 for the identification of the *S₁*-haplotype and SHLM3 and SHLM4 for the identification of the *S₇* haplotype (Table 2, Figure 1). For *S₁* identification, *Taq* DNA polymerase (Qiagen, Hilden, Germany) was used with a temperature profile of an initial step of 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 62 °C for 1 min and 30 s and 72 °C for 2 min, and a final extension of 72 °C for 5 min. For *S₇* identification, the amplification was performed using Phusion® High-Fidelity DNA Polymerase (Thermo Scientific, St Leon-Rot, Germany) with the following temperature profile: 30 s at 98 °C, 35 cycles of 10 s at 98 °C, 30 s at 51 °C, and 1 min at 72 °C, with a 5 min final extension step at 72 °C.

4.4. Sequencing of Genomic PCR Products

In order to design *S*₇-specific primers, a fragment of 900 bp obtained by using the primers PruC2/PruC4R was isolated using the NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany). Cloning was performed using CloneJET PCR Cloning Kit (Thermo Scientific, St Leon-Rot, Germany) and by electroporation in *E. coli* Single-Use JM109 Competent Cells (Promega Biotech Ibérica SL, Madrid, Spain). The search for similarities in the sequences of the NCBI database was performed with BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>, version 2.2.10).

4.5. Pollination Experiments

Pollination crosses were carried out with the five selections in which the identification of the *S*₈ and *S*_c alleles was not possible by PCR amplification. To establish self-(in)compatibility, self-pollinations and cross-pollinations were carried out in semi-in vivo culture of flowers in the laboratory and pollen tube growth was observed under fluorescence microscopy. As control, flowers of each cultivar were pollinated with pollen from the cultivar “Katy”, which is known as the universal pollinizer for apricot (Table 3) [54].

For each self- and cross-pollination, a group of 20–25 flowers was collected from the trees at the balloon stage (Figure 4A), corresponding to stage 58 on the BBCH scale for apricot [55]. Flowers were emasculated in the laboratory to avoid self-pollination, placed on florist foam in water (Figure 4B) at laboratory temperature, and hand pollinated with the help of a paintbrush 24 h after emasculatation. Pollen was obtained from flowers at the same balloon stage by removing and drying the anthers at laboratory temperature during 24 h. Pollen grains were sieved by using a fine mesh (0.26 mm) and then used immediately or frozen at −20 °C until further use at laboratory temperature [56]. After 72 h, when the pollen tubes had enough time to reach the ovary [57], pistils were fixed in ethanol (95%)/acetic acid (3:1, *v/v*) during 24 h, and conserved at 4 °C in 75% ethanol [57]. Pollen viability was also evaluated after each pollination. Pollen was scattered on a solidified pollen germination medium [58] and observed under the microscope 24 h after pollen germination. Pollen grains were considered to be viable when the length of the growing pollen tube was higher than the pollen grain diameter.

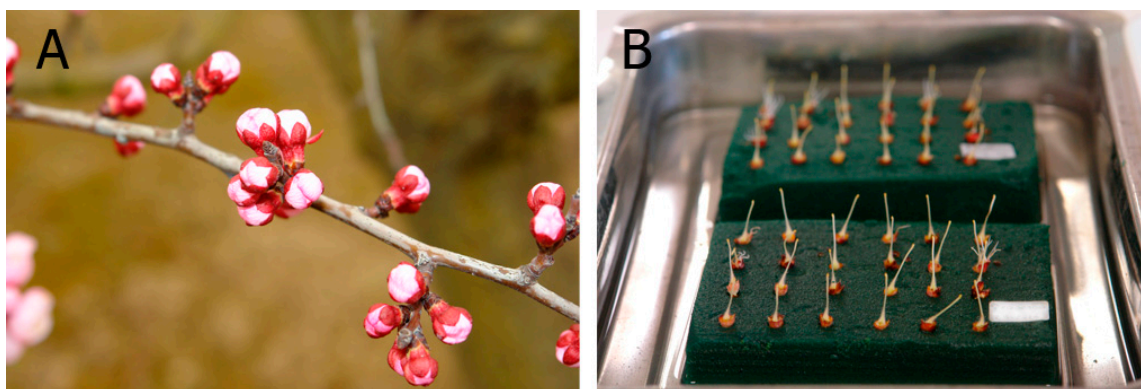


Figure 4. Apricot flowers at balloon stage in the field (A) and pistils on florist foam in water in the laboratory (B).

For histochemical preparations, the pistils were washed three times for 1 h with distilled water and left in 5% sodium sulphite at 4 °C. After 24 h, they were autoclaved at 1 kg/cm² during 10 min in sodium sulphite [59] to soften the tissues, squashed, and stained with 0.1% (*v/v*) aniline blue in 0.1 N K₃PO₄ [60] to stain callose deposition during pollen tube growth under the microscope [21,22,24,56,61]. Pollen tube growth along the style was observed by a Leica DM2500 microscope (Cambridge, UK) with UV epifluorescence using 340–380 bandpass and 425 longpass filters.

Pollen tube growth was recorded on 8–18 pistils in each self-pollination and at least 4–18 pistils in control crosses. Cultivars were considered as self-compatible when most of the pistils displayed at least one pollen tube reaching the base of the style. Self-incompatibility was considered in self-pollinated flowers when pollen tube growth was arrested along the style in all pistils.

Supplementary Materials: Supplementary materials can be found at <http://www.mdpi.com/1422-0067/19/11/3612/s1>.

Author Contributions: S.H., J.I.H., J.L. and J.R. conceived the study. S.H., J.L., J.R. and J.I.H. designed the experiments and wrote the paper. J.L. performed the sequencing of genomic PCR products. S.H. performed most of the experiments and prepared the final data and figures.

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Abbreviations

GSI	Gametophytic Self-Incompatibility System
SSI	Self-Incompatibility System
SI	Self-Incompatibility

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MATERIAL SUPLEMENTARIO. Identification of Self-Incompatibility Alleles by Specific PCR Analysis and S-*RNase* Sequencing in Apricot

El material suplementario puede ser encontrado y descargado en:

<http://www.mdpi.com/1422-0067/19/11/3612/s1>.

CATATCAATTAGACAGCGCATTAGTTGAATGAGGGCGAATTTAGGACGGGAAAGTG GTATGTATTGTT
 TCAAATATTTTTTTTCTTTTCATATTGCTCTTAGAAAATTAGATTGTCATATGAAGATAATACATTTTATTT
 AATAAGCCATGGTCTTGGATAAAATTTTGATGTTTGTCTCTGCTAGGCACATTATTTTGAATCTCTTTTG
 AAATAGGGGAAATATATGGCTAAGTACATTTATATTCATAGGTATTGATGTATATGTCAAGTCAGAATC
 GTGGGAAATAAAAAAACACACATAATTTGACACACTTTTTAATCTTGAGATGAATCACATTGAAAATC
 ATTTACCATTTAAGAGAAATGCATTTCTTTTATTACTTCATTGAAAACCCAAATACATTTAATTATGTATA
 CTCTCTCACTTCCCTTTACCTATTATCATTTTATTCAAAGCAACCTCCTTTATTACTCACCACCTAAGAGT
 ATTCTGACTCTCTAAACCACCTTATTAGATAAAATTTAAAAAGGAAGTGAGAAAACCTCATCTCTAACCAT
 GCTTTTTATCCAGCTCATAGGGAGACCTTTACGAGCTTTTAAATCTGAGGAGAGAGAATGGACCCCTAA
 TGGCTCCATATAATATATTGGTGCCTTATTTAATGAATATTTTAAACCTTTAATTAATATTGCCTATTTTTT
 ATATAGAGATGGACATTTATGTTGAATTAAAAAAGAATTATAACATTTATGACTCCCTAAAGAATATG
 ATGGAAGTTGAAATTTTATAGATAGCTCTAAAATAGATTTTGTGTGTTTTAGCTAAATTTTAACTACAA
 AAATAGAAAGAATGATTCCATGTGCATAATCATAATACTTGACACCTATCCACCATCCTTAATACTTGAT
 ACGTGTCCATATGCATAATCATGGTTTCATATACAAAATAAAAAGTTAATCATGGTTATTCATATGAACA
 CATGTCAAGTGTTGAAAATAATAATGAGGATATACACTGGGTAAAAAGGTGAGTAAAAAATGCTCAC
 CTTTCTTCATTGTCTTCCCTTCCCTGTGCCATGGGTAGCCATAGTCTCTTCATCCCCAACGTTCCCTTCT
 ACCTTACCACCCATACTCCCCCTTCGCTACATTATTCCTCTTAAAAATATTTTTCTCTTAAACCCCTTCAGA
 ACCCATAAAAATAAAAAATAAAAAAAGAACCCCAAATGCACACCCCTCTCCCCGGTAGGACCAGGGT
 CCACATCACACCGCGTTTCTGCACCACCACCAGATCTACCATTTTATAGGATCCTACATTATTGGCTTGTGT
 GGTAACACAGCCACTTGATCCATCTTACTAGACCCATTGGAGCTACCGCATGTCTAGCTCCACCAAGAG
 AGAGTAACAGGTAGGAAACAGTATAAAGGAGGAGAGAAAAAATAAGGGACATTTTGAAAAAGCAA
 AAGTATATAAATTTTTTATATGTTTCTAAGAAGTAAAAAAACATGTTAATTTTTTATTTAAAAAATAC
 TCATGACAGATACATTTTGAGGCATTAATAATGTGTATATAAATTTTTGGTTTTTGTGCTCATCGATCCT
 GATCTATTTTATAGTAATGGATATGATCATCTAATTAAGTACCTACCATTTTGTGCTTGTATTCAAA
 ATATTGTACCTAATGAAAGAATGAAAATGGTAATAACAATCTTATACAAAAATGAACTCTAACTATCCC
 TTACGTTTTTACTTTTTCTCCAATTATGTATATTTGCTTGATGTCTCAGTACCTCAGTTGCGAACCAA
 ACTGAAGAAATCTTGGCCGGACGTGGAAAGTGGGAATGATACAAAATTTTGGGAAGGCGAATGGAAC
 AAACATGGTACATGTTCTGAAGAGAACTAAACCAAATGCAATACTTCGAGAGATCCCACAACATGTG
 GAGGTCGTACAATATTACAGAGATCCTTAAAAACGCTTCAATCAA

Figure S1. *S₂₀/S₅₅* allele. Sequence of 2002 bp obtained from the genotype 'T007 using the primers Pru-C2 and PruC4R. Predicted exons and intron are showed in red and black, respectively.

CTTTGGCCAAGTAATTATTCAAACCCAAGGAAGCCTAGTAATTGCAATGGGTCACAATTTGACGCAAGG
 AAAGTGGTACGTATTGTTTCATTATTTTATATCTTACTCTTTGGCATTAGTTTTTTAGGTTTTTTTATATA
 TAGGAATTAGTGTTTAGAAAATTAGATTGTCATGTGAAGATTTTAATAAAATAAAACCTTTTTCAATA
 AGCCTTGGGTGTTATAGATTAAATTTTGATGTTGGTCTTAGTTAGACACATTATTTGAATATATAGTT
 AAGTACAAAATGGCAAGTACATATTAATATACTTTGAAAATATAATGGATCTGCTCATCTAATACCATT
 TTGTACTAATGCATATATGCAAAACATTGTACATCAAATCTTTTTAAAGCAAGGCTATAATATATTATTG
 GAGATTAAACTCAAATTAATGCTCGGATTTAATGAGACAAAAACAATCTTTGTATTTTAGTACAAG
 CGACAATATAAATTACATGAGAATGAGTTTCTCACATACACATTATCATAGTGTCATAGAAGTTGGAATT
 TAAGACTAGAGATCTACAAATCAAGACTCTTTTTATATTGGGCTAGACTCCGTTAACGACAAAACTCA
 ATAATATTCAAGAATGAAAATCTAATTATCATTTATTGATTACTTTTTCTCAAATATGTGTCTACATTGT
 TTGGATGTCCCAGTCCCCTCAATTGCGATCAAACTGAAGATATCTTGGCCCGACGTGGAAGGTGGCAA
 TGATACACAATTTTGGGAAGGCGAATGGAACAAACATGGTACTTGTTCCGAAGAGACACTTGACCAAA
 CGCAATACTTCGCGCGATCCCACGCGTTTTGGAACATGCGCAATATTACGGAGGTCCTTAAAAACGCTT
 CAATCG

Figure S2. S_7 , S_{13} and S_{46} allele. Sequence of 915 bp obtained from the cultivar 'Charisma' using the primers Pru-C2 and PruC4R. Predicted exons and intron are showed in red and black respectively.

CAPÍTULO 4. Self-Incompatibility in Apricot: Identifying Pollination Requirements to Optimize Fruit Production

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CAPÍTULO 4

Self-Incompatibility in Apricot: Identifying Pollination Requirements to Optimize Fruit Production

Auto(in)compatibilidad en albaricoquero: Identificación de las necesidades de polinización para optimizar la producción frutal

RESUMEN

En los últimos años se está produciendo una importante renovación de las variedades de albaricoquero a nivel mundial, con la introducción de muchas nuevas obtenciones. Algunos genotipos autoincompatibles tolerantes a la enfermedad de sharka causada por el plum pox virus (PPV), que puede reducir severamente la producción y la calidad de la fruta, están siendo utilizados como parentales en la mayoría de los programas de mejora. Como consecuencia, el rasgo de auto(in)compatibilidad se puede transmitir a la descendencia, lo que ha llevado al desarrollo de nuevas variedades autoincompatibles. Esta situación puede afectar considerablemente al manejo del albaricoquero, ya que las necesidades de polinización tradicionalmente no se consideraban un problema en este cultivo y se carece de información para muchas variedades. El objetivo de este trabajo fue determinar las necesidades de polinización de un grupo de nuevas variedades mediante la identificación molecular de los alelos *S* a través de la amplificación por PCR de las regiones de los genes *RNasa* y *SFB* con diferentes combinaciones de cebadores. Se ha descrito el genotipo *S* de 66 variedades de albaricoquero, 41 de ellas por primera vez. Cuarenta y nueve variedades se consideraron autocompatibles y 12 autoincompatibles, que fueron asignadas en sus correspondientes grupos de incompatibilidad. Además, la información disponible fue revisada y agregada a los nuevos resultados obtenidos, dando como resultado una compilación de las necesidades de polinización de 235 variedades de albaricoquero. Esta información permitirá una selección eficiente de los parentales en programas de mejora genética, el diseño adecuado de nuevas plantaciones, así como la identificación y solución de problemas de producción asociados a la falta de cuajado de frutos en plantaciones ya establecidas. La diversidad en el locus *S* observada en las variedades desarrolladas en los programas de mejora indica un posible cuello de botella debido al uso de un número reducido de parentales.

Article

Self-Incompatibility in Apricot: Identifying Pollination Requirements to Optimize Fruit Production

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Abstract: In recent years, an important renewal of apricot cultivars is taking place worldwide, with the introduction of many new releases. Self-incompatible genotypes tolerant to the sharka disease caused by the plum pox virus (PPV), which can severely reduce fruit production and quality, are being used as parents in most breeding programs. As a result, the self-incompatibility trait present in most of those accessions can be transmitted to the offspring, leading to the release of new self-incompatible cultivars. This situation can considerably affect apricot management, since pollination requirements were traditionally not considered in this crop and information is lacking for many cultivars. Thus, the objective of this work was to determine the pollination requirements of a group of new apricot cultivars by molecular identification of the *S*-alleles through PCR amplification of *RNase* and *SFB* regions with different primer combinations. The *S*-genotype of 66 apricot cultivars is reported, 41 for the first time. Forty-nine cultivars were considered self-compatible and 12 self-incompatible, which were allocated in their corresponding incompatibility groups. Additionally, the available information was reviewed and added to the new results obtained, resulting in a compilation of the pollination requirements of 235 apricot cultivars. This information will allow an efficient selection of parents in apricot breeding programs, the proper design of new orchards, and the identification and solution of production problems associated with a lack of fruit set in established orchards. The diversity at the *S*-locus observed in the cultivars developed in breeding programs indicates a possible genetic bottleneck due to the use of a reduced number of parents.

Keywords: apricot; pollen tube; pollination; *Prunus armeniaca*; *S*-alleles; self-incompatibility



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1. Introduction

Apricot (*Prunus armeniaca* L.) belongs to the genus *Prunus* in the Rosaceae family. This crop was domesticated in different domestication events ca. 2000–3000 years ago in Central Asia, which is considered the center of origin of the crop [1,2]. Later, apricot was introduced into the Mediterranean Basin from the Caucasus, a secondary center of diversification [3]. Nowadays, apricot world production has reached 3.7 million tons [4] and it is considered one of the most economically important fruit crops in temperate regions [5].

Apricot cultivars have been traditionally classified into six eco-geographical groups according to their geographical origin: Central Asian, East Chinese, North Chinese, Dzhungar-Zailij, Irano-Caucasian, and European [6]. Apricot cultivars from the Central Asian, which is the oldest and most diverse, the Dzhungar-Zailij, and the Iranian-Caucasian groups, are mostly self-incompatible. Commercial cultivars of Europe, North America, South Africa, and Australia are mainly self-compatible and belong to the European group [7], which has two main gene pools: Continental Europe and Mediterranean Europe [8].

Gametophytic Self-Incompatibility (GSI) is a mechanism to prevent self-fertilization and promote outcrossing present in the Rosaceae [9]. It is controlled by a multiallelic locus named *S* that contains several genes involved in the pollen-pistil recognition. The *S-RNase* gene encodes pistil-expressed glycoproteins with ribonuclease activity that act as highly selective cytotoxins that cause rejection of pollen when its *S*-allele is the same as either of the two *S*-alleles expressed in the pistil [10,11]. Consequently, pollen tube growth is arrested in the style preventing fertilization [12]. The *SFB* gene, which codifies an F-box protein, is specifically expressed in the pollen, and determines the pollen allele specificity [13]. Nowadays, there are self-incompatible and self-compatible apricot cultivars, as in other *Prunus* species such as almond (*Prunus dulcis*), sweet cherry (*Prunus avium*), Japanese plum (*Prunus salicina*), European plum (*Prunus domestica*) and sour cherry (*Prunus cerasus*) [7]. In recent years, the system seems to be more complicated, with the report of different modifier genes involved in the incompatibility response [14,15].

The pollination requirements of cultivars can be established by evaluation of fruit set after self- and cross-pollination under field conditions [16,17], by pollen tube growth observations in self- and cross-pollinated flowers under fluorescence microscopy with the advantage of avoiding failures caused by adverse weather conditions [18–22], and by molecular techniques based on PCR and sequencing approaches of the *S*-locus [23]. In apricot, 33 *S*-alleles (*S*₁ to *S*₂₀, *S*₂₂ to *S*₃₀, *S*₅₂, *S*₅₃, *S*_v, and *S*_x) have been identified so far, including one allele linked to self-compatibility (*S*_c) [16,24–27].

In recent years, an important renewal of apricot cultivars is taking place worldwide, with the introduction of many new releases in response to productive and industrial changes in the crop [28]. The main objectives of breeding programs for the development of new commercial cultivars include sharka-tolerance/resistance, climate adaptability and improved organoleptic properties fruit such as firmness, skin and fresh color, and aroma [29]. The sharka disease was reported for the first time in plum around 1915 and, since then, it has become the most economically important virus disease of *Prunus* species [30]. PPV is usually transmitted by aphids, and probably spread by the propagation of infected plant material [31]. Sharka disease causes discoloration on leaves, petals and fruits, severely reducing fruit production and quality [31].

Self-(in)compatibility and inter-(in)compatibility relationships in apricot have been characterized in traditional and local cultivars from different regions such as China [26], Hungary [32], Morocco [33], North America and Spain [16,21,34], Tunisia [17,35], and Turkey [32,36,37]. Although self-(in)compatibility of new apricot releases are evaluated in some public breeding programs (‘Centro de Edafología y Biología Aplicada del Segura’ (CEBAS-CSIC) in Murcia [38,39], and the ‘Instituto Valenciano de Investigaciones Agrarias’ (IVIA) in Valencia [40], both in Spain; ‘Institut National de la Recherche Agronomique’ (INRA) in France [41–43]; University of Bologna and University of Milan in Italy [44]; ‘Agricultural Research Service’ in Parlier, CA, and Rutgers University in New Brunswick, NJ, in the USA [45]), the pollination requirements of many cultivars are still unknown.

In this work, we evaluate the hypothesis that a significant proportion of new apricot cultivars are self-incompatible and that knowing the incompatibility relationships between cultivars is needed to select appropriate pollinating cultivars in the design of orchards. For this purpose, the *S*-genotype of 66 apricot cultivars was analyzed; of them, 49 were self-compatible and the other 12 were self-incompatible. The results allowed allocating the self-incompatible cultivars in their corresponding incompatibility groups according to their *S*-alleles. In addition, a compilation of the available *S*-genotype data was carried out to evaluate the distribution of the *S*-alleles in the main apricot cultivars grown worldwide and to assess their current genetic diversity.

2. Results

2.1. *S*-Alleles and Incompatibility Groups

PCR analysis based on the amplification of *RNase* and *SFB* genes allowed identifying the *S*-genotype in 66 apricot cultivars, 41 of them reported for the first time (Tables 1 and 2). Firstly,

the combination of the primers SRc-F/SRc-R amplified the first intron of the *RNase*, and the different alleles were classified according to the sizes of the fragments previously established by Vilanova et al. [25] (Figure 1A; Supplementary Table S1). To our knowledge, the sequence of the first intron has only been reported in the S_1 , S_2 , S_4 , S_6 , S_7 and S_c -alleles [22]. The PruC2/PruC4R primer combination amplified the second intron and was used to differentiate the S_6 and S_9 alleles in 15 genotypes, whose sequences were also previously reported [21,46]. The SHLM1 and SHLM2 primer combination amplified a fragment of 650 bp in 17 cultivars, indicating the presence of the S_1 -allele. A 413 bp fragment, that corresponds to the S_7 -allele, was only detected in ‘Charisma’ and ‘Ninfa’ using the primer pair SHLM3/SHLM4 [22].

Table 1. Incompatibility group (I.G.) and S-genotype of 103 apricot cultivars.

I.G. (S-Genotype)	Cultivars Analyzed in This Study	Cultivars Analyzed in Previous Studies
I (S_1S_2)	Muñoz ^b , Pandora ^b	AC1 [21], Castleton [16], Farmingdale [47], Giovanniello [47], Goldrich [48], Hargrand [48], Lambertin-1 [48]
II (S_8S_9)		Ceglédi óriás [24], Cologlu [32], Ligeti óriás [24], Perlecot [21], Pinkcot [21], Szegedi M. [16]
III (S_2S_6)		ASF0401 [21], Avirine (Bergarouge) [21], Monique [49]
IV (S_2S_7)		Ouardi [35], Priana [34]
V (S_2S_8)		Alyanak [32], Holly Cot [21]
VIII (S_6S_9)	Apribang (ASF0405) ^a	ASF0402 [21], Cataloglu [32], Cheyenne [22], Fera Cot [21], Flashcot [50], JNP [21], Ninja [50], Orangered [16,21], Soganci [32], Stark Early Orange [16,21], Sunny Cot [21], Wonder Cot [21]
X (S_7S_{12})	IPS23214 ^a , Monred ^a	Bedri Ahmar [35], Oud Rhayem [35]
XI (S_9S_{13})		Haci Haliloglu [32], Kabaasi [32]
XII ($S_{11}S_{13}$)		Voski [24]
XIII (S_6S_{19})		Levent [32]
XV (S_7S_8)		Ouled El Oud [35]
XVI (S_7S_{11})	Samourai ^{a,c}	Bouk Ahmed [35], Hamidi [35]
XVII (S_8S_{12})		Adedi Ahmar [35]
XVIII (S_1S_3)		Cooper Cot [21], Perfection [48]
XIX (S_2S_3)		Mayacot [21], Sun Glo [49]
XX (S_2S_9)		Goldstrike 02 [21], Hasanbey [32], Magic Cot [21]
XXI (S_3S_8)	Farely ^b , Megate ^b , Monster Cot ^b , Priabel ^b	Lilly Cot [21], Spring Blush [21]
XXII (S_3S_9)		Almadulce [21], Flodea [21], Henderson [16,21], Kosmos [22], Tsunami [50]
XXIII (S_7S_9)		Goldbar [21], Kurukabuk [32]
XXVI (S_1S_6)		Primaya [22]
XXV (S_1S_9)		Almater [50], Aurora [50], Medaga [50]
XXVI (S_6S_8)		Robada [50]
Group 0	Harcot ^b (S_1S_4) [49]	Bouthani Ben Friha ($S_{12}S_{13}$) [35], Cow-1 (S_1S_{31}) [16], Cow-2 ($S_{20}S_{31}$) [16], Estrella (S_1S_7) [51], Harlayne (S_3S_{20}) [16], Harmat ($S_{10}S_{11}$) [24], Korai zamatos ($S_{12}S_{13}$) [24], Mariem (S_7S_{20}) [16], Martinet (S_2S_2) [16], Oud Hmida (S_2S_{12}) [35], Perla (S_2S_{20}) [16], Portici (S_2S_{20}) [16], Shalakh (Erevani) (S_5S_{11}) [16], Velázquez (S_5S_{20}) [16]
Unclassified		
S_1	Dama taronja ^a , Tornado ^a , Vitillo ^a	IBCOT 18-2 [50]
S_2	Fuego ^a	Cyrano [22], IBCOT 29-5 [50], Veecot [21]
S_3	Mogador ^a	Colorado [21], Mikado [22]
S_6		Stella [21]
S_8		Vanilla Cot [21]
S_9		Goldstrike 01 [21]

^a *S*-*RNase* genotypes first reported in this study; ^b S-genotype completed in cultivars in which previously only one allele could be identified [21]; ^c S_c/S_8 allele identified using fluorescence microscopy; ^d S_c/S_8 allele confirmed using the primers AprFBC8-F/AprFBC8-R.

PCR amplification of the S_c - and S_8 -alleles using the primers designed based on the first and second introns of the *RNase* sequence produced identical size fragments [52]. Thus, the AprFBC8-F/AprFBC8-R primers for the *SFB* region were used for distinguishing both *S*-alleles [32]. This primer combination amplified a fragment of 150 bp, allowing to identify the S_8 -haplotype in 3 cultivars, whereas a 500 bp fragment from V2 region was amplified to characterize the S_c -allele in 47 cultivars (Figure 1B; Supplementary Table S1). However, the primers AprFBC8-F/AprFBC8-R did not produce amplification fragments in two cultivars (‘Samourai’ and ‘Water’). Thus, controlled pollinations were carried out to differentiate the S_c - and S_8 -alleles in these two cultivars. All self-pollinated pistils showed germinated pollen grains on the stigma in both cultivars (Figure 2A). ‘Water’ behaved as self-compatible since all the self-pollinated pistils ($n = 17$) showed pollen tubes arriving to the base of the style (Figure 2B). In ‘Samourai’, pollen tubes reached the upper third of the style in all the self-pollinated pistils analyzed (Figure 2C), but pollen tube growth ceased in the middle part of the style, forming a callose tip (Figure 2D), with a mean percentage of style traveled by the pollen tubes of 62.5% ($n = 10$). Thus, ‘Samourai’ was considered as

self-incompatible. In addition, all the examined pistils cross-pollinated with ‘Katy’ showed pollen tubes at the base of the style in ‘Water’ ($n = 20$) and ‘Samourai’ ($n = 5$).

Table 2. *S*-genotype of 153 self-compatible apricot cultivars.

<i>S</i> -Genotype	Cultivars Analyzed in This Study	Cultivars Analyzed in Previous Studies
S_1S_c	Big Red ^a , Dama Rosa ^a , Flavorcot ^a , Rojo Pasión ^a , Rubissia ^a , Water ^{a,c}	Mauricio [34]
S_2S_c	Bergecot ^d [21], Canino ^d [21,34], Harval ^a , Justo Cot ^a , Paviot ^d [21], Primidi ^d [21], Tilton [47]	Berdejo [21,50], Bergeron [52], Budapest [52], Dulcinea [16], Galta Vermella Valenciana [16], Kalao [22], Konservnyi Pozdnii [52], Mamaia [52], Mandulakajski [52], Mediva [21,50], Peñaflo 02 [21,50], Pepito del Rubio [49,50], Rakovszky [52], Regibus [22], Roxana [52], Rózsakajski [16], Sandy cot [21,50], Trevatt [16]
S_3S_c	Rubista ^d [21]	Pricia [21], Rambo [22]
S_5S_c	Búlida [16]	
S_6S_c	Fartoly ^{b,d} [21], Ladycot ^{b,d} [21], Medflo ^d [21], Mediabel ^d [21]	Aprix20 [21,50], Aprix9 [21,50], Bebecou [47], Faralia [21,50], Farlis [21,50], Lito [16]
S_7S_c	Charisma ^d [21], Ninfa [16]	Beliana [34], Sayeb [35]
S_8S_c	Gönci Magyarkajski [52], Luizet ^{b,d} [21]	Andornaktályai magyarkajski [52], Cacansko zlato [52], Callatis [52], Crvena ungarska [52], Darunec malahoyeva [52], Effect [52], Kásna ungarska [52], Krimskyi Amur [52], Nagygyümölcsü magyarkajski [52], Nikitskyi [52], Paksi magyarkajski [52], Pisana [52], Venus [52]
S_9S_c	Alba ^a , Aprisweet (ASF0409) ^a , Micaelo ^a , Tadeo ^b [21]	AC2 [21,50], Ceglédi arany [52], Ceglédi bíborkajski [52], Flopria [21], Lido [22], Tom Cot [21]
$S_{13}S_c$		Modesto [24]
$S_{19}S_c$		Mari de Cenad [16]
$S_{20}S_c$		Cristali [16], Gavatzet [16]
$S_{24}S_c$		Ezzine [16]
S_cS_c		Ananasnyi ciurpinski [52], Asli [35], Borsi-féle kései rózsza [52], Ceglédi kedves [52], Currot [34], Galtaraja [16], Gandia [16], Ginesta [25], Grandir [47], Manri [16], N/A-8 [52], Nyujtó Ferenc emléke [52], Palabras [16], Palau [25], Pannónia [52], Pasinok [52], Patterson [47], Raki [35], Rojo Carlet [16], Sirena [52], Sulmona [52], Tiryntos [16], Xirivello [16], Zaposdolye [52]
S_c	Aprix 116 ^a , Cebas Red [53], Cocot ^a , Corbato ^d [21], Delice cot ^d [21], Fantasma ^a , Farhial ^d [21], IPS21512 ^a , IPS2712 ^a , Laguna ^a , Merino ^a , Mirlo anaranjado ^d [21,54], Mirlo blanco ^d [21,54], Mirlo Rojo [54], Mitger ^d [21], Orange rubis ^a , Precoc de Tiryntos ^a , Primorosa [53], Soledane ^d [21], Tardorange ^a , Valorange ^a	Aprix 33 [21,50], ASF0404 (Apriqueen) [21,50], Dorada [22], Faclo [21,50], Farbaly [21,50], Farbela [21,50], Fardao [21,50], Farfia [21,50], Farius [21,50], IBCOT 13-12 [50], IPS16121 [50], Kioto [50], Memphis [22], Murciana [22], Oscar [22], Playa cot [21,50], Rouge cot [21], Rubely [50], Sherpa [22], Swired [21,50]
S_1S_2	Katy [55]	Lorna [21], Palsteyn [21]
S_2S_9		Victor 1 [21]
S_1		IPS20390 [50], Rubilis [50]
S_3		Golden Sweet [21]

^a *S*-*RNase* genotype first reported in this study; ^b *S*-genotype completed in cultivars in which previously only one allele could be identified [21]; ^c S_c/S_8 allele identified using fluorescence microscopy; ^d S_c/S_8 allele confirmed using the primers AprFBC8-F/AprFBC8-R.

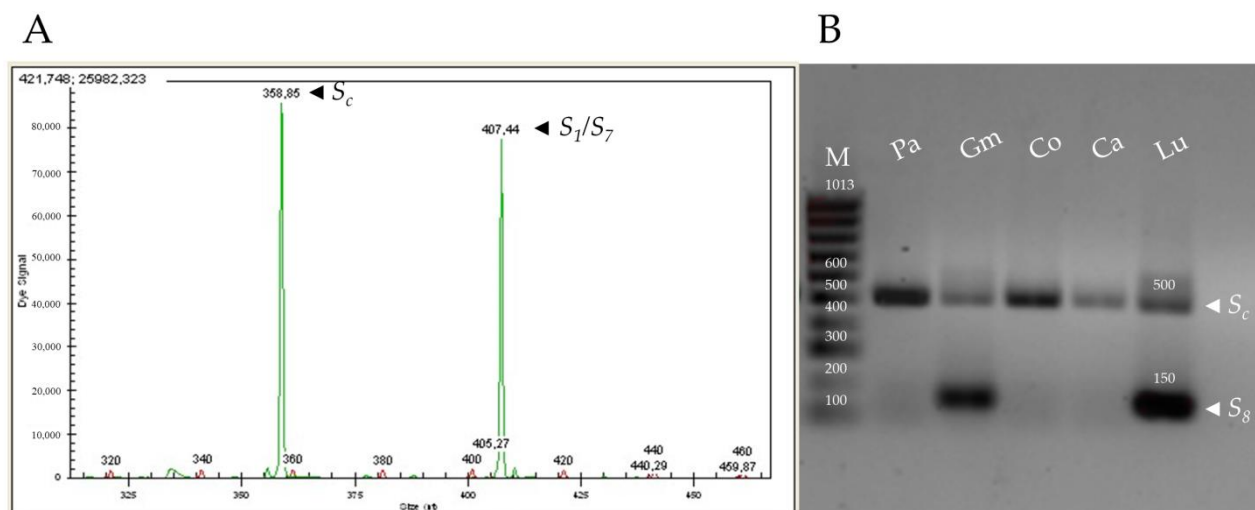


Figure 1. Size of the PCR amplification fragments using different primer pair combinations for the identification of *S*-alleles. (A) Gene analyzer output for the *S*Rc-(F/R) primers showing the size of the two amplified fragments of the *RNase* first intron region corresponding to the *S*-alleles S_c (358 bp, left) and S_1/S_7 (408 bp, right) in apricot cv. ‘Rojo Pasión’. (B) PCR amplification with the AprFBC8-(F/R) primers for identifying S_c - and S_8 -alleles in five apricot cultivars (Pa: ‘Paviot’, Gm: ‘Gönci Magyarkajski’, Co: ‘Corbato’, Ca: ‘Canino’, and Lu: ‘Luizet’). M: 100 bp DNA Ladder.

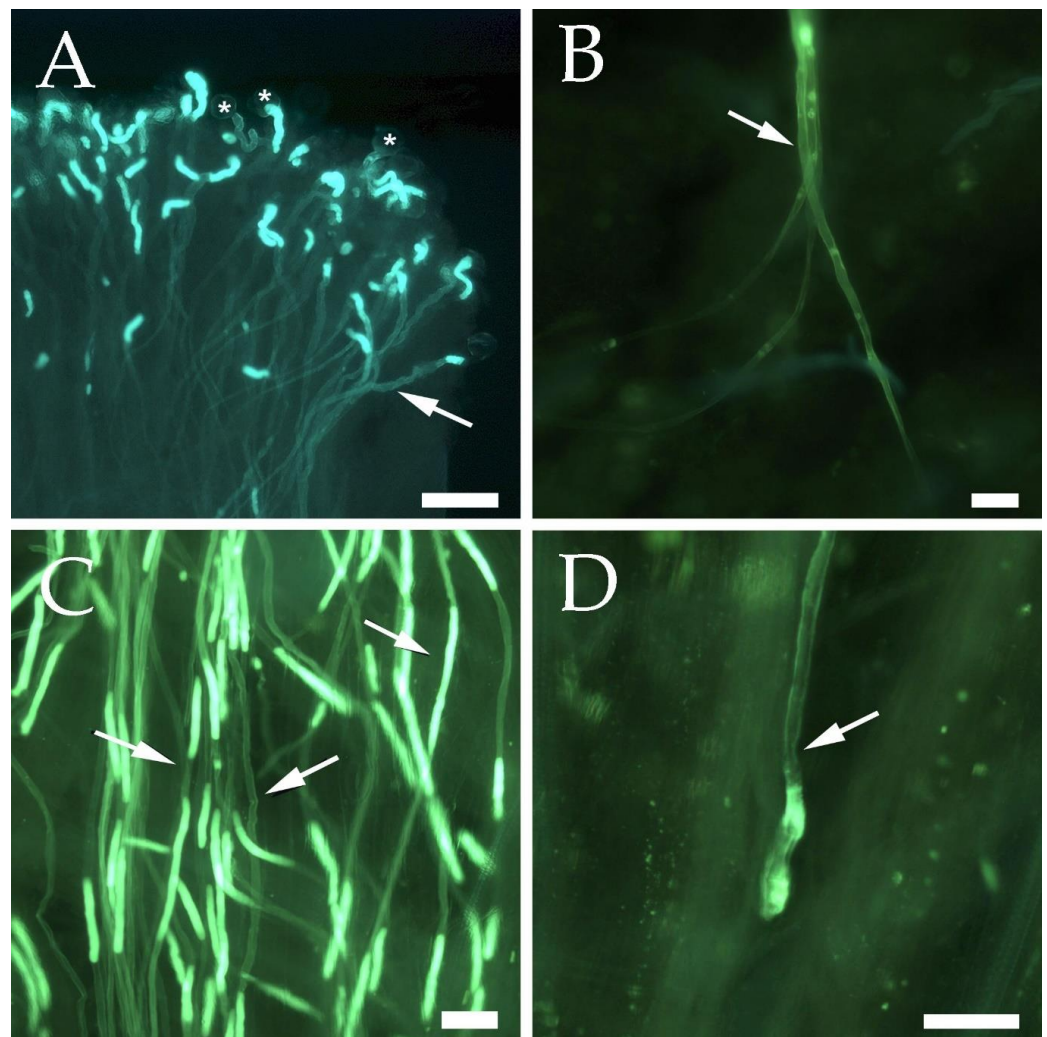


Figure 2. Pollen germination and pollen tube growth in self-pollinated apricot flowers observed under the microscope. In Gametophytic Self-Incompatibility (GSI), both compatible and incompatible pollen grains germinate on the stigma. The pollen grain carries one of the two *S*-alleles of the original genotype. In self-incompatible cultivars, if the *S*-allele of the pollen grain matches one of the two *S*-alleles of the pistil, pollen tube growth is inhibited in the middle part of the style. (A) Pollen grains (*) germinating at the stigma surface with pollen tubes emerging towards the style the style (arrow) in the self-compatible cultivar ‘Water’. (B) Pollen tubes (arrow) reaching the base of the style (down) in the self-compatible cultivar ‘Water’. (C) Pollen tubes (arrows) growing along the style in the self-compatible cultivar ‘Water’. (D) Pollen tube (arrow) arrested in the middle part of the style in the self-incompatible cultivar ‘Samourai’. Aniline blue staining for callose of squash preparations. Scale bars = 100 μm .

The *S*-genotype of eleven cultivars, in which a unique allele could be previously characterized [21], has been completed using several primer combinations. The specific primers SHLM1 and SHLM2 allowed to identify the *S*₁-allele in five cultivars (‘Farely’ (*S*₁*S*₉), ‘Harcot’ (*S*₁*S*₄), ‘Megatea’ (*S*₁*S*₉), ‘Monster Cot’ (*S*₁*S*₉), and ‘Priabel’ (*S*₁*S*₉)). Primers PruC2 and PruC4R enabled the identification of a second *S*-allele, *S*₆ in ‘Pandora’ (*S*₂*S*₆), ‘Muñoz’ (*S*₂*S*₆), ‘Fartoly’ (*S*₆*S*₆), ‘Lady Cot’ (*S*₆*S*₆), and *S*₉ in ‘Tadeo’ (*S*₆*S*₉). The genotype of ‘Luizet’ (*S*₆*S*₈) was identified using the AprFBC8-F/AprFBC8-R primer combination. Additionally, the presence of the *S*₆- and *S*₈-alleles was confirmed in 19 cultivars using the AprFBC8-F and AprFBC8-R primers. In these 19 cultivars, observations of pollen tube growth in pollination experiments were previously used to establish self(in)compatibility; the *S*₆-allele was assigned to self-compatible cultivars, and the *S*₈-allele to self-incompatible accessions [21]. Moreover, the

results allowed to confirm the S -genotype of eight cultivars; ‘Búlida’ (S_5S_c) [16], ‘Canino’ (S_2S_c) [34], ‘Cebas Red’ (S_c) [53], ‘Gönci magyarkajszai’ (S_8S_c) [52], ‘Harcot’ (S_1S_4) [49], ‘Katy’ (S_1S_2) [55], ‘Ninfa’ (S_7S_c) [16] and ‘Tilton’ (S_2S_c) [47]; a single S_c -allele amplification was obtained in ‘Mirlo rojo’ and ‘Primorosa’, which were reported previously as self-compatible (S_cS_c) [53,54].

In order to establish the compatibility relationships among cultivars, the 12 self-incompatible cultivars were allocated in their corresponding incompatibility groups according to their S -genotypes. In five cultivars (‘Dama Toronja’, ‘Tornado’, ‘Vitillo’, ‘Fuego’, ‘Mogador’), self-(in)compatibility could not be established by their S -genotype because only one allele other than S_c could be identified and, consequently, they were considered unclassified (Table 1).

2.2. Diversity in the S -Locus Region

The S -genotypes of 235 apricot accessions, including the 60 cultivars analyzed herein in which two S -alleles could be identified together with 175 accessions previously reported (Tables 1 and 2; Supplementary Table S2) were used to assess the diversity and differentiation at the gametophytic self-incompatibility S -locus.

Fourteen S -alleles in 32 S -locus combinations were identified within 70 traditional apricot accessions, whereas 36 S -genotype combinations with 17 S -alleles were found in the group of 157 releases from breeding programs (Tables 3 and 4). Both groups of accessions showed the same value of the average number of alleles per country ($N_a = 4$). Thirteen S -alleles were present in both groups (Figure 3). However, the alleles S_4 , S_{10} , S_{24} , and S_{31} were only identified in the commercial cultivars ‘Ezzine’ (S_{24}), ‘Harmat’ (S_{10}), ‘Harcot’ (S_4), ‘Cow-1’ (S_{31}), and ‘Cow-2’ (S_{31}), and the S_5 -allele was only found in the traditional cultivars ‘Búlida’ and ‘Velázquez’, from Spain, and ‘Shalakh’, from Armenia.

Table 3. Genetic parameters of apricot traditional cultivars including landraces and local selections. Number of cultivars, number of alleles (N_a), allelic richness (A_r), and number of private alleles (P_a) for each country of origin. SD: standard deviation; SE: standard error.

Country	Number of Cultivars	Number of Alleles (N_a)	Allelic Richness (A_r)	Number of Private Alleles (P_a)
Armenia	1	2	1.67	-
Australia	1	2	1.67	-
France	2	2	1.57	-
Greece	5	3	1.67	-
Hungary	7	4	1.75	-
Italy	4	4	1.70	-
Romania	1	2	1.67	1 (S_{19})
Spain	26	7	1.61	-
Tunisia	9	7	1.80	1 (S_{12})
Turkey	8	6	1.77	-
Ukraine	1	2	1.67	-
The USA	5	6	1.84	1 (S_3)
Total	70	47		3
Mean $\pm$ SD		4 $\pm$ 2	1.70 $\pm$ 0.08	
SE		0.63	0.02	

In both traditional cultivars and cultivars released from breeding programs, S_c was the most frequent S -allele as it was found in 38 and 101 cultivars, respectively. The S_c -allele was not detected in Armenian and Turkish germplasm but was present in more than 50% of the genotypes from Australia, France, Greece, Romania, Spain, and Ukraine (Figure 3A; Supplementary Table S3). A similar trend was observed in cultivars from breeding programs, except for cultivars from North America (Canada and the USA), in which the S_c -allele appeared in less than 20% of the cultivars (Figure 3B; Supplementary Table S4).

Table 4. Genetic parameters of apricot cultivars released from breeding programs. Number of cultivars, number of alleles (N_a), allelic richness (A_r), and number of private alleles (P_a) for each country of origin. SD: standard deviation; SE: standard error.

Country	Number of Cultivars	Number of Alleles (N_a)	Allelic Richness (A_r)	Number of Private Alleles (P_a)
Bulgaria	1	2	1.67	-
Canada	4	6	1.87	1 (S_4)
France	58	10	1.83	1 (S_{31})
Hungary	13	8	1.72	3 (S_{10}, S_{11}, S_{12})
Italy	2	3	1.71	-
Macedonia	1	2	1.67	-
Romania	5	3	1.48	-
Serbia	1	2	1.67	-
South Africa	1	2	1.67	-
Spain	33	7	1.76	-
Switzerland	2	2	1.71	-
Tunisia	6	5	1.70	1 (S_{24})
Turkey	1	2	1.67	1 (S_{19})
Ukraine	4	2	1.50	-
Unknown	1	2	1.67	-
The USA	24	9	1.84	-
Total	157	67		7
Mean $\pm$ SD SE		4 $\pm$ 3.05 0.76	1.70 $\pm$ 0.10 0.03	

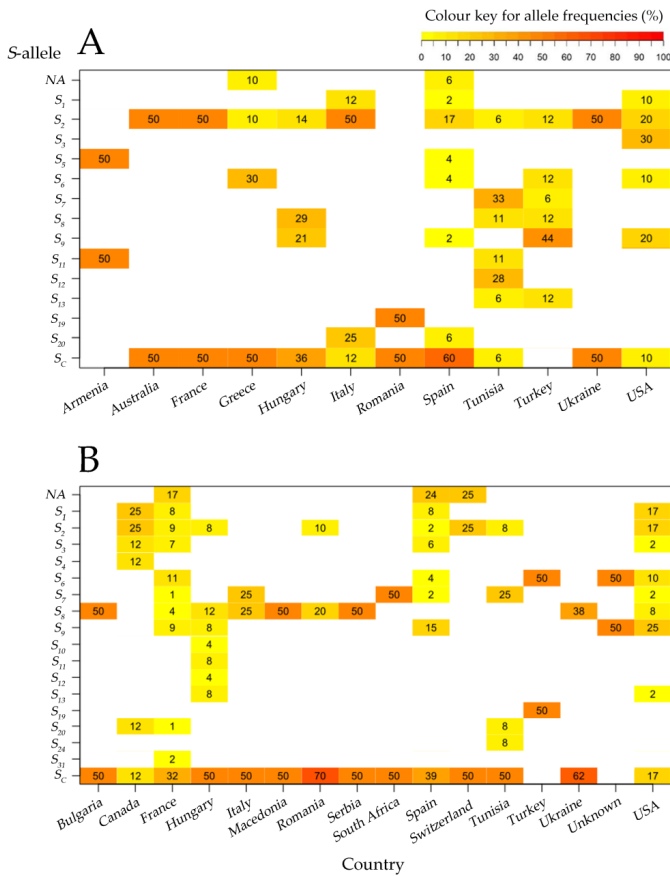


Figure 3. Heatmaps of allele frequencies in traditional cultivars (A) and cultivars from breeding programs (B) for each country of origin using “PopGenReport” v. 3.0.4 R package. Cell color indicates the proportion of the total number of alleles, and the numbers within a cell show the percentage of the number of alleles in each country. The frequency of each S-allele was calculated in each country within each group of accessions, showing statistically significant relationships ($p < 0.05$) between S-alleles and countries in the groups of both traditional cultivars and releases from breeding programs.

In traditional cultivars, S_2 was the second most frequent S -allele ($n = 24$), followed by S_9 ($n = 13$), S_6 and S_8 ($n = 8$), S_7 ($n = 7$), S_{12} and S_{20} ($n = 5$), S_1 , S_3 , S_5 , S_{11} , and S_{13} ($n = 3$), and S_{19} ($n = 1$). In cultivars from breeding programs, S_9 was the second most frequent S -allele ($n = 35$) followed by S_2 ($n = 26$), S_1 ($n = 24$), S_6 ($n = 23$), S_8 ($n = 21$), S_3 ($n = 14$), S_7 ($n = 8$), S_{13} and S_{20} ($n = 3$), S_{11} and S_{31} ($n = 2$) and S_4 , S_{10} , S_{12} , S_{19} , S_{24} ($n = 1$) (Supplementary Tables S2–S4).

A higher percentage of single alleles was observed in cultivars from breeding programs compared to traditional cultivars: 17% in France, 24% in Spain, and 25% in Switzerland for the cultivars from breeding programs compared to 10% in Greece and 6% in Spain for the traditional cultivars (Figure 3).

Regarding the allele frequencies, the chi-squared tests showed a statistically significant relationship ($p < 0.05$) between S -alleles and countries in the groups of both traditional cultivars and releases from breeding programs (Supplementary Tables S3 and S4).

The existence of private alleles, S -alleles that are found only in a single population, was used as an indicator of genetic differentiation between both groups. Three alleles (P_a) were found in the group of traditional cultivars. S_3 was only found in ‘Perfection’ and ‘Sun Glo’ from the USA, S_{12} in ‘Bedri Ahmar’, ‘Bouthani Ben Friha’, ‘Oud Rhayem’, and ‘Oud Hmida’, from Tunisia, and S_{19} in ‘Mari de Cenad’ from Romania. In the group of releases from breeding programs, seven private alleles were identified, three of them, S_{10} , S_{11} and S_{12} , present in Hungarian accessions (‘Harmat’, ‘Korai zamatos’, and ‘Voski’).

Although the number of genotypes in the group of cultivars from breeding programs was nearly twice than those in the group of traditional cultivars, the same value of average allelic richness ($A_r = 1.70$) was found in both groups. Comparisons of traditional cultivars with cultivars from breeding programs revealed a slight loss of diversity for the S -locus in Hungarian, Tunisian, and Turkish modern cultivars. On the contrary, higher allelic richness in cultivars from breeding programs than in traditional cultivars were observed in France, Italy, and Spain.

3. Discussion

3.1. Self- and Cross-Incompatibility in Apricot

The S -genotype of 66 apricot cultivars was reported herein, 41 for the first time. A total of 49 cultivars were characterized as self-compatible since their genotype contained the S_c -allele, which is associated with self-compatibility [56]. However, the self-compatibility of two cultivars was determined through controlled pollinations due to mismatching of PCR primers resulting in no amplification. When self(in)compatibility cannot be determined by identifying the S -genotype, laboratory pollination experiments have proven to be an accurate method because they avoid weather-related failures under field conditions [23,57,58].

Here, the S -genotype has been characterized in 11 cultivars [‘Pandora’ and ‘Muñoz’ (S_2S_6), ‘Farely’, ‘Megatea’, ‘Monster Cot’, and ‘Priabel’ (S_1S_9), ‘Harcot’ (S_1S_4), ‘Fartoly’ and ‘Ladycot’ (S_6S_c), ‘Luizet’ (S_8S_c), and ‘Tadeo’ (S_9S_c)], in which only one S -allele could be previously identified [21], probably by the mismatching of PCR primers or preferential amplification of the detected allele. The use of specific primers for the S_1 -allele [22] allowed the identification of this allele in 15 cultivars for the first time. The S_c -allele was confirmed in 19 cultivars in which self-compatibility was previously assessed by cross-pollinations [21]. AprFBC8-(F/R) primers allowed to distinguish between the S_c - and S_8 -alleles, since an insertion of 358 bp in the SFB gene causes a loss of the incompatibility that has been observed in the SFB_c gene but not in the sequence of SFB_8 [52,56]. Therefore, the cultivars carrying the S_8 -allele but not the S_c -allele, such as ‘Sweet Cot’, might be considered as self-incompatible. A single S_c -allele was identified in 21 genotypes. As this allele is associated with self-compatibility [56], these cultivars could present homozygosity, as it has been considered in previous reports for some cultivars that have been characterized as S_cS_c [16,25,34,35,47,52]. Our results agree with previous reports of the S -genotype for ‘Búlida’ [16], ‘Cebas Red’ [53], ‘Canino’ [34], ‘Gönci magyarkajszí’ [52], ‘Harcot’ [49], ‘Katy’ [55], ‘Ninfa’ [16] and ‘Tilton’ [47]. According to Egea et al. [59], ‘Rojo Pasión’ resulted

from a cross between ‘Orange Red’ (S_6S_9 [16,21]) and ‘Currot’ (S_cS_c [34]). However, our results for the S -genotype (S_1S_c) differ from this pedigree. Additionally, our results for three cultivars, ‘Alba’, ‘Corbato’, and ‘Tadeo’, differ from the S -genotype previously reported [16].

According to their S -allele composition, 12 self-incompatible cultivars were allocated in their corresponding cross-incompatibility groups together with the 77 self-incompatible cultivars previously analyzed [16,21,22,24,32,34,35,47–50]. To date, a total of thirty-five incompatibility groups (I to XXXV) have been described in apricot [17,21,22,32,35,48]. Self-incompatible cultivars within the same incompatibility group have the same S -genotype and are genetically incompatible with each other. On the other hand, cultivars from different incompatibility groups are inter-compatible, since at least one of the S -alleles of their genotype is different [60]. Although lower yields have been related to semi-compatible pollinizers (when one S -allele is identical and the other differs) in Japanese plum and sweet cherry [61–63], there is no information on the effects of semi-compatibility in apricot. In addition, 16 cultivars are included in group 0 since no other cultivars with the same S -genotype have been reported until now. Thus, the cultivars from group 0 as well as the self-compatible cultivars could act as universal pollinizers. This could be highly valuable information since the knowledge of incompatibility relationships aims to help breeders to choose parental genotypes for breeding programs and fruit growers to select compatible pollinizers coincident at flowering time.

3.2. Current Genetic Diversity at the S -Locus

To provide an overview of the genetic diversity at the S -locus of currently grown apricot cultivars, results herein have been combined with previous results [16,21,22,24,25,32,34,35,39,47–50,52,54,55] to analyze the frequency and distribution of the S -alleles. A total of 33 S -alleles (S_1 to S_{20} , S_{22} to S_{30} , S_{52} , S_{53} , S_c , S_v , and S_x) have been described in apricot cultivars [16,24–27]. Fourteen S -alleles were detected within the group of 70 traditional cultivars from Armenia, Australia, France, Greece, Hungary, Italy, Romania, Spain, Tunisia, Turkey, Ukraine, and the USA, whereas 17 S -alleles were identified in the group of cultivars from breeding programs, reflecting lower S -allele diversity in the group of traditional cultivars. Twenty S -alleles (20) were reported in a group of 67 cultivars from Europe and North America [16], but fewer S -alleles were found when local accessions or landraces were studied separately in Tunisia [17,35], Turkey [32,36,37], and Morocco [33].

Four S -alleles (S_{10} , S_{24} , S_{31} and S_4) were exclusively found in the group of cultivars from breeding programs, which could be related to the use of landraces instead of commercial cultivars in some breeding programs. Two of these S -alleles showed a clear relationship with a specific breeding program: S_{24} was found in ‘Ezzine’ from INRAT (Tunisia) [64], and S_{31} was found in ‘Cow-1’ and ‘Cow-2’ from INRA (France) [65]; S_4 was present in the North American cultivar ‘Harcot’ [49]. Although this cultivar has been used as a parental genotype in several breeding programs to introduce Sharka-resistance to new releases [29], the S_4 -allele has not been found in any recent releases. On the other hand, the S_5 -allele has been previously reported only in some traditional cultivars from Spain and Armenia. Our results differed in the S -genotype of the Spanish landrace ‘Corbato’ in which this allele has been previously reported, (S_c vs. S_2S_5 [16]). The presence of the S_5 -allele in this cultivar, as well as in other traditional cultivars from Spain and Armenia, has been reported by Muñoz-Sanz et al. [16]. They suggested that the presence of the S_5 -allele in those populations could be the result of a connection between Southern-Spanish accessions with the Armenian and Eastern-Turkish accessions; in addition, they suggested that Moroccan accessions are a part of the Southwest-Mediterranean apricot diffusion route.

Traditionally, the main marker for self-compatibility in apricot has been S_c [49]. For this reason, one of the main objectives of breeding programs has been to introduce this S -allele into new releases [11]. Our results showed that S_c was the most frequent S -allele in both the groups of traditional and cultivars from breeding programs. Although we did not detect the S_c -allele in traditional cultivars from Armenia and Turkey, previous reports found some self-compatible Turkish cultivars carrying this S -allele [32,37]. The S_c -allele might

have evolved in Southeastern Turkey as a result of a pollen-part mutation within *SFB*₈, causing the pollen with the mutated *S*₈ haplotype to be self-compatible [52]. Subsequently, it is hypothesized that the allele *S*_c was disseminated to the Mediterranean Basin since the cultivars from Central Asia, the center of origin of apricot, are self-incompatible [52,66]. The *S*_c-allele was present in cultivars from breeding programs in most countries except for Turkey, probably due to the low number of new releases from this country studied in this work. However, the low presence of the *S*_c-allele in North American cultivars (the USA and Canada) is probably due to the fact that most of these cultivars are self-incompatible [28,67].

The presence of just one allele has been observed in a high number of cultivars, mostly cultivars released from breeding programs, mainly as genotype *S*_c- (Supplementary Table S2) that would presumably correspond to *S*_c*S*_c. Homozygote cultivars can arise as a result of self- and cross-pollinations with self-compatible parents in breeding programs. Although this genotype has been frequently found in apricot cultivars from most European countries [16,25,34,52], results herein as well as those from previous studies could not confirm homozygosity since sequencing would be needed [22].

The distribution of *S*-alleles varied considerably between countries, with a significant association ($p < 0.05$) between *S*-alleles and geographical origin, both in the group of traditional accessions and in the group of breeding program releases. Within traditional cultivars, the *S*₃-allele was found exclusively in North American cultivars [16]. The *S*₁₂-allele was only found in four cultivars from Tunisia [35], being one of the most frequent *S*-alleles in this country [17]. The *S*₁₉-allele was only found in one cultivar from Romania, 'Mari de Cenad', despite having been associated with Hungarian and Turkish local apricots [32]. Muñoz-Sanz et al. [16] reported that the *S*₁₉-allele found in this cultivar could be *S*₂₀; however, further sequencing would be required to confirm this.

Alleles *S*₁₀ to *S*₁₄ have Armenian origin [24] but they have also been described in cultivars from Eastern Europe [32], Morocco [33], Tunisia [17,35] and Turkey [32,37]. Our results showed three of these alleles (*S*₁₀, *S*₁₁, and *S*₁₂) in three Hungarian traditional cultivars, 'Harmat', 'Korai zamatos', and 'Voski'.

Thus, in addition to providing useful information to know the self-compatibility of cultivars, *S*-genotyping can be a valuable tool in elucidating the evolution and dissemination of the crop.

3.3. Self-Compatibility and Diversity

Self-compatibility has been described as a cause of loss of genetic diversity since it promotes inbreeding [68]. Self-incompatibility not only reduces inbreeding by preventing self-fertilization but also reduces mating between close relatives, ensuring the exchange of genetic material [69]. It has been suggested that the loss of genetic diversity affecting the *S*-locus is due to crop dissemination [16]. In fact, a bottleneck has been observed in apricot diversity as a consequence of the domestication and diffusion of the apricot throughout the history of the crop [8]. Additionally, a decrease in genetic diversity from the eastern (Iran-Caucasian area) to the south-western (North Mediterranean Basin and South Mediterranean Basin areas) distribution of the crop has been detected, analyzing local cultivars from Algeria, France, Italy, Morocco, Spain, Tunisia, and Turkey [70]. A lower number of *S*-alleles were found in accessions from Moroccan oases as compared to the whole allele pool in this country, probably due to the pressure to increase production and self-compatibility in the genotypes, allowing a higher level of endogamy [33]. A similar situation was described in landraces from Central Europe [52].

Apricots belonging to the European group have been traditionally considered to be self-compatible [71]. However, the number of self-incompatible commercial cultivars in the European group increased rapidly over the last two decades due to the use of self-incompatible North American cultivars as parentals in breeding programs [21,28]. Recent studies show that about half of the new releases are self-compatible: 51.1% (47 of 92) [21] and 49.6% (61 of 123) [20].

Recently, additional sources of self-compatibility have been described in apricot in addition to the S_c -allele. Thus, some works have reported the existence of an additional mutation in the M -locus not linked to the S -locus, which causes a loss of pollen S -activity [16,55,56,72]. Pollen-part mutations (PPMs) in the M -locus were mapped at the distal end of chromosome three in ‘Canino’ (called m [72]) and in ‘Katy’ (called m' [55]). Muñoz-Sanz et al. [73] proposed the *ParMDO* gene as a relevant gene involved in pollen part SI function. In order to optimize the screening of self-compatible genotypes, a new useful method based on both loci has been recently developed [74].

Although a clear trend towards releasing self-compatible cultivars is shown, our results exhibited similar allelic richness values in both groups, traditional cultivars, and cultivars from breeding programs. A clear differentiation between apricot landraces and cultivars from breeding programs was recently revealed using SSR markers, showing an unexpected higher diversity in cultivars from breeding programs, which was related to the use of North American genotypes as parents [75]. Our results showed a slight loss of diversity for the S -locus in Hungarian, Tunisian, and Turkish cultivars comparing landraces with releases from breeding programs. However, this situation was not observed in the countries included in the North Mediterranean Basin group such as France, Italy, and Spain. This could be due to the higher number of new releases from breeding programs of these countries.

Although there is no evidence of a reduction in the diversity at the S -locus in cultivars developed in breeding programs, results herein suggests that the use of a reduced number of parents in breeding programs can lead to a genetic bottleneck.

4. Materials and Methods

4.1. Plant Material

Young leaves from 66 apricot cultivars, including traditional cultivars (landraces and local selections) and releases from breeding programs of several origins (Tables 1 and 2), were collected in spring from germplasm collections and orchards in Spain. Moreover, flowers were collected from two cultivars (‘Samourai’ and ‘Water’) for pollination experiments to establish the self-(in)compatibility by microscopic observations. The apricot accessions analyzed originated from 12 countries: Armenia, Australia, France, Greece, Hungary, Italy, Romania, Spain, Tunisia, Turkey, Ukraine, and the USA.

4.2. DNA Extraction and S -Allele Identification

Genomic DNA of each sample was extracted using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to Hormaza [76] and quantified by NanoDrop™ ND-1000 spectrophotometer (Bio-Science, Budapest, Hungary).

The S -genotype of each cultivar was identified through PCR amplification of *RNase* and *SFB* regions with different primer combinations (Table 5) [23]. The first intron of the *S-RNase* gene was amplified with the fluorescently labeled primer combination SRc-(F/R) [25,77]. PCR amplifications were carried out in 15 μ L reaction volumes, containing 10 $\times$ NH_4 Reaction Buffer, 25 mM $MgCl_2$, 2.5 mM of each dNTP, 10 μ M of each primer, 100 ng of genomic DNA and 0.5 U of BioTaq™ DNA polymerase (Bioline, London, UK). The temperature profile used had an initial step of 3 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 55 °C and 3 min at 72 °C, and a final step of 5 min at 72 °C. The amplified fragments were analyzed in a CEQ™ 8000 capillary electrophoresis DNA analysis system (Beckman Coulter, Fullerton, CA, the USA) and classified according to Vilanova et al. [25] and Herrera et al. [21].

Table 5. SSR primers used in this study for the identification of *S*-alleles in apricot (*Prunus armeniaca*).

Amplified Region	Name	Specificity	Primer Sequence (5' → 3')	Reference
<i>S</i> - <i>RNase</i> 1st intron	SRc-(F/R)		F: CTCGCTTTCCTTGTTCTTGC	[77]
			R: GGCCATTGTTGCACCCCTTG	
<i>S</i> - <i>RNase</i> 2nd intron	Pru-C2/C4R		F: CTTTGGCCAAAGTAATTATTCAAACC	[78]
	SHLM1/SHLM2	<i>S</i> ₁ -allele	R: GGATGTGGTACGATTGAAGCG	[22]
			F: GGTGGAGGTGATAAGGTAGCC	
	SHLM3/SHLM4	<i>S</i> ₇ -allele	R: GGCTGCATAAGGAAGCTGTAGG	[22]
			F: TATATCTTACTCTTTGGC	
<i>SFB</i>	AprFBC8-(F/R)		R: CACTATGATAATGTGTATG	[32]
			F: CATGGAAAAAGCTGACTTATGG	
			R: GCCTCTAATGTCATCTACTCTTAG	

Because two pairs of alleles, *S*₆/*S*₉ and *S*₁/*S*₇, showed similar fragment sizes, specific primers based on the second intron of the *RNase* were used to distinguish between them. For the identification of the *S*₆- and *S*₉-alleles, the PruC2/PruC4R primer combination designed from *P. avium* *S*-*RNase*-cDNA sequences [78] was used to differentiate both alleles in 15 genotypes. Specific primers SHLM1/SHLM2 and SHLM3/SHLM4 were required to distinguish between *S*₁ and *S*₇, respectively [22]. PCR reactions were carried out according to Vilanova et al. [25], but with the addition of 10 cycles and using 55 °C of annealing temperature [79]. The amplified fragments were separated on 1% (*w/v*) agarose gels and the DNA bands were visualized using the nucleic acid stain SYBR Green (Thermo Fisher Scientific, St Leon-Rot, Germany). For the identification of the *S*₁-allele, the specific primers SHLM1 and SHLM2 were used following the protocol described by Herrera et al. [22] for *Taq* DNA polymerase (Qiagen, Hilden, Germany). Primers SHLM3 and SHLM4 were used for *S*₇-allele identification. PCR reactions were performed with Phusion® High-Fidelity DNA Polymerase (Thermo Fisher Scientific, St Leon-Rot, Germany) according to Herrera et al. [22].

Since PCR amplification of the *S*₆- and *S*₈-alleles using the primers SRc-(F/R) provides a fragment of similar size [24,56], the specific primers AprFBC8-F and AprFBC8-R, designed based on the V2 and HVb variable region of *SFB* gene, were used to distinguish between both alleles [32]. The PCR amplifications were carried out using the program previously described by Halász et al. [32].

4.3. Pollination Experiments

In cultivars ‘Samourai’ and ‘Water’, no amplification was produced with the primers AprFBC8-F/AprFBC8-R. Thus, controlled pollinations were carried out in these two cultivars to differentiate the *S*₆- and *S*₈-alleles. Self-(in)compatibility was established by laboratory-controlled pollinations and the observation of pollen tube growth under fluorescence microscopy [23]. Self-pollinations were carried out in the two cultivars. Pollen of the cultivar ‘Katy’, known as universal pollinizer for apricot [55], was used to pollinize another set of flowers of each cultivar as control.

Flowers from each cultivar were collected at the balloon stage one day before anthesis and emasculated to avoid self-pollination. Pistils were placed on wet florist foam and maintained at laboratory temperature. After 24 h, a group of 20–25 flowers were hand-pollinated with the help of a paintbrush for each self- and cross-pollination [80]. Pollen was obtained from flowers at the same balloon stage by removing and drying the anthers at laboratory temperature during 24 h. Pollen grains were then sieved by using a fine mesh (0.26 mm) and used immediately or frozen at −20 °C until further use. Seventy-two hours after pollination, pistils were fixed in ethanol (95%)/acetic acid (3:1, *v/v*) during 24 h, and conserved at 4 °C in 75% ethanol. After hand pollinations, pollen viability was evaluated. Pollen from each pollen donor was scattered on a solidified pollen germination medium [81] and pollen germination was observed under the microscope after 24 h. Pollen

grains were considered viable when the length of the growing pollen tube was higher than the pollen grain diameter.

For histochemical preparations, the fixed pistils were washed three times for 1 h with distilled water and left in 5% sodium sulphite in distilled water at 4 °C for 24 h. Then, they were autoclaved at 1 kg/cm² during 10 min in sodium sulphite to soften the tissues [82]. Pistils were squashed and stained with 0.1% (*v/v*) aniline blue in 0.1N K₃PO₄ [83] to observe callose. Examination of pollen tube growth was carried out by fluorescence microscopy using by a Leica DM2500 microscope (Cambridge, UK) with UV epifluorescence using 340–380 bandpass and 425 longpass filters.

Pollen tube behavior was observed in at least 10 pistils in each self-pollination. Cultivars were considered as self-incompatible when pollen tube growth was arrested along the style in most self-pollinated pistils. On the other hand, when the pollen tube reached the base of the style in most self-pollinations, the cultivars were considered as self-compatible.

4.4. S-Allele Diversity Analysis

In order to analyze the *S*-allele genetic diversity, the *S*-*RNase* genotypes of the 60 cultivars identified herein were compiled with those of the 175 cultivars previously reported (Tables 1 and 2; Supplementary Table S2). Cultivars were first classified into two groups according to the pedigree origin of the accessions, traditional, and releases from breeding programs. Statistical analyses were performed using the R programming environment (R Core Team, 2022, version 4.1.0, Vienna, Austria). The *S*-genetic profiles were stored in a csv file which was converted into a matrix of allelic frequencies stored in a *genind* class with the “*loci2genind*” function using the R package “*pegas*” version 1.0–1 [84]. Missing data (<0.1%) were replaced with the mean frequency of the corresponding allele, which avoids adding artefactual between-group differentiation [85].

The number of alleles (*N_a*), allelic richness (*A_r*), and private alleles (*P_a*) were calculated for all countries on the traditional and cultivars from breeding programs using the *ade-genet* 2.1.3 [85], and *PopGenReport* 3.0.4 [86] packages. Additionally, the frequency of each *S*-allele was calculated in each country within each group and the results were plotted as a heatmap with the R package *PopGenReport* version 3.0.4 [86]. To analyze the relationship between the *S*-alleles and their distribution by country, a contingency table of absolute frequencies of alleles by country was created and a chi-square test was performed with the “*chisq.test*” function using the R package “*stats*” v. 4.1.2. Due to the low number of observations in some countries, a Monte Carlo simulation with 2000 replicates was indicated.

5. Conclusions

Results reveal that a significant proportion of new apricot releases are self-incompatible and, therefore, require cross-pollination to produce fruit. Knowing the incompatibility relationships between cultivars will help breeders to select suitable parental genotypes in crosses. This information, combined with the flowering dates in each geographical area, will allow the selection of appropriate inter-compatible pollinizers for self-incompatible cultivars in the design of new orchards. The identification of the *S*-alleles, in addition to the determination of the pollination requirements of the cultivars, can elucidate missing gaps in the evolution, domestication, and dissemination of the crop. The diversity at the *S*-locus observed in the cultivars developed in breeding programs indicates a possible genetic bottleneck due to the use of a reduced number of parents in breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/plants11152019/s1>, Table S1: Sizes of PCR fragment amplification using five primer pair combinations for the identification of *S*-alleles and *S*-genotype of the analyzed apricot accessions; Table S2: Origin and *S*-genotype of the 235 analyzed apricot accessions; Table S3: Contingency table of absolute allele frequencies by country in apricot traditional cultivars including landraces and local selections and Pearson’s chi-squared test with simulated *p*-value (based on 2000 replicates); Table S4: Contingency table of absolute allele frequencies by country in apricot

cultivars released from breeding programs and Pearson's chi-squared test with simulated p -value (based on 2000 replicates).

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MATERIAL SUPLEMENTARIO. Self-Incompatibility in Apricot: Identifying Pollination Requirements to Optimize Fruit Production

El material suplementario puede ser encontrado y descargado en:
<https://www.mdpi.com/article/10.3390/plants11152019/s1>.

Table S1: Sizes of PCR fragment amplification using five primer pair combinations for the identification of *S*-alleles and *S*-genotype of the analyzed apricot accessions.

Cultivar	SRc- (F/R) (bp)	PruC2- PruC4R (bp)	SHLM1- SHLM2 (bp)	SHLM3- SHLM4 (bp)	AprFBC8- (F/R) (bp)	<i>S</i> -genotype
Alba	358/420	700			500	<i>S</i> ₉ <i>S</i> _c
Apribang (ASF0405)	420	700/1386				<i>S</i> ₆ <i>S</i> ₉
Aprisweet (ASF0409)	358/420	700			500	<i>S</i> ₉ <i>S</i> _c
Aprix 116	358				500	<i>S</i> _c
Bergecot	334/358				500	<i>S</i> ₂ <i>S</i> _c
Big Red	358/408		650		500	<i>S</i> ₁ <i>S</i> _c
Búlida	358/383				500	<i>S</i> ₅ <i>S</i> _c
Canino	334/358				500	<i>S</i> ₂ <i>S</i> _c
Cebas Red	358				500	<i>S</i> _c
Charisma	358/408			413	500	<i>S</i> ₇ <i>S</i> _c
Cocot	358				500	<i>S</i> _c
Corbato	358				500	<i>S</i> _c
Dama Rosa	358/408		650		500	<i>S</i> ₁ <i>S</i> _c
Dama toronja	408		650			<i>S</i> ₁
Delice cot	358				500	<i>S</i> _c
Fantasme	358				500	<i>S</i> _c
Farely	408/420	700	650			<i>S</i> ₁ <i>S</i> ₉
Farhial	358				500	<i>S</i> _c
Fartoly	358/420	1386			500	<i>S</i> ₆ <i>S</i> _c
Flavorcot	358/408		650		500	<i>S</i> ₁ <i>S</i> _c
Fuego	334					<i>S</i> ₂
Gönci Magyarkajsz	358				150/500	<i>S</i> ₈ <i>S</i> _c
Harcot	247/408		650			<i>S</i> ₁ <i>S</i> ₄
Harval	334/358				500	<i>S</i> ₂ <i>S</i> _c
IPS21512	358				500	<i>S</i> _c
IPS23214	274/408		650			<i>S</i> ₁ <i>S</i> ₃
IPS2712	358				500	<i>S</i> _c
Justo Cot	334/358				500	<i>S</i> ₂ <i>S</i> _c
Katy	334/408		650			<i>S</i> ₁ <i>S</i> ₂
Ladycot	358/420	1386			500	<i>S</i> ₆ <i>S</i> _c
Laguna	358				500	<i>S</i> _c

Cultivar	SRc- (F/R) (bp)	PruC2- PruC4R (bp)	SHLM1- SHLM2 (bp)	SHLM3- SHLM4 (bp)	AprFBC8- (F/R) (bp)	S-genotype
Luizet	358				150/500	S ₈ S _c
Medflo	358/420	1386			500	S ₆ S _c
Mediabel	358/420	1386			500	S ₆ S _c
Megatea	408/420	700	650			S ₁ S ₉
Merino	358				500	S _c
Micaelo	358/420	700			500	S ₉ S _c
Mirlo anaranjado	358				500	S _c
Mirlo blanco	358				500	S _c
Mirlo Rojo	358				500	S _c
Mitger	358				500	S _c
Mogador	274					S ₃
Monred	274/408		650			S ₁ S ₃
Monster Cot	408/420	700	650			S ₁ S ₉
Muñoz	334/420	1386				S ₂ S ₆
Ninfa	358/408			413	500	S ₇ S _c
Orange rubis	358				500	S _c
Pandora	334/420	1386				S ₂ S ₆
Paviot	334/358				500	S ₂ S _c
Precoz de Tiryntos	358				500	S _c
Priabel	408/420	700	650			S ₁ S ₉
Primidi	334/358				500	S ₂ S _c
Primorosa	358				500	S _c
Rojo Pasión	358/408		650		500	S ₁ S _c
Rubissia	358/408		650		500	S ₁ S _c
Rubista	274/358				500	S ₃ S _c
Samourai	274/358					S ₃ S ₈ *
Soledane	358				500	S _c
Sweet Cot	334/358				150	S ₂ S ₈
Tadeo	358/420	700			500	S ₉ S _c
Tardorange	358				500	S _c
Tilton	334/358				500	S ₂ S _c
Tornado	408		650			S ₁
Valorange	358				500	S _c
Vitillo	408		650			S ₁
Water	358/408		650			S ₁ S _c *

* S_c / S₈ allele identified using fluorescence microscopy.

Table S2: Origin and S-genotype of 235 analyzed apricot accessions.

Cultivar	Allele1	Allele2	Country	Origin	Cultivar	Allele1	Allele2	Country	Origin	Cultivar	Allele1	Allele2	Country	Origin
AC1	S1	S2	USA	B.P.	Bedri Ahmar	S7	S12	Tunisia	L.S.	Cologlu	S8	S9	Turkey	L.S.
AC2	S9	Sc	USA	B.P.	Beliana	S7	Sc	Tunisia	Landrace	Cooper Cot	S1	S3	France	B.P.
Adedi Ahmar	S8	S12	Tunisia	L.S.	Berdejo	S2	Sc	Spain	Landrace	Corbato	Sc	NA	Spain	Landrace
Alba	S9	Sc	Spain	B.P.	Bergecot	S2	Sc	France	B.P.	Cow-1	S1	S31	France	B.P.
Almadulce	S3	S9	Spain	B.P.	Bergeron	S2	Sc	France	Landrace	Cow-2	S20	S31	France	B.P.
Almater	S1	S9	Spain	B.P.	Big Red	S1	Sc	France	B.P.	Cristalí	S20	Sc	Spain	Landrace
Alyanak	S2	S8	Turkey	L.S.	Borsi-féle kései rózsza	Sc	Sc	Hungary	Landrace	Crvena ungarska	S8	Sc	Macedonia	B.P.
Ananasnyi ciurpinski	Sc	Sc	Ukraine	Unknown	Bouk Ahmed	S7	S11	Tunisia	L.S.	Currot	Sc	Sc	Spain	Landrace
Andornaktályai magyarkajszai	S8	Sc	Hungary	L.S.	Bouthani Ben Friha	S12	S13	Tunisia	L.S.	Dama Rosa	S1	Sc	Spain	B.P.
Apribang (ASF0405)	S6	S9	France	B.P.	Budapest	S2	Sc	Hungary	B.P.	Darunec malahoyeva	S8	Sc	Ukraine	Unknown
Apriqueen (ASF0404)	Sc	NA	France	B.P.	Búlida	S5	Sc	Spain	Landrace	Delice cot	Sc	NA	France	B.P.
Aprisweet (ASF0409)	S9	Sc	France	B.P.	Cacansko zlato	S8	Sc	Serbia	B.P.	Dorada	Sc	NA	Spain	B.P.
Aprix 116	Sc	NA	Spain	B.P.	Callatis	S8	Sc	Romania	B.P.	Dulcinea	S2	Sc	Italy	Landrace
Aprix 33	Sc	NA	Spain	B.P.	Canino	S2	Sc	Spain	Landrace	Effect	S8	Sc	Ukraine	B.P.
aprix20	S6	Sc	Spain	B.P.	Castleton	S1	S2	USA	B.P.	Estrella	S1	S7	Spain	B.P.
Aprix9	S6	Sc	Spain	B.P.	Cataloglu	S6	S9	Turkey	L.S.	Ezzine	S24	Sc	Tunisia	B.P.
ASF0401	S2	S6	France	B.P.	Cebas Red	Sc	NA	Spain	B.P.	Faclo	Sc	NA	France	B.P.
ASF0402	S6	S9	France	B.P.	Ceglédi arany	S9	Sc	Hungary	B.P.	Fantasme	Sc	NA	France	B.P.
Asli	Sc	Sc	Tunisia	B.P.	Ceglédi	S9	Sc	Hungary	B.P.	Faralia	S6	Sc	France	B.P.
Aurora	S1	S9	USA	B.P.	bíborakajski	S9	Sc	Hungary	B.P.	Farbaly	Sc	NA	France	B.P.
Avirine (Bergarouge)	S2	S6	France	B.P.	Ceglédi kedves	Sc	Sc	Hungary	B.P.	Farbela	Sc	NA	France	B.P.
Bebecou	S6	Sc	Greece	Landrace	Ceglédi óriás	S8	S9	Hungary	L.S.	Fardao	Sc	NA	France	B.P.
					Charisma	S7	Sc	South Africa	B.P.	Farely	S1	S9	France	B.P.
					Cheyenne	S6	S9	Spain	B.P.	Farfia	Sc	NA	France	B.P.
					Cocot	Sc	NA	France	B.P.	Farhial	Sc	NA	France	B.P.

CAPÍTULO 4 | MATERIAL SUPLEMENTARIO

Cultivar	Allele1	Allele2	Country	Origin	Cultivar	Allele1	Allele2	Country	Origin	Cultivar	Allele1	Allele2	Country	Origin
Farius	Sc	NA	France	B.P.	Hasanbey	S2	S9	Turkey	L.S.	Magic Cot	S2	S9	USA	B.P.
Farlis	S6	Sc	France	B.P.	Henderson	S3	S9	USA	Landrace	Mamaia	S2	Sc	Romania	B.P.
Farmingdale	S1	S2	USA	B.P.	Holly Cot	S2	S8	France	B.P.	Mandulakajsi	S2	Sc	Hungary	B.P.
Fartoly	S6	Sc	France	B.P.	IBCOT13-12	Sc	NA	Spain	B.P.	Manrí	Sc	Sc	Spain	Landrace
Feria Cot	S6	S9	France	B.P.	IPS16121	Sc	NA	France	B.P.	Mari de Cenad	S19	Sc	Romania	L.S.
Flashcot	S6	S9	USA	B.P.	IPS21512	Sc	NA	France	B.P.	Mariem	S7	S20	Tunisia	B.P.
Flavorcot	S1	Sc	USA	B.P.	IPS23214	S1	S3	France	B.P.	Martinet	S2	S2	Spain	Landrace
Flodea	S3	S9	Spain	B.P.	IPS2712	Sc	NA	France	B.P.	Mauricio	S1	Sc	Spain	Landrace
Flopria	S9	Sc	Spain	B.P.	JNP	S6	S9	Unknown	B.P.	Mayacot	S2	S3	France	B.P.
Galta Vermella	S2	Sc	Spain	Landrace	Justo Cot	S2	Sc	France	B.P.	Medaga	S1	S9	France	B.P.
Valenciana					Kabaasi	S9	S13	Turkey	L.S.	Medflo	S6	Sc	France	B.P.
GaltaRoja	Sc	Sc	Spain	Landrace	Kalao	S2	Sc	Spain	B.P.	Mediabel	S6	Sc	France	B.P.
Gandía	Sc	Sc	Spain	Landrace	Kâsna ungarska	S8	Sc	Bulgaria	B.P.	Mediva	S2	Sc	France	B.P.
Gavatxet	S20	Sc	Spain	Landrace	Kioto	Sc	NA	France	B.P.	Megatea	S1	S9	Spain	B.P.
Ginesta	Sc	Sc	Spain	Landrace	Konservnyi	S2	Sc	Ukraine	L.S.	Memphis	Sc	NA	Spain	B.P.
Giovanniello	S1	S2	Italy	Landrace	Pozdnii					Merino	Sc	NA	Spain	Landrace
Goldbar	S7	S9	USA	B.P.	Korai zματος	S12	S13	Hungary	B.P.	Micaelo	S9	Sc	Spain	B.P.
Goldrich	S1	S2	USA	B.P.	Kosmos	S3	S9	Spain	B.P.	Milord	Sc	NA	Spain	B.P.
Goldstrike 02	S2	S9	USA	B.P.	Krimskyi Amur	S8	Sc	Ukraine	B.P.	Mirlo anaranjado	Sc	NA	Spain	B.P.
Gönci					Kurukabuk	S7	S9	Turkey	Landrace	Mirlo blanco	Sc	NA	Spain	B.P.
magyarkajsi	S8	Sc	Hungary	B.P.	Ladycot	S6	Sc	France	B.P.	Mirlo Rojo	Sc	NA	Spain	B.P.
Grandir	Sc	Sc	Unknown	Unknown	Laguna	Sc	NA	Unknown	Unknown	Mitger	Sc	NA	Spain	Landrace
Haci Haliloglu	S9	S13	Turkey	L.S.	Lambertín-1	S1	S2	USA	B.P.	Modesto	Sc	S13	USA	B.P.
Hamidi	S7	S11	Tunisia	L.S.	Levent	S6	S19	Turkey	B.P.	Moniqui	S2	S6	Spain	Landrace
Harcot	S1	S4	Canada	B.P.	Lido	S9	Sc	Spain	B.P.	Monred	S1	S3	Spain	Unknown
Hargrand	S1	S2	Canada	B.P.	Ligeti óriás	S8	S9	Hungary	L.S.	Monster Cot	S1	S9	USA	B.P.
Harlayne	S3	S20	Canada	B.P.	Lilly Cot	S3	S8	USA	B.P.	Muñoz	S2	S6	Spain	Landrace
Harmat	S10	S11	Hungary	B.P.	Lito	S6	Sc	Greece	Landrace	Murciana	Sc	NA	Spain	B.P.
Harval	S2	Sc	Canada	B.P.	Luizet	S8	Sc	France	B.P.					

Cultivar	Allele1	Allele2	Country	Origin	Cultivar	Allele1	Allele2	Country	Origin	Cultivar	Allele1	Allele2	Country	Origin
Nagygyümölcsű magyarkajszai	S8	Sc	Hungary	B.P.	Pisana	S8	Sc	Italy	B.P.	Sherpa	Sc	NA	Spain	B.P.
Nikitskyi	S8	Sc	Ukraine	B.P.	Playa cot	Sc	NA	France	B.P.	Sirena	Sc	Sc	Romania	B.P.
Ninfa	S7	Sc	Italy	B.P.	Portici	S2	S20	Italy	Landrace	Soganci	S6	S9	Turkey	L.S.
Ninja	S6	S9	France	B.P.	Precoz de Tiryntos	Sc	NA	Greece	Landrace	Soledane	Sc	NA	France	B.P.
NJA-8	Sc	Sc	USA	B.P.	Priabel	S1	S9	France	B.P.	Spring Blush	S3	S8	France	B.P.
Nyújtó F.	Sc	Sc	Hungary	B.P.	Priana	S2	S7	France	B.P.	Stark E.Orange	S6	S9	USA	Landrace
Orange rubis	Sc	NA	France	B.P.	Pricia	S3	Sc	France	B.P.	Sulmona	Sc	Sc	Romania	B.P.
Orangered	S6	S9	USA	B.P.	Primaya	S1	S6	France	B.P.	Sun Glo	S2	S3	USA	Landrace
Oscar	Sc	NA	Spain	B.P.	Primidi	S2	Sc	France	B.P.	Sunny Cot	S6	S9	USA	B.P.
Ouardi	S2	S7	Tunisia	B.P.	Primorosa	Sc	NA	Spain	B.P.	Sweet Cot	S2	S8	USA	B.P.
Oud Hmida	S2	S12	Tunisia	L.S.	Raki	Sc	Sc	Tunisia	B.P.	Swired	Sc	NA	Switzerland	B.P.
Oud Rhayem	S7	S12	Tunisia	L.S.	Rakovszky	S2	Sc	Hungary	L.S.	Szegedi M.	S8	S9	Hungary	Landrace
Oueld El Oud	S7	S8	Tunisia	L.S.	Rambo	S3	Sc	Spain	B.P.	Tadeo	S9	Sc	Spain	Landrace
Paksi magyarkajszai	S8	Sc	Hungary	B.P.	Regibus	S2	Sc	Switzerland	B.P.	Tardorange	Sc	NA	Spain	B.P.
Palabras	Sc	Sc	Spain	Landrace	Robada	S6	S8	USA	B.P.	Tilton	S2	Sc	USA	Landrace
Palau	Sc	Sc	Spain	Landrace	Rojo Carlet	Sc	Sc	Spain	Landrace	Tiryntos	Sc	Sc	Greece	Landrace
Pandora	S2	S6	Greece	Landrace	Rojo Pasi6n	S1	Sc	Spain	B.P.	Tom Cot	S9	Sc	USA	B.P.
Pann6nia	Sc	Sc	Hungary	B.P.	Rouge cot	Sc	NA	France	B.P.	Trevatt	S2	Sc	Australia	Landrace
Pasinok	Sc	Sc	Ukraine	B.P.	Roxana	S2	Sc	Afghanistan	Unknown	Tsunami	S3	S9	France	B.P.
Patterson	Sc	Sc	USA	B.P.	R6zsakajszai	S2	Sc	Hungary	Landrace	Valorange	Sc	NA	Spain	B.P.
Paviot	S2	Sc	France	Landrace	Rubely	Sc	NA	France	B.P.	Vel6zquez	S5	S20	Spain	Landrace
Pe6aflor 02	S2	Sc	Spain	Landrace	Rubissia	S1	Sc	France	B.P.	Venus	S8	Sc	Romania	B.P.
Pepito del Rubio	S2	Sc	Spain	Landrace	Rubista	S3	Sc	France	B.P.	Voski	S11	S13	Hungary	B.P.
Perfection	S1	S3	USA	Landrace	Samourai	S3	S8	France	B.P.	Water	S1	Sc	Unknown	Unknown
Perla	S2	S20	Italy	Landrace	Sandy cot	S2	Sc	France	B.P.	Wonder Cot	S6	S9	USA	B.P.
Perlecot	S8	S9	USA	B.P.	Sayeb	S7	Sc	Tunisia	B.P.	Xirivello	Sc	Sc	Spain	Landrace
Pinkcot	S8	S9	France	B.P.	Shalakh	S5	S11	Armenia	L.S.	Zapodolye	Sc	Sc	Ukraine	Unknown

L.S.: Local selection; B.P.: Breeding program

Table S3: Contingency table of absolute allele frequencies by country in traditional apricot cultivars, including landraces and local selections, and Pearson's chi-squared test with simulated p-value (based on 2000 replicates).

	Armenia	Australia	France	Greece	Hungary	Italy	Romania	Spain	Tunisia	Turkey	Ukraine	USA
NA	0	0	0	1	0	0	0	3	0	0	0	0
S_1	0	0	0	0	0	1	0	1	0	0	0	1
S_2	0	1	2	1	2	4	0	9	1	2	1	2
S_3	0	0	0	0	0	0	0	0	0	0	0	3
S_5	1	0	0	0	0	0	0	2	0	0	0	0
S_6	0	0	0	3	0	0	0	2	0	2	0	1
S_7	0	0	0	0	0	0	0	0	6	1	0	0
S_8	0	0	0	0	4	0	0	0	2	2	0	0
S_9	0	0	0	0	3	0	0	1	0	7	0	2
S_{11}	1	0	0	0	0	0	0	0	2	0	0	0
S_{12}	0	0	0	0	0	0	0	0	5	0	0	0
S_{13}	0	0	0	0	0	0	0	0	1	2	0	0
S_{19}	0	0	0	0	0	0	1	0	0	0	0	0
S_{20}	0	0	0	0	0	2	0	3	0	0	0	0
S_c	0	1	2	5	5	1	1	31	1	0	1	1
Chi-squared	372.96											
df	NA											
p-value	0.0004998											

Table S4: Contingency table of absolute allele frequencies by country in apricot cultivars released from breeding programs and Pearson's chi-squared test with simulated p-value (based on 2000 replicates).

	Bulgaria	Canada	France	Hungary	Italy	Macedonia	Romania	Serbia	South Africa	Spain	Switzerland	Tunisia	Turkey	Ukraine	Unknown	USA
NA	0	0	20	0	0	0	0	0	0	16	1	0	0	0	0	0
S_1	0	2	9	0	0	0	0	0	0	5	0	0	0	0	0	8
S_2	0	2	10	2	0	0	1	0	0	1	1	1	0	0	0	8
S_3	0	1	8	0	0	0	0	0	0	4	0	0	0	0	0	1
S_4	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S_6	0	0	13	0	0	0	0	0	0	3	0	0	1	0	1	5
S_7	0	0	1	0	1	0	0	0	1	1	0	3	0	0	0	1
S_8	1	0	5	3	1	1	2	1	0	0	0	0	0	3	0	4
S_9	0	0	10	2	0	0	0	0	0	10	0	0	0	0	1	12
S_{10}	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
S_{11}	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
S_{12}	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
S_{13}	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1
S_{19}	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
S_{20}	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0
S_{24}	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
S_{31}	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
S_c	1	1	37	13	2	1	7	1	1	26	2	6	0	5	0	8
Chi-squared	513.39															
df	NA															
p-value	0.006997															

CAPÍTULO 5. Male Meiosis as a Biomarker for Endo- to Ecodormancy Transition in Apricot

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CAPÍTULO 5

Male Meiosis as a Biomarker for Endo- to Ecodormancy Transition in Apricot

La meiosis masculina como biomarcador para la transición de endo- a ecodormancia en albaricoquero.

RESUMEN

El reposo es una estrategia adaptativa de las plantas para sobrevivir en condiciones climáticas desfavorables durante el invierno. En las regiones templadas, la mayoría de los árboles frutales necesitan una exposición a un cierto período de bajas temperaturas para superar la endodormancia. Después de la salida de la endodormancia, se necesita una exposición a temperaturas cálidas para florecer (ecodormancia). Las necesidades de frío y de calor están determinadas genéticamente y, por lo tanto, son específicas para cada especie y variedad. La falta de suficiente frío invernal puede provocar fallos en la floración y la fructificación, comprometiendo así la cosecha. Por tanto, el conocimiento de las necesidades de frío y de calor es esencial para optimizar la selección de variedades para cultivarlas en diferentes condiciones edafoclimáticas. Sin embargo, la falta de marcadores fenológicos o biológicos vinculados a las fases de reposo dificultan establecer el final de la endodormancia. Esto ha llevado a estimaciones indirectas que normalmente no son válidas en diferentes condiciones agroclimáticas. El incremento en el número de inviernos más templados causados por el cambio climático y el continuo desarrollo de nuevas variedades enfatizan la necesidad de un marcador biológico adecuado que permita determinar la transición de endo- a ecodormancia para una estimación precisa de las necesidades agroclimáticas (NA) de cada variedad. En este trabajo se evalúa la meiosis masculina como biomarcador para determinar la salida de endodormancia y estimar las necesidades de frío y calor en albaricoquero. Para ello, se caracterizó histoquímicamente el desarrollo del polen en 20 variedades durante 8 años, y las etapas de desarrollo se relacionaron con el reposo. Los resultados se compararon con tres enfoques que estiman indirectamente la salida del reposo: una metodología experimental mediante la evaluación del crecimiento de yemas en varetas recolectadas periódicamente durante los meses de invierno y trasladadas a cámaras con condiciones controladas durante 3 años, y dos enfoques estadísticos que relacionan las temperaturas estacionales y las fechas de floración en una serie de 11 a 20 años por correlación y regresión de mínimos cuadrados parciales. Los resultados revelan que la meiosis masculina es un biomarcador válido para determinar el final de la endodormancia y estimar las NA en albaricoquero.



Male Meiosis as a Biomarker for Endo- to Ecodormancy Transition in Apricot

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Dormancy is an adaptive strategy in plants to survive under unfavorable climatic conditions during winter. In temperate regions, most fruit trees need exposure to a certain period of low temperatures to overcome endodormancy. After endodormancy release, exposure to warm temperatures is needed to flower (ecodormancy). Chilling and heat requirements are genetically determined and, therefore, are specific for each species and cultivar. The lack of sufficient winter chilling can cause failures in flowering and fruiting, thereby compromising yield. Thus, the knowledge of the chilling and heat requirements is essential to optimize cultivar selection for different edaphoclimatic conditions. However, the lack of phenological or biological markers linked to the dormant and forcing periods makes it difficult to establish the end of endodormancy. This has led to indirect estimates that are usually not valid in different agroclimatic conditions. The increasing number of milder winters caused by climatic change and the continuous release of new cultivars emphasize the necessity of a proper biological marker linked to the endo- to ecodormancy transition for an accurate estimation of the agroclimatic requirements (AR) of each cultivar. In this work, male meiosis is evaluated as a biomarker to determine endodormancy release and to estimate both chilling and heat requirements in apricot. For this purpose, pollen development was characterized histochemically in 20 cultivars over 8 years, and the developmental stages were related to dormancy. Results were compared to three approaches that indirectly estimate the breaking of dormancy: an experimental methodology by evaluating bud growth in shoots collected periodically throughout the winter months and transferred to forcing chambers over 3 years, and two statistical approaches that relate seasonal temperatures and blooming dates in a series of 11–20 years by correlation and partial least square regression. The results disclose that male meiosis is a possible biomarker to determine the end of endodormancy and estimate AR in apricot.

Keywords: chilling requirements, heating requirements, *Prunus armeniaca*, dormancy, modeling, pollen development, dynamic model, flower buds

INTRODUCTION

Temperate woody perennials survive at low temperatures in winter by entering a dormant stage. This mechanism is characterized by the absence of visible growth in any plant structure containing a meristem and is associated with a slowdown of biological activity, a modification in hormonal balance, and extensive metabolic remodeling (Horvath et al., 2003; Rohde and Bhalerao, 2007).

However, dormancy is not just a survival strategy (Rohde and Bhalerao, 2007). Indeed, the temperature is a conditional factor in woody plants in temperate climates (Cooke et al., 2012; Ríos et al., 2014). Thus, chilling accumulation is required for proper flowering since the lack of winter chill during the winter period can cause physiological disorders such as bud burst delay and erratic and insufficient flowering negatively affecting yield (Viti et al., 2008; Campoy et al., 2011).

Although the need to accumulate chill for flowering and fruiting has been known for over 2 centuries (Knight, 1801), the biological basis behind remains elusive (Lloret et al., 2018; Goeckeritz and Hollender, 2021). However, the importance of chilling requirements (CR) in fruit trees is growing due to the increased release of new cultivars in many fruit tree crops, the expansion to warmer areas, and the lower winter chilling temperatures caused by climate change in many regions of the world (Luedeling et al., 2011).

Lang et al. (1987) proposed three different stages in dormancy: endodormancy, referred to when the regulation of dormancy is triggered by physiological factors; ecodormancy that refers to dormancy regulated by environmental factors; and paradormancy, which is used when growth inhibition arises from another part of the plant (e.g., apical dominance). The key factor to estimate agroclimatic requirements (AR; chilling and heat requirements) of particular cultivars is determining when the tree has overcome the phase of endodormancy and fulfilled its CR. Several approaches have been developed to estimate CR. For this purpose, the date of breaking of endodormancy has been experimentally determined by sequentially collecting shoots from dormant trees during the winter, exposing them to warm temperatures in controlled conditions, and quantifying chilling temperatures up to shoot collecting time when flower buds are considered capable to resume growth (Fadón et al., 2020a). Alternatively, the end of endodormancy has been determined by statistical correlations (Alonso et al., 2005) or partial least squares (PLS) regressions (Luedeling et al., 2013) with data of flowering dates and temperatures from long series of years. Both types of approaches are time-consuming, and in many cases, the results are not directly comparable under different climatic conditions (Fadón and Rodrigo, 2018). In the experimental approach, the use of cut shoots cannot entirely reflect the behavior of adult trees in field conditions, and several factors may affect the establishment of the date of endodormancy release and hence the estimations of CR (Fadón and Rodrigo, 2018). Likewise, statistical correlations require data from long series of years that are only available for a few sets of cultivars that exclude new cultivars, and the results may not fit equally well in different climates and latitudes (Dennis, 2003; Fadón et al., 2020a).

Several models have been used to quantify chilling and heating accumulation through hourly temperature data. The dynamic model has been reported as the most accurate model of chilling quantification (Ruiz et al., 2007; Luedeling and Brown, 2011; Campoy et al., 2012b, 2019; Luedeling and Gassner, 2012). However, CR has also been calculated using Utah (Richardson et al., 1974) and Weinberger (1950) models to compare the results with those of the previous studies.

The dynamic model assumes that chilling accumulation is a two-step process, in which previous warm temperatures can counteract the effect of low temperatures during endodormancy. Initially, cold temperatures would promote the accumulation of an intermediate product. However, warm temperatures during this period of accumulation can stimulate the degradation of this precursor and reverse the process. Once the accumulation of this product reaches a critical concentration, it converts to a chill portion (CP), a stable factor, which cannot be destroyed, and promotes the break of the endodormancy (Fishman et al., 1987). The Utah model establishes temperature ranges that contribute differently to chilling accumulation, which is measured as chill units (CU) (Richardson et al., 1974). The chill accumulation takes place within a temperature range of 1.5–12.4°C (Richardson et al., 1974). Finally, the Weinberger model proposes that only temperatures below 7.2°C (45°F) are useful and have an effect in breaking the endodormancy (chilling hours, CH) (Weinberger, 1950).

Nowadays, CR in most cultivars is unknown, mainly due to the problems associated with the existing methods and the lack of a biological marker to determine the chilling fulfillment of a tree in field conditions (Fadón and Rodrigo, 2018). In previous works in apricot, another differentiation has been related to winter dormancy since it sets a boundary between sporogenous tissue and pollen grain formation stages (Julian et al., 2011). The stamens remain in this anatomically quiescent stage during endodormancy to resume growth and complete the development of pollen grains in ecodormancy (Rodrigo and Herrero, 2002). However, male meiosis takes place once CR has been fulfilled but flower buds do not show external signs of development (Bartolini et al., 2006; Julian et al., 2014).

On the other hand, pollen development is a well-characterized and highly conserved process in angiosperms. Prior to meiosis, microspore mother cells become isolated by a wall with the deposition of a callose layer (McCormick, 2004). Callose is a linear homopolymer widely found in higher plants with important roles in plant growth and development (Chen and Kim, 2009). During pollen development, callose may act as a physical molecular filter involved in stress responses (Dong et al., 2005; Blackmore et al., 2007).

In this work, we evaluate male meiosis as a biological marker of breaking dormancy for estimating AR by histochemical detection of callose deposition around microspore mother cells. For this purpose, chilling and heating requirements of a group of 20 apricot cultivars have been estimated by quantifying chill accumulation until the date of male meiosis and heat accumulation from male meiosis to flowering for over 4 years. To validate the results with those obtained with other existing

methods, endodormancy breaking has also been experimentally estimated by studying cut shoots under forcing conditions, by two statistical models that estimate the correlation between blooming dates and seasonal temperatures and the determination of the daily influence of chill accumulation in blooming dates by PLS regression.

MATERIALS AND METHODS

Plant Material

Plant material was obtained from an experimental orchard located at the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) in Zaragoza (Spain) at 41°44'30" N, 0°47'00" W and 220 m altitude. Twenty apricot cultivars with different flowering dates were selected. Phenological observations of flower buds were carried out two times a week in each cultivar from bud break to flowering. Blooming dates were recorded according to Baggiolini stages, considering full flowering (F50) when 50% of flower buds were in stage F (Baggiolini, 1952) that corresponds to stage 65 (full bloom) in the BBCH scale (Meier, 2001; Pérez-Pastor et al., 2004). Daily temperatures during 1998–2021 were acquired from a meteorological station close to the orchard.

Determination of Endodormancy Breaking

Four different procedures were carried out to estimate the chilling and heating requirements for flowering.

Forcing Test: Cutting Shoots Under Forcing Conditions

Flower bud growth was evaluated in response to warm conditions after exposure to chill in the field. Four shoots of each cultivar were collected weekly from November to March over 3 years (2016–2017, 2017–2018, and 2019–2020). The shoots with lengths of around 40 cm were placed in a growth chamber with controlled conditions and maintained on a wet florist foam. The end of the shoot was cut underwater to avoid cavitation and the water was renovated periodically. The shoots were maintained at $22 \pm 1^\circ\text{C}$ with a 12-h light photoperiod for 8 days. To determine the end of endodormancy, 10 flower buds were randomly picked and weighed on the first and last day in the growth chamber. Endodormancy was considered overcome when a significant bud weight increase (30%) was observed after the period under controlled conditions (Brown and Kotob, 1957; Tabuenca, 1964; Ruiz et al., 2007). The end of endodormancy for each cultivar was established for each year.

Correlation of Long Term Records of Blooming Dates and Daily Winter Temperatures

A statistical model based on the temperature effects on blooming dates in 13 years was carried out for each cultivar. Two matrixes were defined to determine the end of endodormancy. One of them was based on blooming dates composed of two axes: years and genotypes. The other one was defined by the number of days from October 1 (day 1) to April 30 (day 212) and the

average of the mean daily temperature measured at 15 day periods (Alonso et al., 2005). The vector obtained on each day was correlated to the corresponding vector of the blooming dates matrix and the Pearson correlation coefficient (PCC) was calculated. The endodormancy to ecodormancy period was established when no significant correlation coefficients were found, i.e., from the last day significantly positive ($\text{PCC} = 0.553$) to the first day significantly negative ($\text{PCC} = -0.553$). The analysis was based on a dataset of 13 years with the Student's *t*-test for 11 df and a two-tailed test at $\alpha = 0.05$. The end of endodormancy was the mean date of the transition period in each cultivar.

Partial Least Squares Regression Analysis of Daily Chill, Heat Accumulation, and Blooming Dates

Partial least squares regression analysis was carried out to establish chilling and heating phases (Luedeling et al., 2013). Blooming dates for a period between 11 and 18 years, depending on the cultivar, were correlated with daily chill and heat accumulation. Chill accumulation was measured as chill portions of the dynamic model whereas growing degree hours (GDH) were used to measure heat accumulation (Fadón et al., 2021).

The PLS analysis calculates model coefficients that indicate the effect of the chill portions in the blooming dates. Negative coefficients have a blooming-advancing effect whereas positive coefficients are related to high temperatures during the chilling period that results in a delay in blooming. The absolute value of the model coefficients indicates the strength of the effect. The variable-importance-in-the-projection (VIP) values were used to define the chilling and heating phases. Values greater than $\text{VIP} = 0.8$ indicate significant importance of model coefficients (Luedeling and Gassner, 2012; Luedeling et al., 2013; Fadón et al., 2021). A single date for the beginning and the end of the chilling and forcing periods was defined per cultivar.

Male Meiosis Characterization

Flower buds were sampled weekly during winter, from November to February, for 8 years. The buds were fixed in ethanol (95%)/acetic acid (3:1, v/v) for 24 h and conserved at 4°C in ethanol 75% (Williams et al., 1999). To determine the stage of the development of pollen, anthers were removed from fixed buds with the help of a scalpel and mounted by the squash technique adding 0.1% aniline blue in 0.1 N K_3PO_4 to stain callose (Currier, 1957). Microscopic preparations were observed under a Leica DM2500 microscope (Cambridge, United Kingdom) with UV epifluorescence using 340–380 bandpass and 425 longpass filters. The formation of a callose wall around the microspore mother cells was established as the date when the CR was fulfilled (Julian et al., 2014). The end of the endodormancy was determined per year in each cultivar.

Quantification of Chilling and Heat Requirements

The Weinberg, Utah, and dynamic models were used to quantify CR. The amount of chill required to overcome endodormancy was calculated from the initial date of chilling accumulation

until the date of endodormancy release established by the different methods. The day considered as the beginning of chilling accumulation varied between the models. According to Weinberger (1950), chilling hours are the number of hours below 7°C from the time in autumn when chill hours start to occur. However, in the Utah model, the day when the temperatures produced the maximum negative effect is considered as the starting date of the accumulation of chill units (Richardson et al., 1974). In the dynamic model, we established 1st September as the starting date of accumulation of portion units (Fishman et al., 1987). That date fits well with the climatic conditions in Zaragoza, where usually no chilling occurs before it.

In the two experimental methods (forcing test and male meiosis), CR was calculated per year and the mean value was established per cultivar. Since the statistical approaches determined a single date for the end of the endodormancy, a unique chilling requirement value was obtained per cultivar.

Heat requirements were expressed as the accumulation of GDH from endodormancy breaking to flowering. The GDH corresponds to an accumulation of hours above 4.5°C from the date established as the end of the endodormancy to the date when 50% of the flowers were open (F50) (Richardson et al., 1974).

Statistical Analyses

All the analyses were performed in the R programming environment v. 4.1.0 (R Core Team, 2022). The PLS analysis was carried out using the *chillR* package (version 0.70.24) (Luedeling, 2019).

The coefficient of variation was used as the indicator of quality within years for each method. To compare the results obtained from the four different methods and evaluate the potential of bias between them, we carried out a difference plot analysis using the mean chill portions values per cultivar through the Bland–Altman method (Bland and Altman, 1986, 1999) and the *MethComp* (Carstensen et al., 2013) packages for R. To assess the level of agreement between a pair of measurement methods, two-by-two analyses were performed to establish a two-sided 95% confidence interval (Shieh, 2018) and whenever 95% of the scatter points fell within that interval both methods were considered to be in agreement.

RESULTS

Chill accumulation started in October in all the years analyzed and increased exponentially during the winter months. However, significant differences were observed between years, ranging from 89.4 chilling portions (CP) (2011–2012) to 112.9 CP (2003–2004) accumulated at the end of March (Figure 1).

Establishment of Endodormancy Breaking by Cut Shoots Subject to Forcing Conditions

For each cultivar and year, the comparison of the evolution of flower bud growth under field and controlled conditions allowed

determining the endodormancy breaking date when the flower buds increased their weight by 30% after 8 days in the growth chamber (Supplementary Table 1). As an example, the date of endodormancy breaking for “Pandora” was 6 January 2019 (Figure 2). The average date of endodormancy breaking for the 3 years analyzed ranged between December 27 and February 8 between cultivars (Table 1).

Determination of the Transition From Endodormancy to Ecodormancy by Correlation Modeling

For each cultivar, the correlation coefficients between the full flowering dates and the winter temperatures were obtained using the mean daily temperature in periods of 15 days over 13 years (Supplementary Figure 1). The transition from endodormancy to ecodormancy was established when the last significant positive correlation coefficient was obtained to the first significant negative correlation coefficient. The dates of endodormancy breaking ranged from January 15 to February 22 (Table 1 and Supplementary Table 2). As an example, the endodormancy breaking date in “Pandora” was estimated on January 18 (Figure 3).

Determination of Chilling and Forcing Periods by Partial Least Squares Regression

Chilling and forcing phases for each cultivar were defined based on the results of the PLS analysis with daily chill accumulation quantified in CP. High daily chill accumulation rates during the chilling phase generally indicated a correlation with early blooming dates. The beginning and the end of the chilling periods extended from negative significant coefficients occurring in September–October, around endodormancy establishment, to negative significant coefficients in December–January, indicating the end of endodormancy. As an example, in “Pandora,” the chilling period was considered from 8th October to 13th January, when a clear trend of negative model coefficients, as well as predominantly significant VIP scores, was shown (Figure 4A). The starting dates in all the cultivars took place in a short period, from 29th September to 9th October (Supplementary Table 3). However, the dates of endodormancy breaking were highly variable, ranging from 17th December to 14th February (Table 1).

The forcing periods were derived from the PLS analysis based on daily heat accumulation. Negative model coefficients and high VIP scores indicated that high daily heat accumulation during this period had a strong effect on bloom-advancing dates. Forcing periods were highly variable between cultivars, lasting between 30 and 71 days. The start date of ecodormancy ranged from early-January to late-February (Table 1). As an example, the forcing phase in “Pandora” was estimated from 24th January to 11th March (Figure 4B).

For all cultivars, the forcing period did not follow directly after the chilling period, but it was preceded by a transition phase during which the coefficients were neither clearly negative nor clearly positive, and they were not considered by the VIP analysis. This transition period occurred between January and

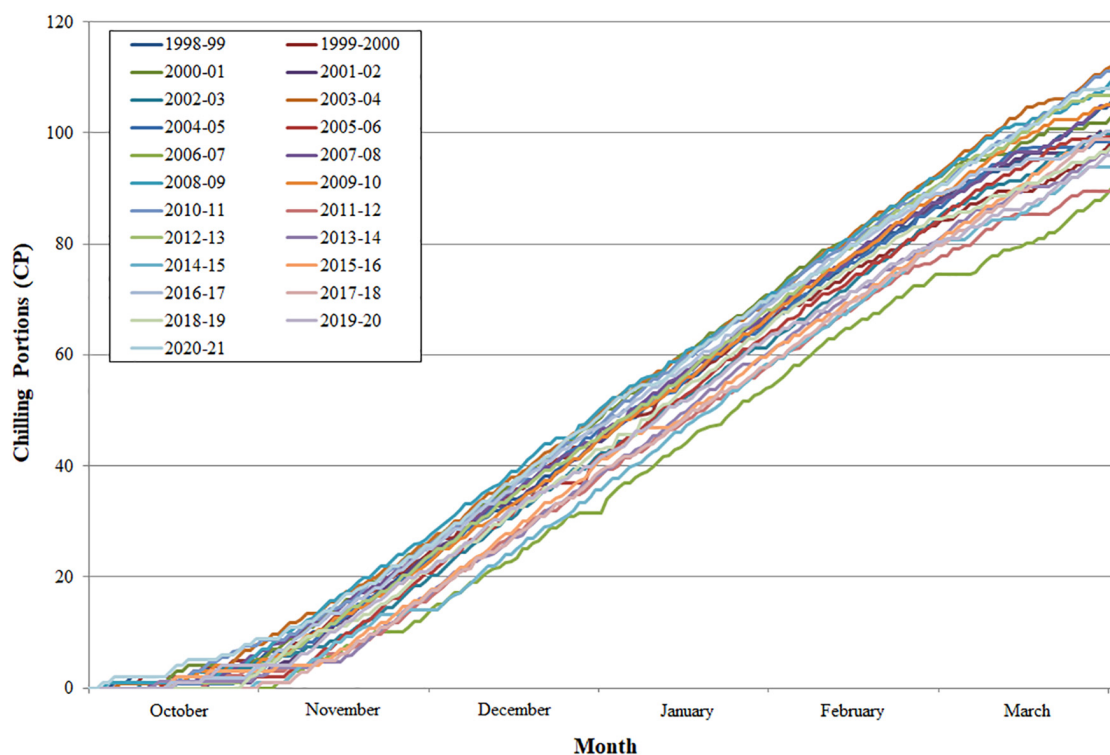


FIGURE 1 | Mean daily chill portion accumulation per year for the period 1998–2021 at Zaragoza (Spain).

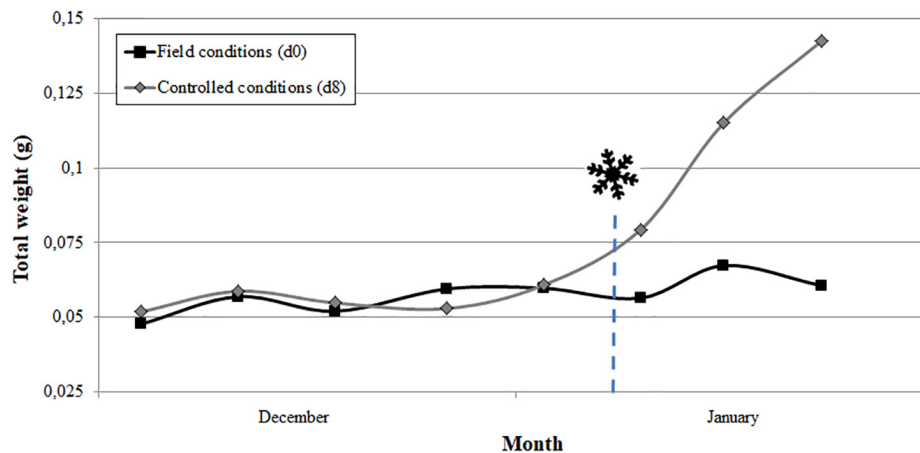


FIGURE 2 | Estimation of breaking of endodormancy date (*) in the apricot cultivar "Pandora" in 2019 by the Forcing test. Flower bud weight in field conditions and after 8 days in the growth chamber. Chilling requirements were considered fulfilled when the increase in the weight of the flower buds in the growth chamber was 30% higher than in field conditions ($n = 10$).

February and ranged from 3 to 20 days depending on the cultivars (Supplementary Figure 2).

Anther Development and Dormancy Phases

Sporogenous tissue (Figure 5A) was observed in the anthers of all the cultivars during several months of the endodormancy phase

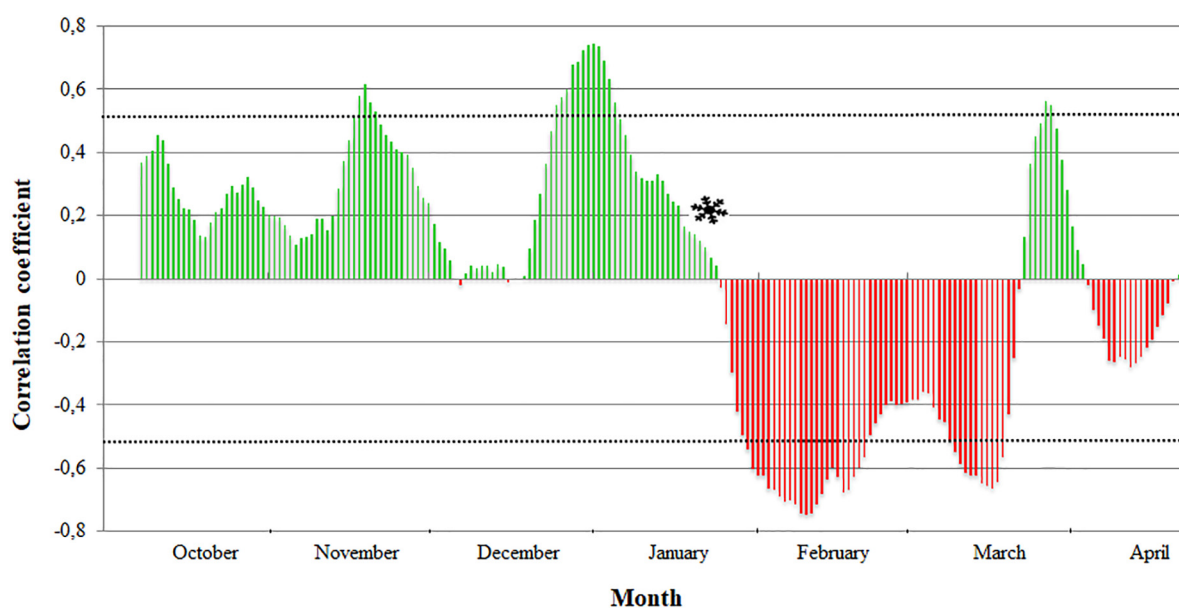
when no signs of development were observed. The deposition of callose around the sporogenous tissue cells was the first sign of reactivation of anther development (Figure 5B). The onset of meiosis began with the accumulation of a callose layer around the microspore mother cells that became isolated (Figure 5C). About a week later, two meiotic divisions occurred rapidly forming the tetrads, a stage of four haploid cells surrounded by callose (Figure 5D). After meiosis, the callose wall dissolved,

TABLE 1 | Dates of endodormancy and chilling requirements of 20 apricot cultivars determined according to four methods (forcing test, correlation, PLS, and male meiosis).

	Chilling requirements (Chilling portions)											
	Forcing test			Correlation model			PLS regression			Male meiosis		
	Endodormancy breaking	Value $\pm$ SD	cv	Endodormancy breaking	Value $\pm$ SD	cv	Endodormancy breaking	Value $\pm$ SD	cv	Endodormancy breaking	Value $\pm$ SD	cv
Berdejo	06 Jan	47.2 $\pm$ 4.5	9.6	18 Jan	57.0 $\pm$ 4.9	8.7	14 Jan	54.2 $\pm$ 4.6	8.5	26 Jan	61.9 $\pm$ 5.1	8.3
Canino	27 Dec	40.2 $\pm$ 6.1	15.1	18 Jan	57.0 $\pm$ 4.9	8.7	15 Jan	54.8 $\pm$ 4.7	8.6	14 Jan	53.2 $\pm$ 6.2	11.6
Corbato	07 Jan	47.5 $\pm$ 1.5	3.1	16 Jan	55.7 $\pm$ 4.8	8.7	15 Jan	54.9 $\pm$ 4.5	8.3	23 Jan	59.7 $\pm$ 4.6	7.6
Goldrich	29 Dec	42.0 $\pm$ 7.7	18.2	17 Jan	56.4 $\pm$ 4.8	8.6	13 Jan	53.6 $\pm$ 3.9	7.3	21 Jan	58.3 $\pm$ 5.5	9.4
Gönci Magyar	25 Jan	60.2 $\pm$ 5.1	8.4	04 Feb	69.1 $\pm$ 5.0	7.2	12 Feb	74.8 $\pm$ 5.1	6.8	02 Feb	66.8 $\pm$ 5.2	7.7
Harcot	03 Jan	45.0 $\pm$ 2.5	5.6	15 Jan	54.8 $\pm$ 4.9	8.9	13 Jan	53.3 $\pm$ 4.1	10.7	23 Jan	59.8 $\pm$ 5.2	8.7
Henderson	05 Feb	69.1 $\pm$ 5.2	7.6	04 Feb	69.1 $\pm$ 5.0	7.2	13 Jan	53.6 $\pm$ 3.9	7.3	07 Feb	70.9 $\pm$ 10.4	14.6
Luizet	07 Jan	48.0 $\pm$ 4.0	8.3	19 Jan	58.0 $\pm$ 4.9	8.5	28 Jan	64.0 $\pm$ 4.5	7.1	25 Jan	61.3 $\pm$ 4.8	7.9
Mitger	07 Jan	47.5 $\pm$ 1.5	3.1	16 Jan	55.7 $\pm$ 4.8	8.7	29 Dec	43.2 $\pm$ 3.9	9.1	21 Jan	58.0 $\pm$ 5.4	9.3
Moniqui 1006	04 Jan	45.8 $\pm$ 3.5	7.6	15 Jan	54.8 $\pm$ 4.9	8.9	17 Dec	33.9 $\pm$ 4.6	13.6	23 Jan	59.6 $\pm$ 5.3	8.9
Moniqui 2113	10 Jan	49.8 $\pm$ 2.4	4.9	16 Jan	55.7 $\pm$ 4.8	8.7	14 Jan	55.0 $\pm$ 4.3	14.0	26 Jan	61.7 $\pm$ 5.9	9.6
Muñoz	05 Jan	46.5 $\pm$ 3.8	8.2	18 Jan	57.0 $\pm$ 4.9	8.7	21 Jan	58.6 $\pm$ 4.4	14.0	22 Jan	59.1 $\pm$ 4.8	8.1
Pandora	04 Jan	45.8 $\pm$ 3.5	7.6	18 Jan	57.0 $\pm$ 4.9	8.7	13 Jan	53.6 $\pm$ 3.9	7.3	20 Jan	60.7 $\pm$ 9.6	15.8
Paviot	06 Jan	47.2 $\pm$ 4.5	9.6	18 Jan	57.0 $\pm$ 4.9	8.7	14 Jan	54.3 $\pm$ 4.4	8.1	25 Jan	61.6 $\pm$ 5.0	8.1
Pepito del Rubio	27 Dec	41.2 $\pm$ 4.1	10.0	16 Jan	55.7 $\pm$ 4.8	8.7	13 Jan	53.3 $\pm$ 4.3	13.2	22 Jan	58.9 $\pm$ 5.1	8.7
Stark Early Orange	25 Jan	60.4 $\pm$ 2.6	4.3	06 Feb	70.7 $\pm$ 5.0	7.1	14 Feb	76.7 $\pm$ 4.2	5.5	08 Feb	71.4 $\pm$ 6.4	9.0
Stella	08 Feb	69.9 $\pm$ 6.7	9.5	22 Feb	81.7 $\pm$ 5.3	6.4	09 Feb	72.9 $\pm$ 4.5	6.1	01 Mar	84.8 $\pm$ 7.5	8.8
Sun Glo	15 Jan	53.9 $\pm$ 3.5	6.4	20 Jan	58.6 $\pm$ 4.9	8.4	13 Jan	53.6 $\pm$ 3.9	7.3	01 Feb	66.3 $\pm$ 7.0	10.6
Tadeo	09 Jan	49.1 $\pm$ 2.4	5.0	10 Feb	73.5 $\pm$ 5.0	6.8	13 Jan	53.6 $\pm$ 3.9	7.3	24 Jan	60.5 $\pm$ 5.3	8.7
Veecot	09 Jan	48.9 $\pm$ 1.7	3.5	19 Jan	58.0 $\pm$ 4.9	8.5	14 Jan	54.6 $\pm$ 4.0	7.3	16 Jan	54.5 $\pm$ 5.4	9.8

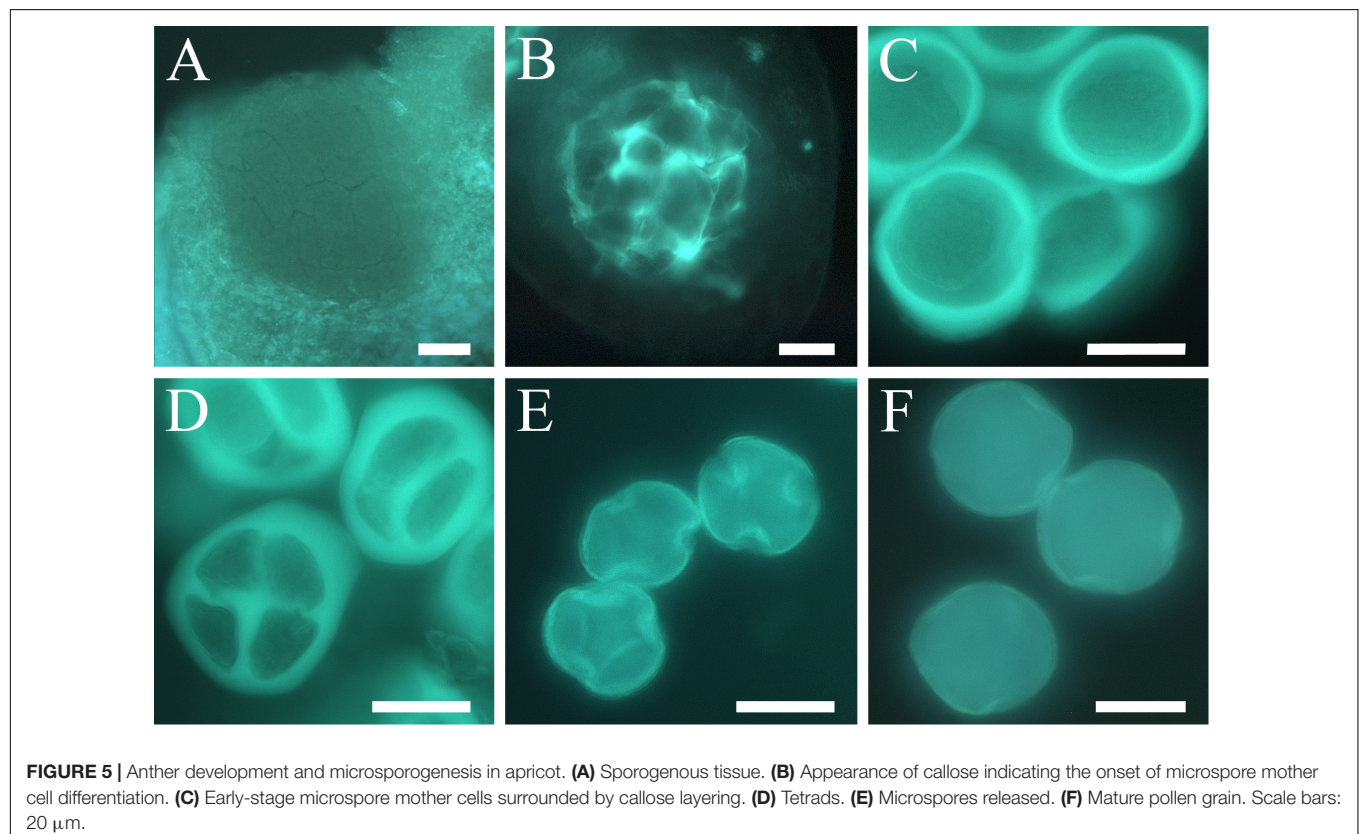
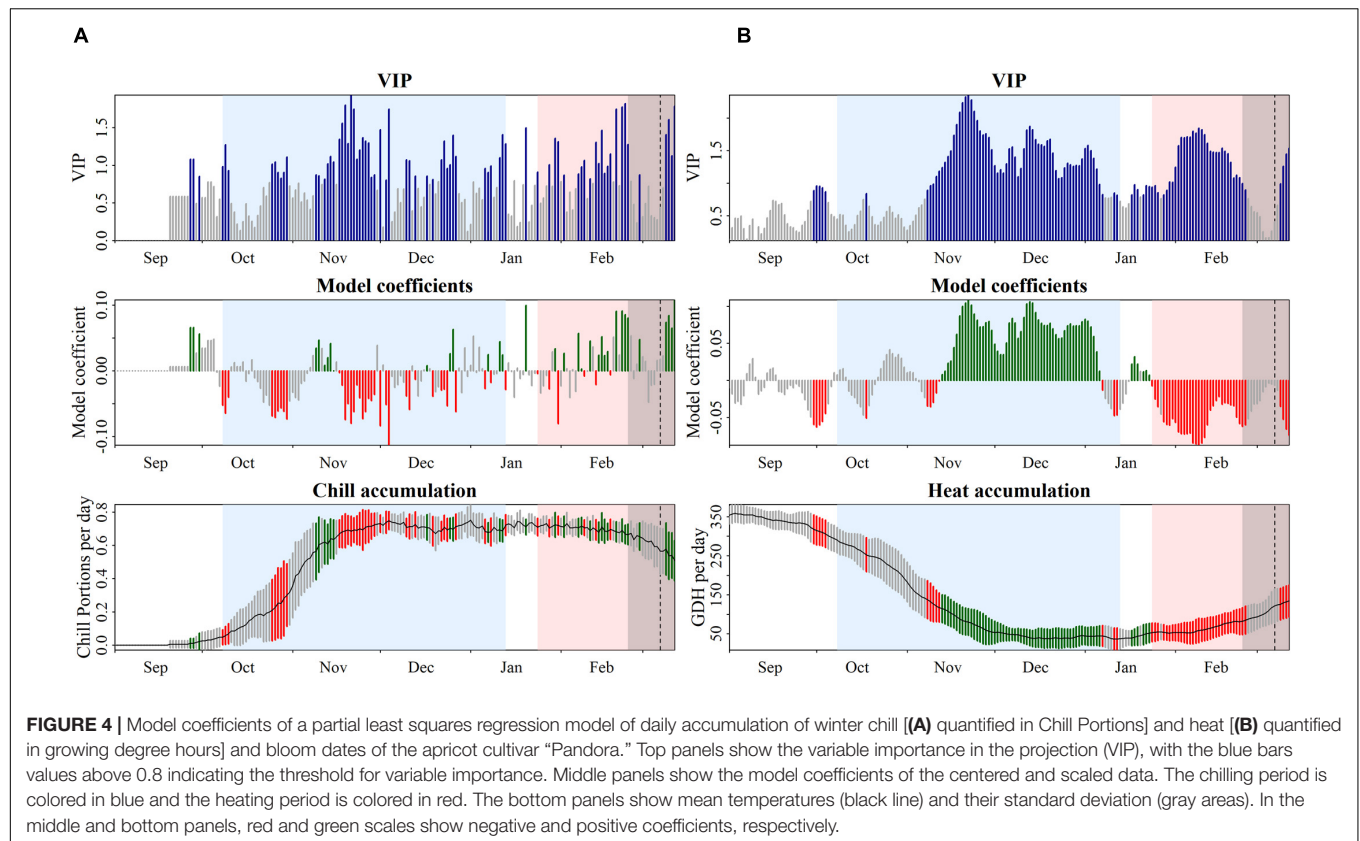
Values are in chill portions.

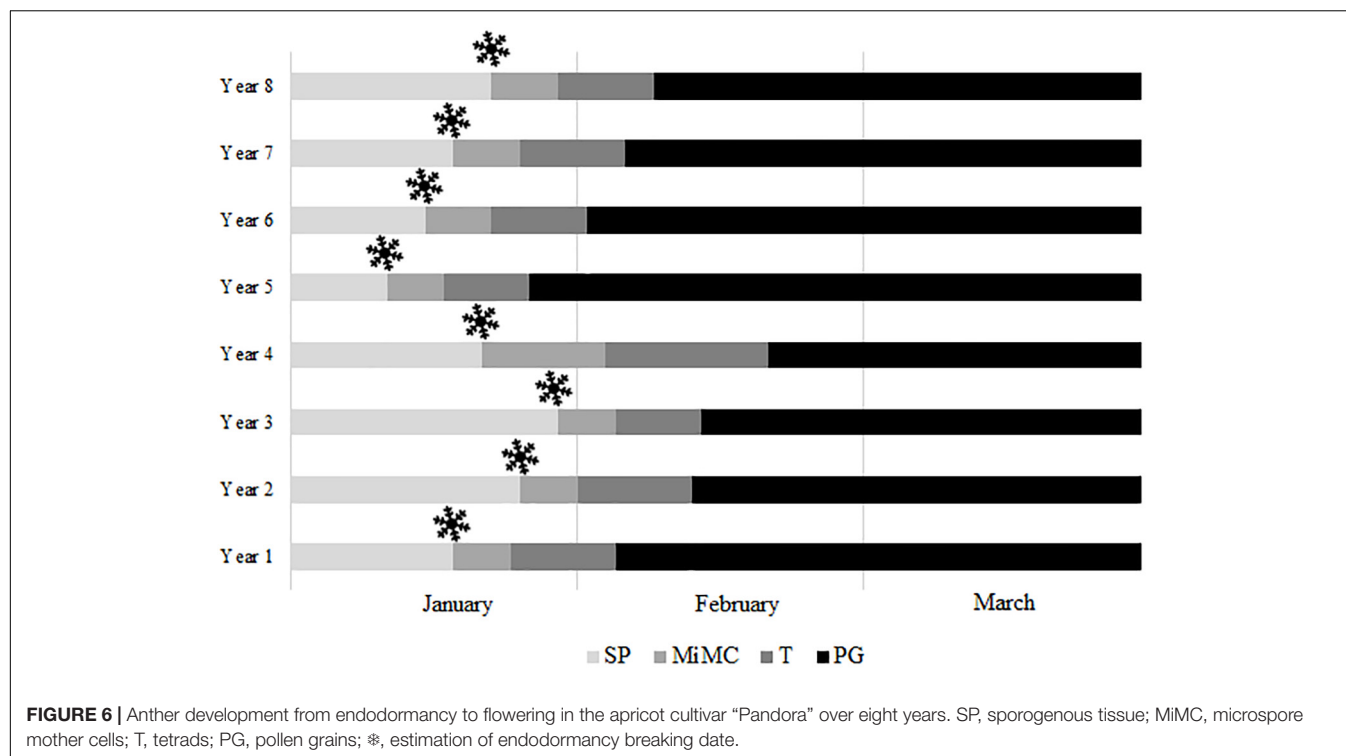
SD, standard deviation; cv, coefficient of variation.

**FIGURE 3 |** Establishment of endodormancy breaking date (*) in the apricot cultivar “Pandora” by analyzing the evolution of the Pearson correlation coefficients between the full flowering dates and the winter temperatures of periods of 15 days. Discontinuous lines delimit the significant values (0.553, -0.553). Green and red scales show positive and negative coefficients, respectively.

closely followed by the release of the young microspores (Figure 5E), which developed into mature pollen grains before anthesis (Figure 5F).

The average dates of breaking of endodormancy, considering the first appearance of callose surrounding the microspore mother cells, were established in all the cultivars from 14th





January to 1st March (Table 1), although differences were observed between years for each cultivar (Supplementary Table 4). As an example, the onset of meiosis in “Pandora” ranged between January 12 and January 30 (Figure 6).

In all the cultivars, the end of the endodormancy took place earlier according to the forcing test than in PLS regression, correlation model, and male meiosis, respectively.

Chilling Requirements

Once the end of the endodormancy was established for each method, the CR was estimated by quantifying the CP accumulated until that date. The lowest values were found in PLS regression (30.9–76.7 CP) which showed a broader range than the other methods [forcing test (40.2–69.9 CP), correlation model (53.2–84.2 CP), and male meiosis (54.8–81.7 CP)]. The cultivars with higher CR (“Gönci Magyar,” “Stark Early Orange,” and “Stella”) were the same in all methods. However, the cultivars with lower CR showed some differences depending on the type of method, experimental or statistical. Both correlation and PLS methods shared a group of lower chilling cultivars (“Harcot,” “Mitger,” “Moniqui 1006,” “Moniqui 2113,” and “Pepito del Rubio”), in addition to “Muñoz” in the PLS method, and “Corbato” in the correlation method. Both experimental methods (forcing test and male meiosis) reported the same lower CR cultivars (“Canino,” “Goldrich,” and “Pepito del Rubio”) but higher values were observed in the male meiosis method.

The Bland–Altman analysis was used to look for potential bias between the four methods by plotting the difference in chill portions per cultivar against the mean in pairwise comparisons (Figure 7) and assessing their agreement level. Most of the

scatter points on the Bland–Altman plots were uniformly scattered between the limits of agreement in all the comparisons, suggesting the absence of bias. About 95% of cultivars would have a difference in chill portions, as measured by any two different methods, within the limits of agreement, suggesting good agreements. The 20 cultivars were classified into three groups according to their CR (Table 2).

Heat Requirements

The heat requirements of cultivars showed differences according to the method used. The highest values were obtained with the forcing test (2,866–5,936 GDH) whereas the PLS method showed the lowest values (3,448–5,468 GDH). In the correlation model, the heating requirements values were uniform between cultivars ranging from 4,022 to 4,953 GDH, except for “Tadeo,” which showed the lowest value (3,000 GDH) (Table 3). The values of heat requirements displayed higher coefficients of variability than those obtained for CR. The 20 cultivars were classified into three groups according to their heat requirements (Table 4).

DISCUSSION

In this work, we have validated male meiosis as a biomarker for the determination of dormancy breaking by comparing the dormancy breaking dates calculated with three other common methodologies in 20 apricot cultivars. The results support our initial hypothesis of male meiosis as a suitable biomarker for endo- to ecodormancy transition.

The AR has been determined in 20 apricot cultivars, for ten of which (“Berdejo,” “Henderson,” “Mitger,” “Muñoz,”

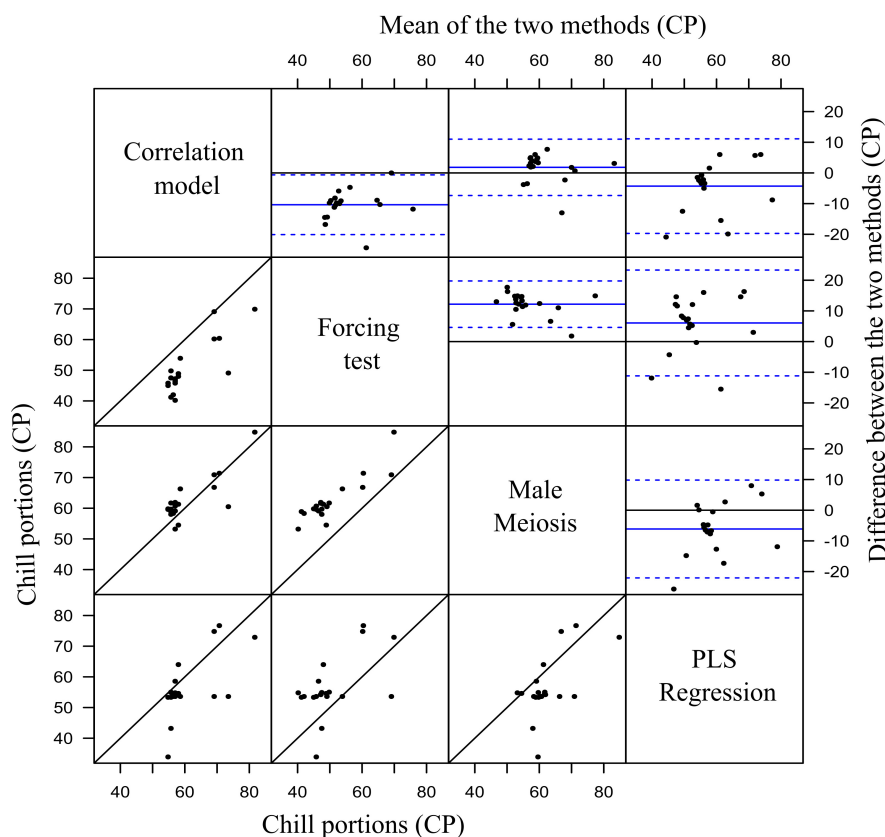


FIGURE 7 | Graphical methods for assessing variability and agreement in paired measurements of chill portion values obtained from four different methods. Scatter diagrams (lower-left grid) show the set of paired values in each pairwise comparison. Bland-Altman plots (upper-right grid) of the difference in chill portions of each value-pair against the mean of each value-pair. Blue horizontal lines show the mean difference (solid lines) and the superior and inferior limits of agreement (± 1.96 -SD), respectively (dotted lines).

“Pandora,” “Pepito del Rubio,” “Stella,” “Sun Glo,” “Tadeo,” and “Veecot”) no previous data were available. This is the first time that a biomarker (male meiosis) and two statistical methods (statistical correlation and PLS regression) have been used to estimate chilling and heat requirements in apricot since all the previous data have been obtained with the experimental method of transferring shoots into a growth chamber sequentially during winter.

The CR reported herein ranged from 30.9 to 84.8 CP (Dynamic model), 470–1,681 CU (Utah model), and 410–1,437 CH (Weinberger model), which are in line with the results previously reported in this species (reviewed in Fadón et al., 2020a). Our estimate of the CR for “Canino” (875.4

CH vs. 750–878 CH), “Luizet” (1,016.3 CH vs. 1,058–1,150 CH), “Moniqui” (983.6 and 1,036.4 CH vs., 799–1,057 CH), and “Paviot” (1,023.7 CH vs. 995–1,296 CH) by using male meiosis as a biomarker of endodormancy breaking was similar to what has been previously determined at the same location (Tabuenca, 1964, 1968, 1979) by using the forcing test (**Supplementary Table 4**). Three cultivars, “Canino,” “Harcot,” and “Goldrich” were previously studied in different regions, Tuscany (Italy) (García et al., 1999; Viti et al., 2010) and South Africa (Campoy et al., 2012a) compared to Murcia (Spain). Although significant differences were reported between locations, our results under forcing conditions were similar to those reported in Murcia (Spain). In addition, our results showed similar CR values for some cultivars evaluated with the forcing test previously in other regions, such as “Moniqui” in Italy (921.7 and 1,003.1 CU vs. 930–1,140 CU; Guerriero et al., 2006), “Gönci Magyar” in Serbia (1,200.8 CU vs. 1,122–1,310 CU; Ruml et al., 2018), and “Goldrich” in Italy and Serbia (854.0 CU vs. 950–1,030 CU and 834–846 CU, respectively; Guerriero et al., 2006; Ruml et al., 2018; **Supplementary Table 1**).

The four methods showed the same group of cultivars with higher CR, and similar values were obtained between methods for each cultivar. However, some differences between methods

TABLE 2 | Classification of 20 apricot cultivars according to their chilling requirements (Chill portions) determined by the male meiosis method.

High chilling requirements (≥ 66 CP)	Henderson, Gönci Magyar, Stark Early Orange, Stella, Sun Glo
Medium chilling requirements (56–65 CP)	Berdejo, Corbato, Goldrich, Harcot, Luizet, Mitger, Moniqui 1006, Moniqui 2113, Muñoz, Pandora, Paviot, Pepito del Rubio, Tadeo
Low-medium chilling requirements (50–55 CP)	Canino, Veecot

TABLE 3 | Heat requirements from the end of endodormancy to full flowering of 20 apricot cultivars determined according to four methods (forcing test, correlation model, PLS, and male meiosis methods).

	Heat requirements (Growing Degree Hours)							
	Forcing test		Correlation model		PLS regression		Male meiosis	
	Value $\pm$ SD	cv	Value $\pm$ SD	cv	Value $\pm$ SD	cv	Value $\pm$ SD	cv
Berdejo	5,278 $\pm$ 706	13.4	4,917 $\pm$ 882	17.9	3,905 $\pm$ 948	24.3	4,170 $\pm$ 498	11.9
Canino	5,026 $\pm$ 906	18.0	4,023 $\pm$ 784	19.5	3,634 $\pm$ 905	24.9	4,212 $\pm$ 765	18.2
Corbato	4,814 $\pm$ 263	5.5	4,520 $\pm$ 864	19.1	3,640 $\pm$ 870	23.9	3,900 $\pm$ 440	11.3
Goldrich	4,749 $\pm$ 1,146	24.1	4,086 $\pm$ 791	19.4	4,004 $\pm$ 976	24.3	3,712 $\pm$ 343	9.2
Gönci Magyar	4,288 $\pm$ 78	1.8	4,861 $\pm$ 887	18.2	3,448 $\pm$ 779	22.6	4,027 $\pm$ 513	12.7
Harcot	4,767 $\pm$ 470	9.9	4,689 $\pm$ 873	18.6	4,209 $\pm$ 937	22.3	3,998 $\pm$ 402	10.1
Henderson	2,866 $\pm$ 666	23.2	4,441 $\pm$ 837	18.8	4,604 $\pm$ 1,083	23.5	4,159 $\pm$ 968	23.3
Luizet	5,076 $\pm$ 407	8.0	4,732 $\pm$ 858	18.1	4,004 $\pm$ 906	22.6	4,120 $\pm$ 437	10.6
Mitger	4,777 $\pm$ 599	12.5	4,638 $\pm$ 860	18.5	5,326 $\pm$ 951	17.8	4,084 $\pm$ 467	11.4
Moniqui 1006	4,921 $\pm$ 399	8.1	4,821 $\pm$ 882	18.3	4,811 $\pm$ 921	19.1	4,045 $\pm$ 241	6.0
Moniqui 2113	5,144 $\pm$ 538	10.5	4,902 $\pm$ 884	18.0	5,305 $\pm$ 953	18.0	4,196 $\pm$ 542	12.9
Muñoz	5,170 $\pm$ 799	15.5	4,650 $\pm$ 845	18.2	3,934 $\pm$ 881	22.4	4,185 $\pm$ 527	12.6
Pandora	4,924 $\pm$ 993	20.2	4,141 $\pm$ 792	19.1	3,723 $\pm$ 926	24.9	3,863 $\pm$ 498	12.9
Paviot	5,119 $\pm$ 551	10.8	4,781 $\pm$ 860	18.0	3,721 $\pm$ 886	23.8	4,135 $\pm$ 411	9.9
Pepito del Rubio	5,296 $\pm$ 646	12.2	4,638 $\pm$ 860	18.5	3,852 $\pm$ 894	23.2	4,019 $\pm$ 312	7.8
Stark Early Orange	4,152 $\pm$ 76	1.8	4,123 $\pm$ 758	18.4	3,607 $\pm$ 819	22.7	4,053 $\pm$ 1,220	30.1
Stella	5,936 $\pm$ 191	3.2	4,511 $\pm$ 638	14.1	5,468 $\pm$ 847	15.5	3,879 $\pm$ 746	19.2
Sun Glo	4,915 $\pm$ 733	14.9	4,953 $\pm$ 944	19.1	5,054 $\pm$ 1,174	23.2	3,789 $\pm$ 472	12.5
Tadeo	4,742 $\pm$ 239	5.1	3,000 $\pm$ 514	17.1	4,604 $\pm$ 1,083	23.5	4,126 $\pm$ 576	14.0
Veecot	4,564 $\pm$ 700	15.3	4,091 $\pm$ 789	19.3	4,660 $\pm$ 1,093	23.5	4,284 $\pm$ 898	21.0

Values are in growing degree hours.

SD, standard deviation; cv, coefficient of variation.

were found for the cultivars with lower CR, “Canino,” “Golrich,” and “Pepito del Rubio” in both experimental methods, and “Harcot,” “Mitger,” “Moniqui 1006,” “Moniqui 2113,” and “Pepito del Rubio” in both statistical methods. Differences between experimental and statistical methods could be due to the different number of years used, 11–18 for statistical methods and 3–8 for experimental methods. Previous studies in apricot using the forcing test have reported differences in CR not only between years, but also between locations, which may be due to a small number of years of experiments (Garcia et al., 1999; Viti et al., 2010; Campoy et al., 2012a).

The classification of the cultivars obtained according to their CR was similar to that obtained by other authors (Ruiz et al., 2007; Campoy et al., 2012a). “Canino” has been considered a medium chill cultivar in previous works (Campoy et al., 2012a), whereas our results showed lower chill portions accumulation in “Canino” and “Veecot” than those in the other cultivars. Thus, we propose an intermediate group that includes cultivars with low-medium CR.

Differences in CR between methods affected heating requirements, showing a significant negative correlation as reported in previous work (Ruiz et al., 2007). Lower values of heat accumulation were found with the male meiosis method whereas higher values were obtained with the forcing test. The heat requirements reported for “Canino” (5,026 GDH) and “Harcot” (4,767 GDH) are in line with those reported in

previous studies in Spain [5,418–6,029 GDH (Garcia et al., 1999; Campoy et al., 2012a)]. Our results showed higher heat accumulation for “Goldrich,” “Moniqui,” and “Gönci Magyar” than in other countries. As an example, for “Goldrich,” 4,479 GDH vs. 3,175–3,432 GDH in Serbia (Ruml et al., 2018) and 3,950 or 485–913 GDH in Italy (Guerriero et al., 2006; Viti et al., 2010).

Differences between methods as well as those in previous reports may also be due to the specific limitations of each method. The determination of endodormancy breaking by monitoring shoots under forcing conditions is the only experimental approach currently available and the most widely used to estimate the AR of fruit tree cultivars for more than 60 years (Brown and Kotob, 1957; Fadón et al., 2020a). However, the variability of the factors affecting the experimental design has often led to inconsistencies in the results previously obtained. In apricot, as in other species, the frequency of sampling of shoots during winter may vary between studies, as well as the temperature and photoperiod conditions in the growth chamber (Tabuenca, 1968, 1979; Andrés and Durán, 1999; Valentini et al., 2004; Ruiz et al., 2007; Bartolini et al., 2020). Furthermore, the determination of the endodormancy breaking date through the evaluation of bud growth can be carried out after different periods in the chamber. Several criteria are also used, such as significant increases in fresh (Brown and Kotob, 1957) or dry weight (Tabuenca, 1968), as well as changes in bud phenology

TABLE 4 | Classification of 20 apricot cultivars according to their heat requirements (growing degree hours) determined by the male meiosis method.

High heating requirements ($\geq 4,101$ GDH)	Berdejo, Canino, Henderson, Luizet, Moniquei 2113, Muñoz, Paviot, Tadeo, Veecot
Medium heating requirements (3,901–4,100 GDH)	Gönci Magyar, Harcot, Mitger, Moniquei 1006, Pepito del Rubio, Stark Early Orange
Low heating requirements ($\leq 3,900$ GDH)	Goldrich, Corbato, Pandora, Stella, Sun Glo

(Bennett, 1949). Moreover, most studies evaluate flower buds, but some use vegetative buds (Fernandez et al., 2019). The lack of standardization has made most of the results not applicable to other regions with different climatic conditions (Campoy et al., 2019; Fadón et al., 2020a).

The statistical approaches estimate the date of chilling fulfillment based on a long series of phenological observations. Both correlations of winter temperatures with flowering dates and the PLS analysis have been widely used for the determination of AR in sweet cherry (Luedeling et al., 2013; Fadón et al., 2021), almond (Alonso et al., 2005; Benmoussa et al., 2017a; Díez-Palet et al., 2019), pistachio (Benmoussa et al., 2017b), and apple (Díez-Palet et al., 2019). In our work, results from both statistical methods have shown results similar to those from experimental methods. However, some works indicate the need for about 20 years of flowering date records to obtain consistent results (Luedeling and Gassner, 2012; Luedeling et al., 2013; Fadón et al., 2021), which is one of the main pitfalls of these methods since there are usually not so many years of data for many cultivars, especially for new releases from breeding programs (Fadón et al., 2020a). In addition, these approaches can estimate average chill and heat periods, but cannot provide information about dormancy dynamics in a particular year.

The limitations of the currently available methods, the increasing number of new releases in many fruit tree crops, as well as the decrease in winter chilling due to global warming have led to a number of recent research efforts focused on identifying suitable biomarkers to determine the transition from endo- to ecodormancy (Martínez-Gómez et al., 2017; Lloret et al., 2018). These include starch accumulation within the ovary primordia cells (Fadón et al., 2018a) and hormone regulation (Chmielewski et al., 2017; Vimont et al., 2019; Guillamón et al., 2020), as well as the expression of the *DORMANCY-ASSOCIATED MAD-BOX* (DAM) and other candidate genes in several *Prunus* species (Bielenberg et al., 2004; Sánchez-Pérez et al., 2014; Rothkegel et al., 2017; Prudencio et al., 2018, 2019; Balogh et al., 2019; Falavigna et al., 2019; Vimont et al., 2019; Quesada-Traver et al., 2020; Lloret et al., 2021). To deepen insights into the genetics of dormancy, transcriptomic studies have been performed by using flower buds at different dormant stages in apricot (Yu et al., 2020; Canton et al., 2021), sweet cherry (Vimont et al., 2019; Canton et al., 2021), almond (Prudencio et al., 2020), or peach (Yu et al., 2020; Canton et al., 2021). Genes involved in stress response, sugar metabolism, and cell wall assembly contribute

to the endodormancy to ecodormancy transition in apricot. Hormone biosynthesis, including ABA catabolism and secondary metabolism, as well as floral and pollen development genes are relevant at ecodormancy (Yu et al., 2020). Sporophytic genes, such as *MALE STERILE1* (*MS1*), *ABORTED MICROSPORES* (*AMS*), and *MYB103* (Canton et al., 2021), as well as other genes involved in sporopollenin synthesis and deposition found in peach (Ríos et al., 2013), could be useful markers of the ecodormancy stage.

In apricot, the characterization of the phases of pollen development in relation to dormancy established that male meiosis is the first detectable sign of development after endodormancy breaking (Julian et al., 2011), when flower buds are still closed with no observable external phenological changes (Fadón et al., 2018b). This led us to use male meiosis as a biomarker to estimate the AR of cultivars for the first time by using the emergence of callose around the microspore mother cells as a boundary between endo- and ecodormancy. Callose deposition can be considered as a physical filter of molecules that have been reported as a response to several stresses (Chen and Kim, 2009) or degeneration processes, such as ovule abortion (Rodrigo and Herrero, 1998). During pollen development, the accumulation of a layer of callose around the microspore mother cells could provide insulation and possible protection against low winter temperatures.

Since the statistical analyses showed good agreement when male meiosis was compared to the other methods, these results strongly endorse the use of male meiosis as a new and alternative method to determine endodormancy release in apricot. The results obtained by this approach showed slightly higher values than those obtained by the other methods because male meiosis occurs in the early stages of ecodormancy, once the endodormancy breaking had already passed. Despite this limitation, this method has an important advantage since it allowed the determination of endo- and ecodormancy in a short time from samples collected directly from the field, avoiding the variability of results caused by the many factors that may affect the experiments under forced conditions (Dennis, 2003; Fadón and Rodrigo, 2018) and without the need to have a large phenological data set available (Luedeling and Gassner, 2012; Fadón et al., 2020a). Despite the important advantages of this method in estimating CR in apricot, endodormancy breaking and male meiosis are not as closely related processes in other species as sweet cherry, in which several months elapse between both processes (Fadón et al., 2019) and this could lead to overestimated calculations of AR (Fadón et al., 2021). This may be because sweet cherry requires a longer forcing period to flower (Fadón et al., 2020b). However, male meiosis as a biomarker of endodormancy could be an applicable method in those species with a shorter forcing phase in which flowering is more conditioned by the chilling phase, such as almond (Egea et al., 2003) or Japanese plum (Ruiz et al., 2018). Although further studies are needed to determine if this method is valid in other species, our results exhibit male meiosis as a valid approach for apricot, not only for determining AR but also

as a basis for physiological and genetic studies of dormancy. In addition, whether a bud is an endo- or ecodormant can be determined in a short time from a sample taken directly from the tree in field conditions. The knowledge of the endo- to ecodormancy transition may provide valuable information to improve and simplify orchard management decisions. The progressive loss of cold hardiness is triggered once the buds have fulfilled their CR (Weiser, 1970a,b) but show no external signs of development (Julian et al., 2007; Hillmann et al., 2021). Determining endodormancy breaking in apricot through the detection of male meiosis could provide insights into whether flower buds are vulnerable to spring frosts, allowing agronomic frost protection decisions to be made. In addition, the date of male meiosis could allow the determination of the optimal application time of agrochemical treatments to advance flowering (Guillamón et al., 2022).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

SH, JH, JL, and JR conceived the study and designed the experiments. SH collected the shoot and bud samples and performed the microscopic analysis. SH and JA performed the correlation analysis. SH and EF performed the PLS analysis. SH and AH performed the statistical analysis. SH, JL, EF, AH, JA, JH, and JR analyzed the results and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.842333/full#supplementary-material>

Supplementary Figure 1 | Establishment of endodormancy breaking date (T) in 20 apricot cultivars by analyzing the evolution of the Pearson correlation coefficients between full flowering dates and winter temperatures of periods of 15 days. Discontinuous lines delimit the significant values (0.553, –0.553). Green and red scales show positive and negative coefficients, respectively.

Supplementary Figure 2 | Model coefficients of Partial Least Squares regression between accumulation rates of agroclimatic metrics (Chill Portions according to the Dynamic Model and Growing Degree Hours) and bloom dates of 20 apricot cultivars. Top panels show the Variable importance in the projection (VIP), with the blue bars values above 0.8 indicating the threshold for variable importance. Middle panels show the model coefficients of the centered and scaled data. Chilling period is colored in blue and heating period is colored in red. Bottom panels show mean temperatures (black line) and their standard deviation (grey areas). In middle and bottom panels, red and green scales show negative and positive coefficients, respectively.

Supplementary Table 1 | Chilling and heat requirements of 20 apricot cultivars determined by the Forcing test. Dates of endodormancy and full flowering, yearly values of chill accumulation expressed in chill portions, chill units, and chill hours below 7°C, yearly values of heat accumulation expressed in Growing Degree Hours (GDH), mean values, standard deviation (SD), and coefficient of variation (cv).

Supplementary Table 2 | Chilling and heat requirements of 20 apricot cultivars determined by the Correlation method. Dates of endodormancy and full flowering, values of chill accumulation expressed in chill portions, chill units, and hours below 7°C, values of heat accumulation expressed in Growing Degree Hours (GDH), standard deviation (SD), and coefficient of variation (cv).

Supplementary Table 3 | Chilling and heating requirements of 20 apricot cultivars determined by the PLS regression method. Chilling and forcing periods are established with Partial Least Squares regression based on the Dynamic Model and the Growing Degree Hours Model. Chill accumulation is expressed in chill portions, chill units, and hours below 7°C, values of heat accumulation are expressed in Growing Degree Hours (GDH), standard deviation (SD), and coefficient of variation (cv).

Supplementary Table 4 | Chilling and heating requirements of 20 apricot cultivars determined by the Male meiosis method. Dates of endodormancy and full flowering, yearly values of chill accumulation expressed in chill portions, chill units, and hours below 7°C, yearly values of heat accumulation expressed in Growing Degree Hours (GDH), mean values, standard deviation (SD), and coefficient of variation (cv).

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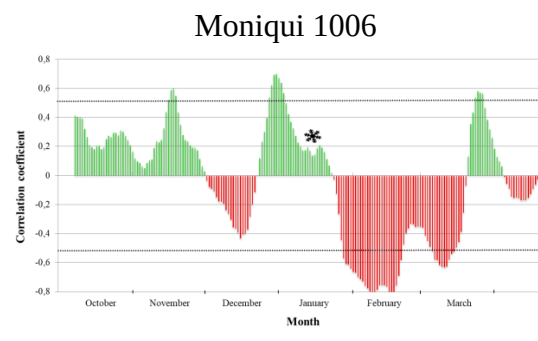
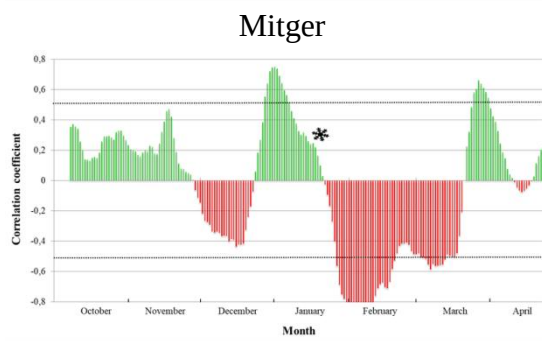
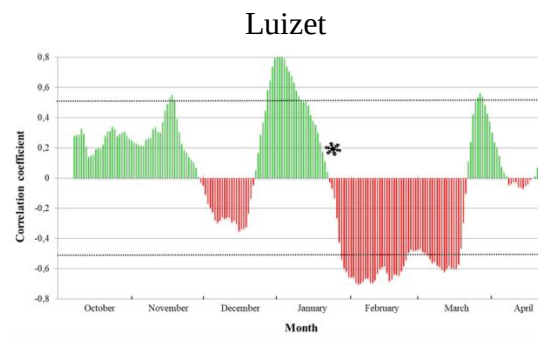
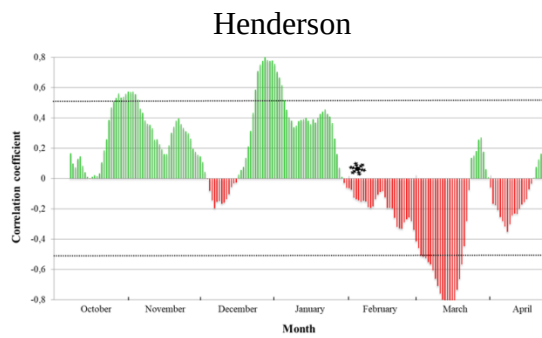
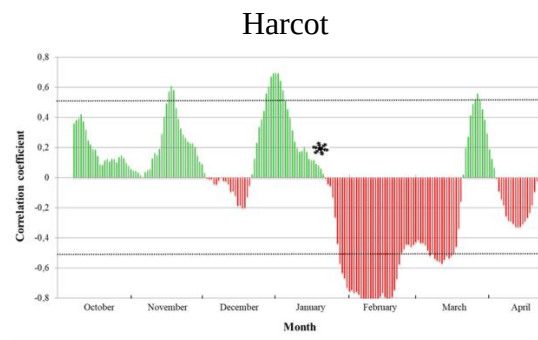
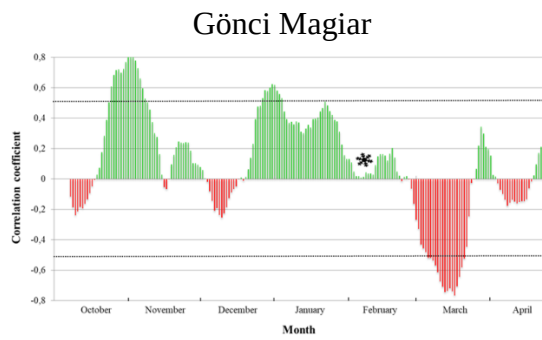
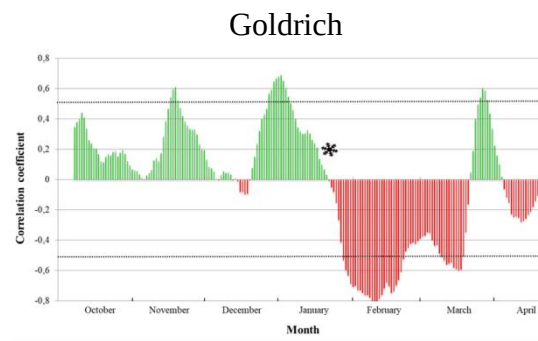
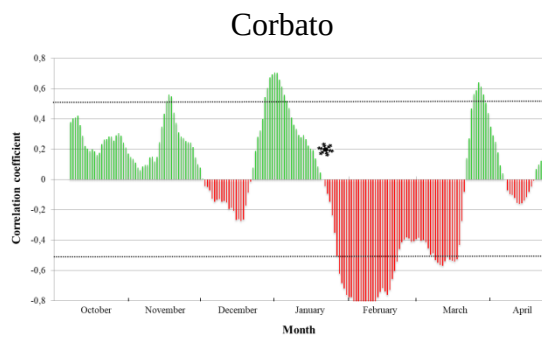
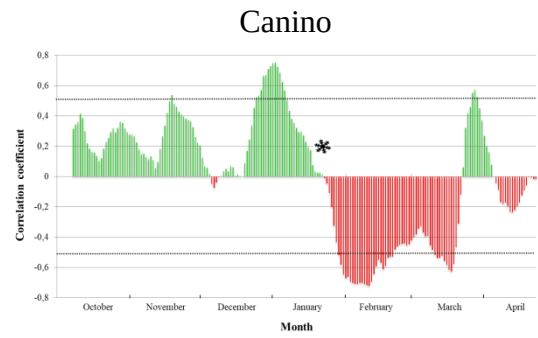
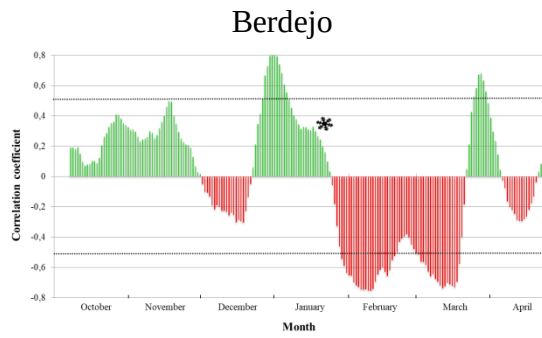
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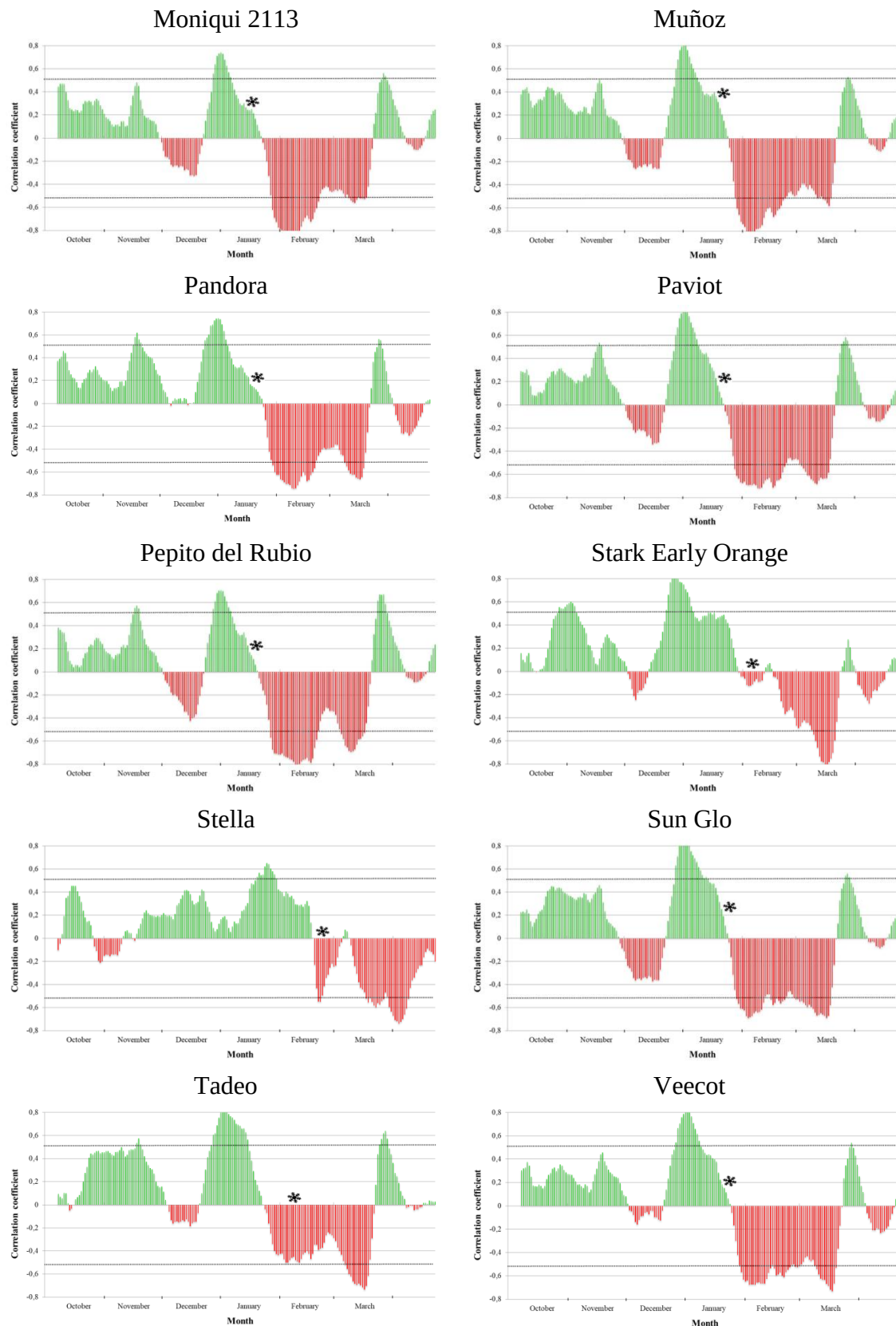
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MATERIAL SUPLEMENTARIO. Male Meiosis as a Biomarker for Endo- to Ecodormancy Transition in Apricot

El material suplementario puede ser encontrado y descargado en:

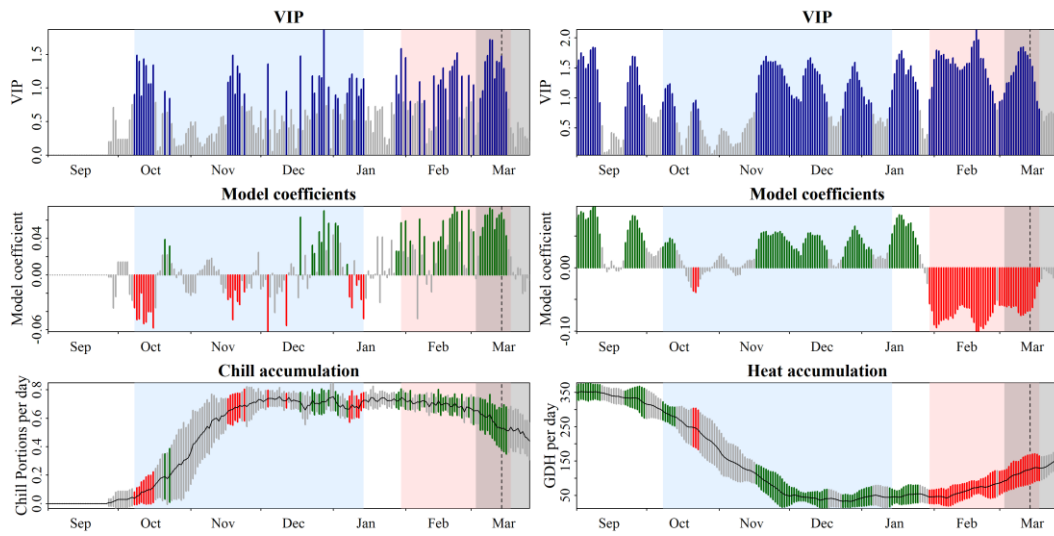
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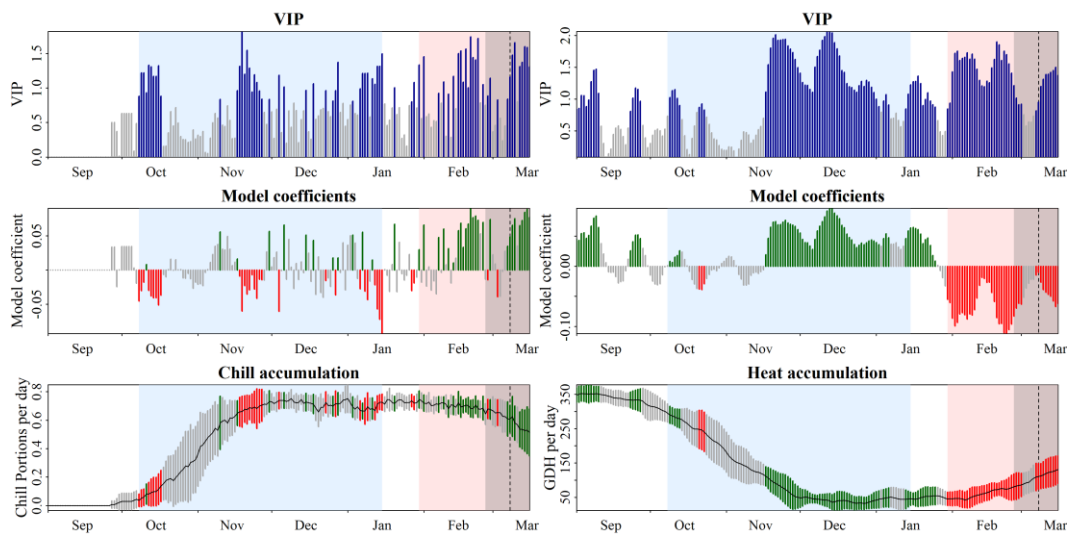


Supplementary Figure 1. Establishment of endodormancy breaking date (T) in 20 apricot cultivars by analyzing the evolution of the Pearson correlation coefficients between full flowering dates and winter temperatures of periods of 15 days. Discontinuous lines delimit the significant values (0.553, -0.553). Green and red scales show positive and negative coefficients, respectively.

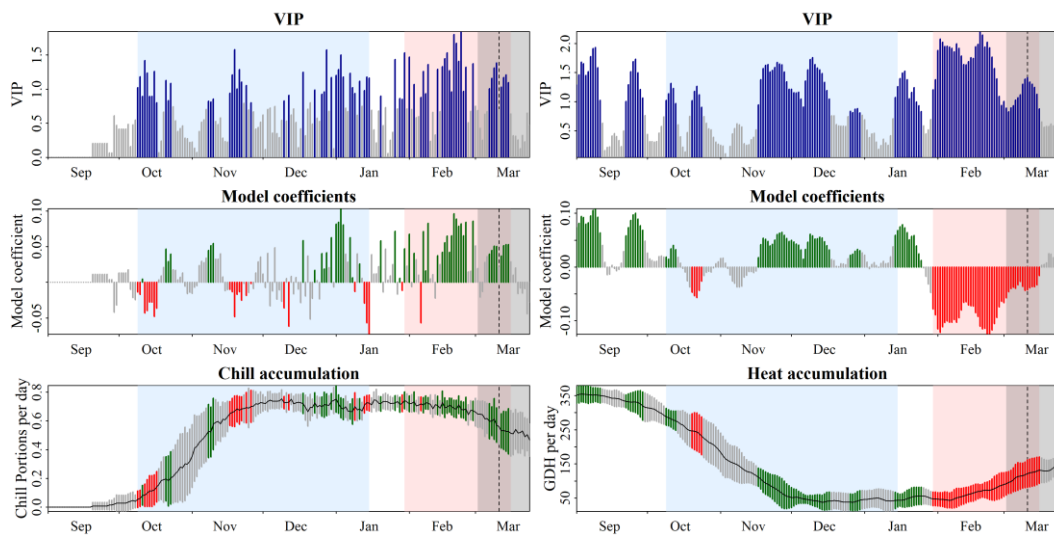
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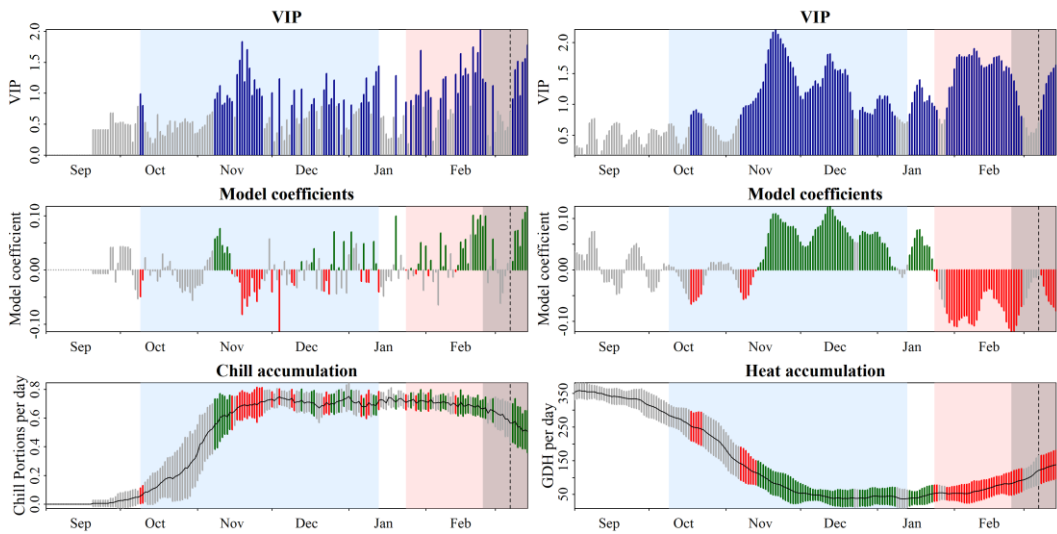
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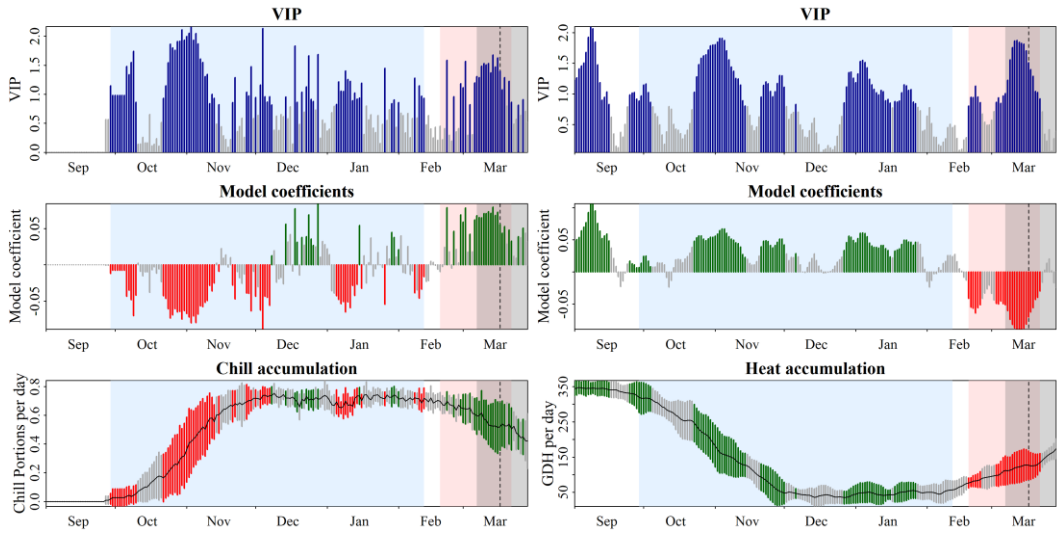
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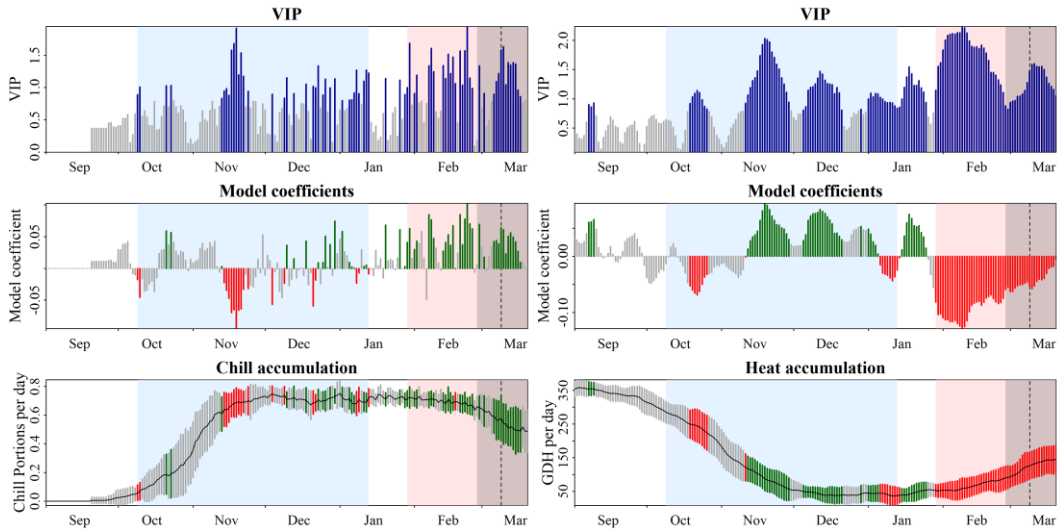
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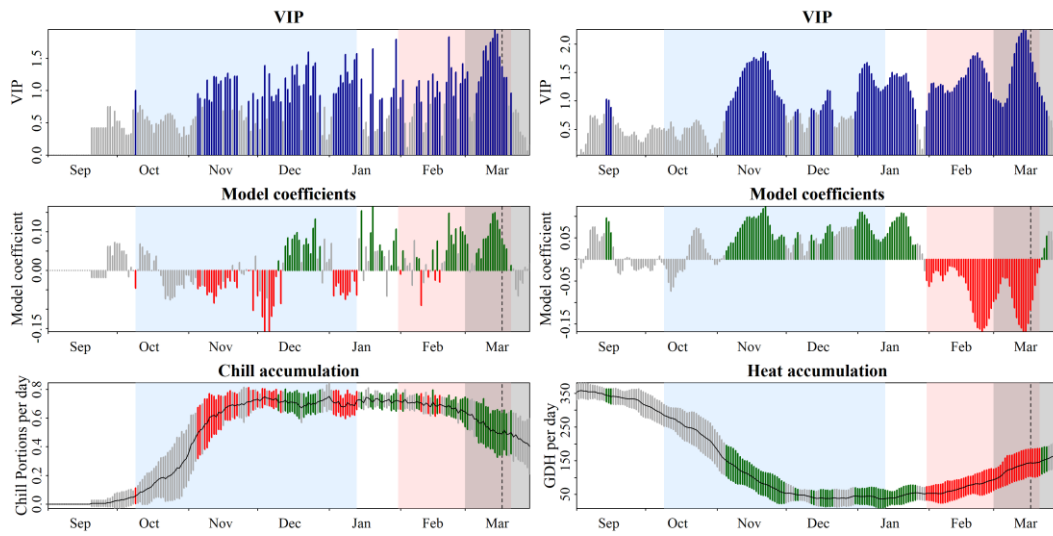
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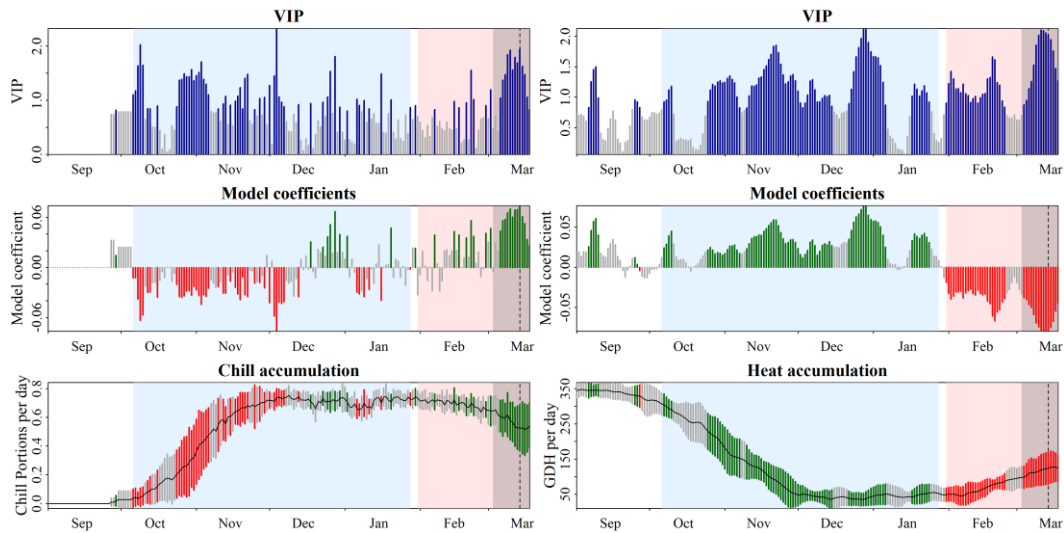
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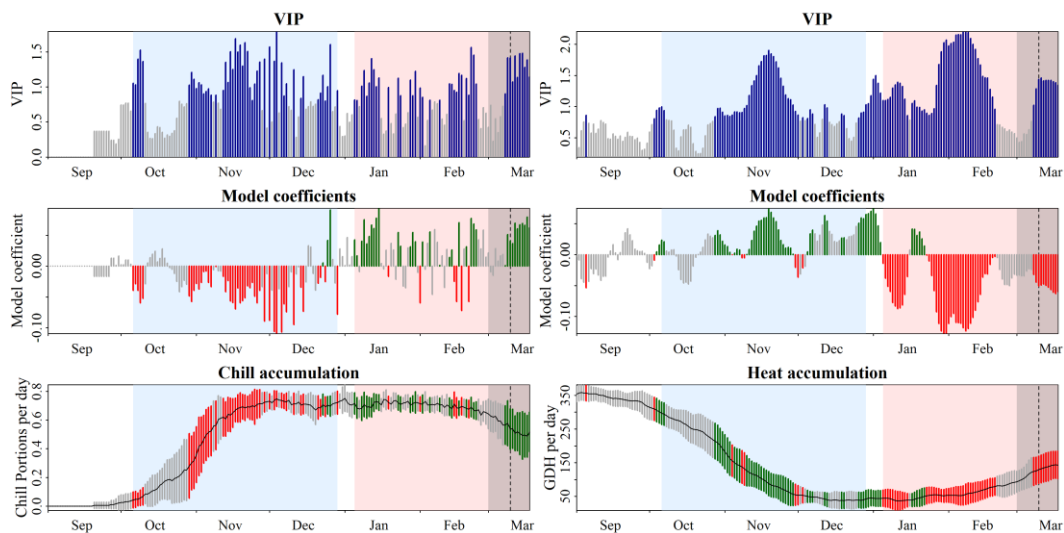
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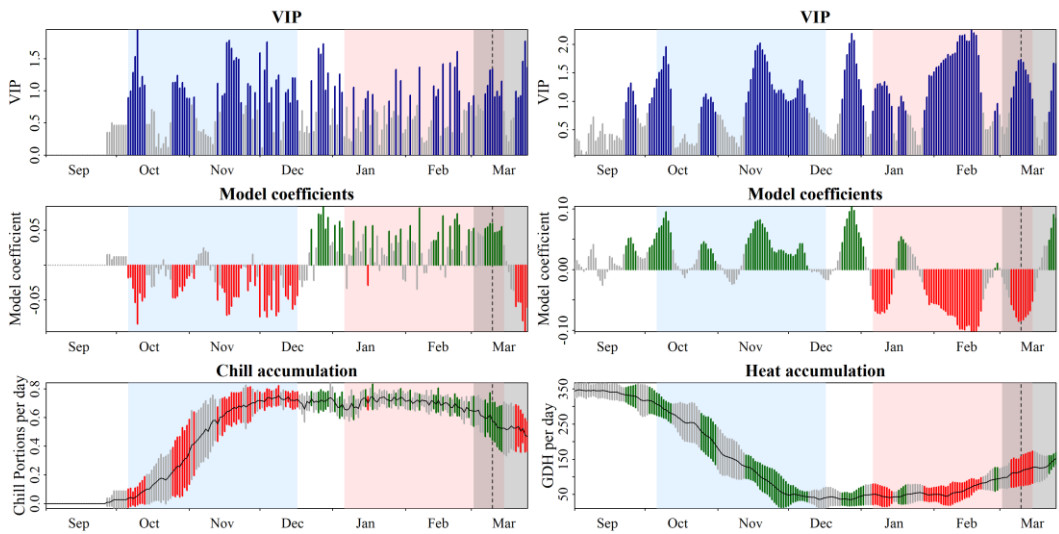
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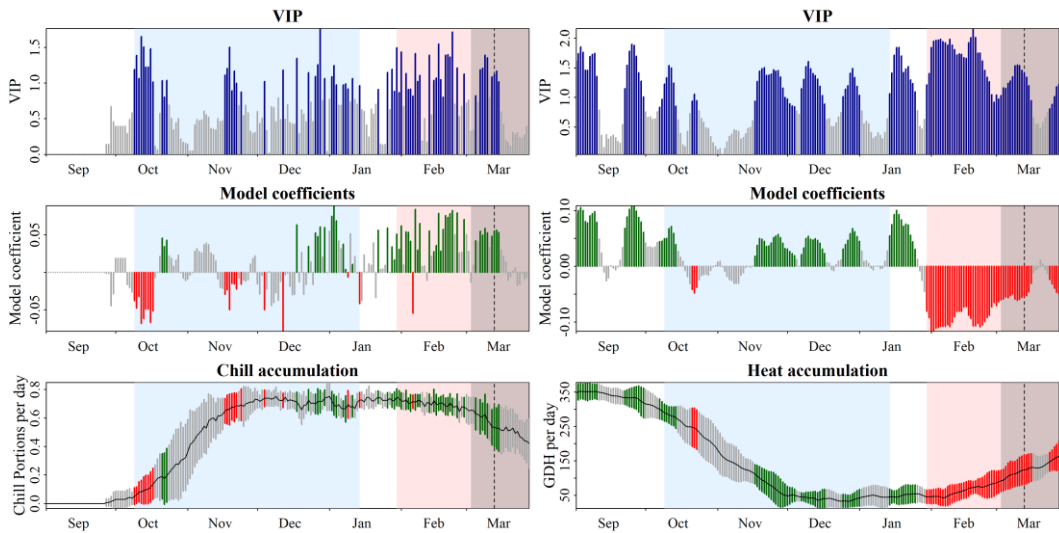
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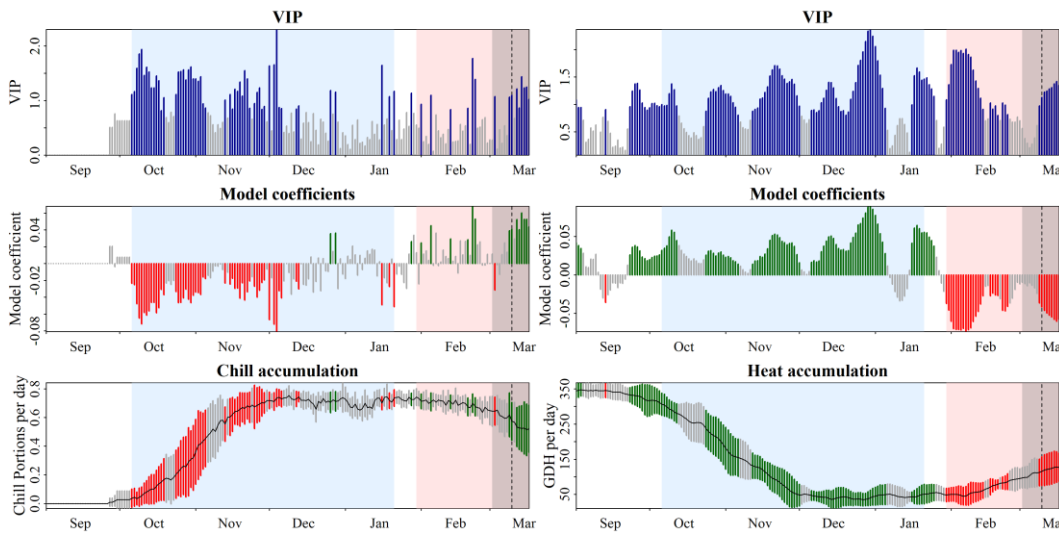
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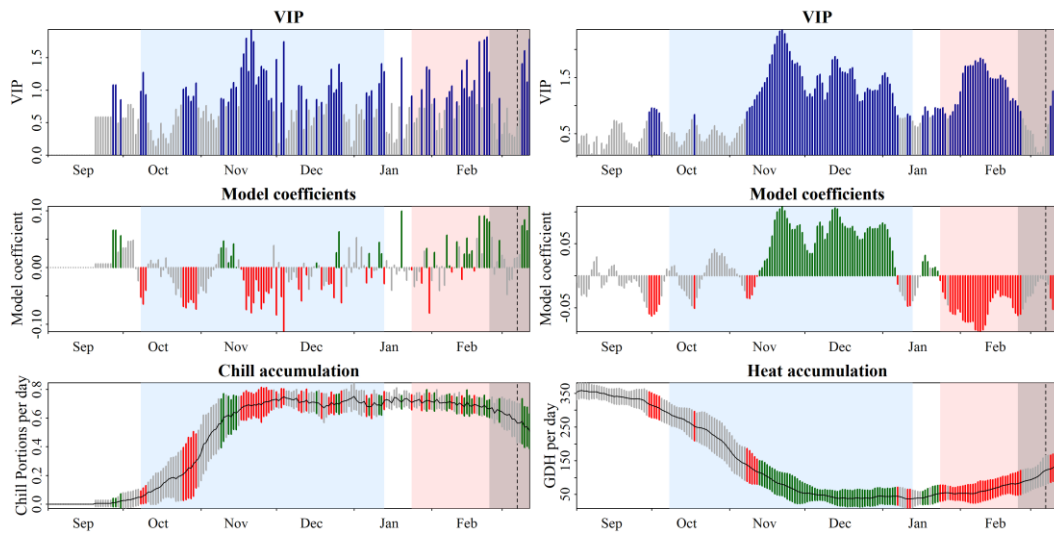
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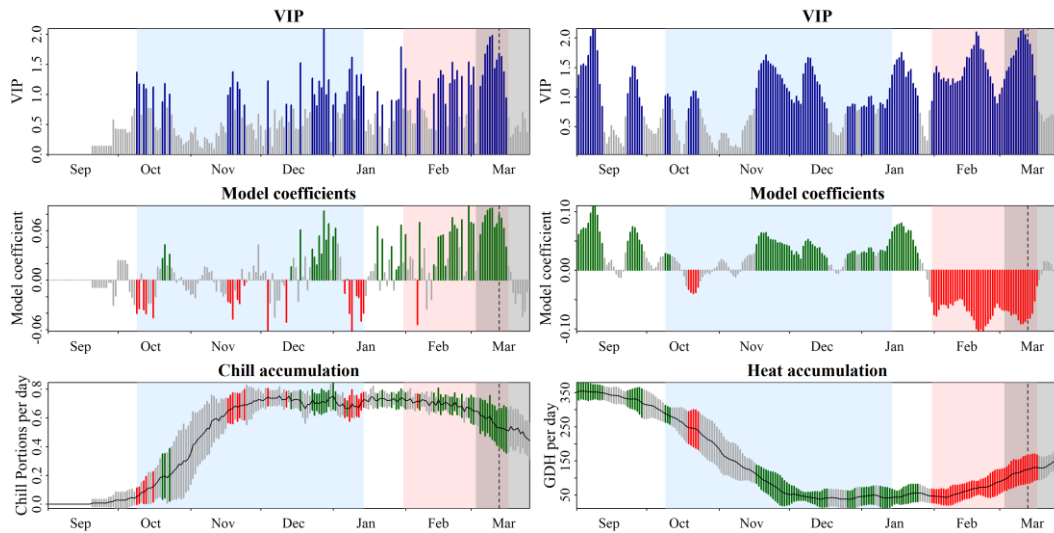
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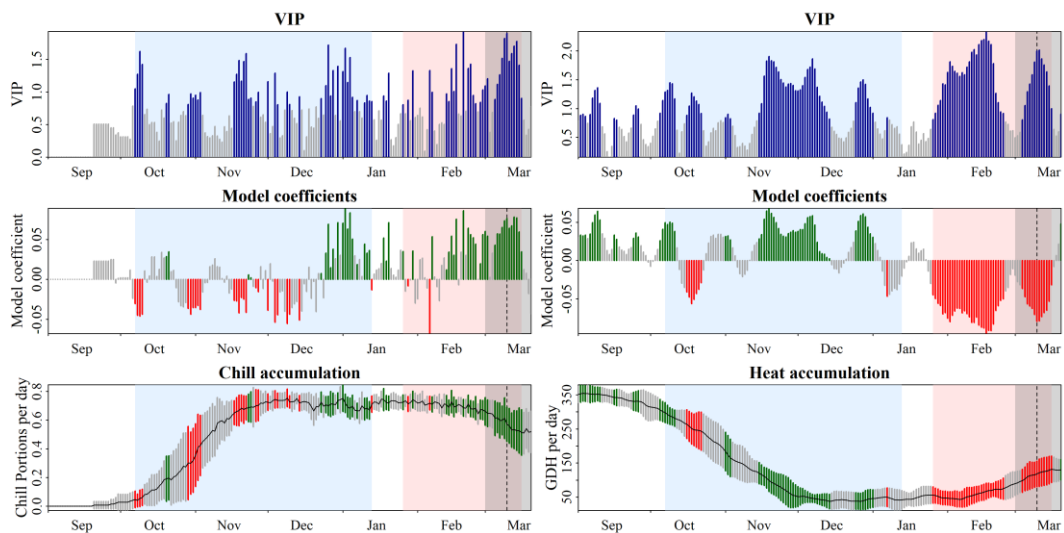
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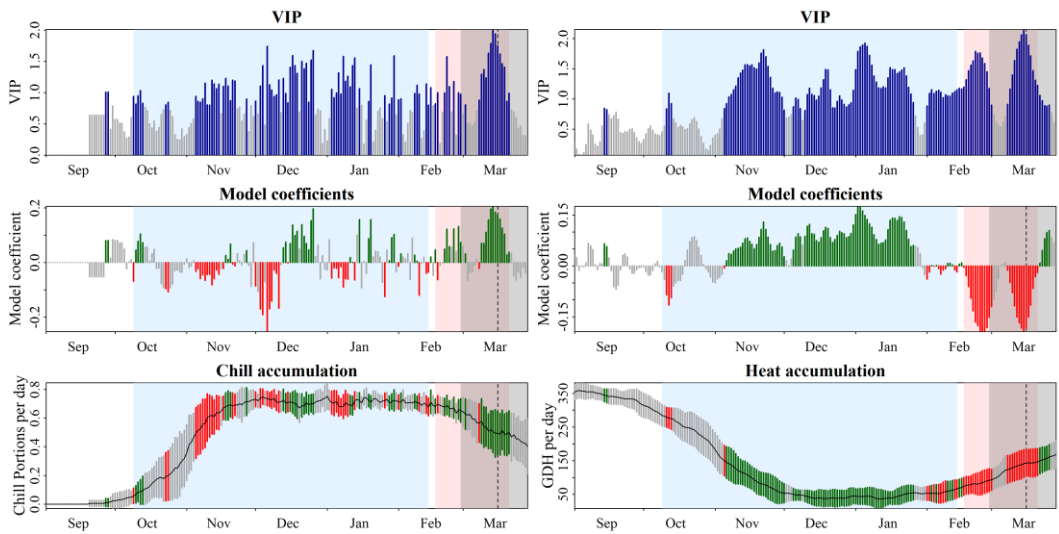
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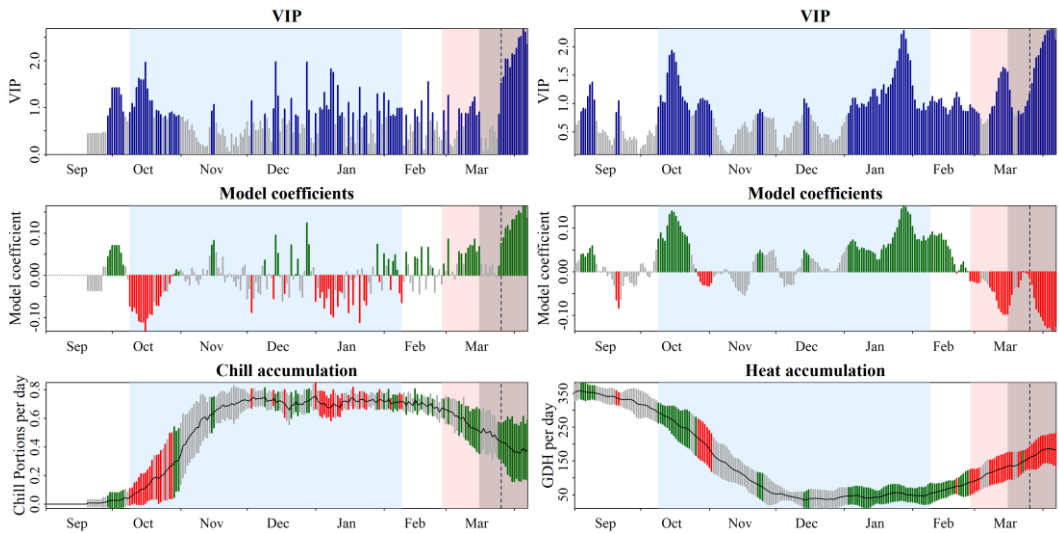
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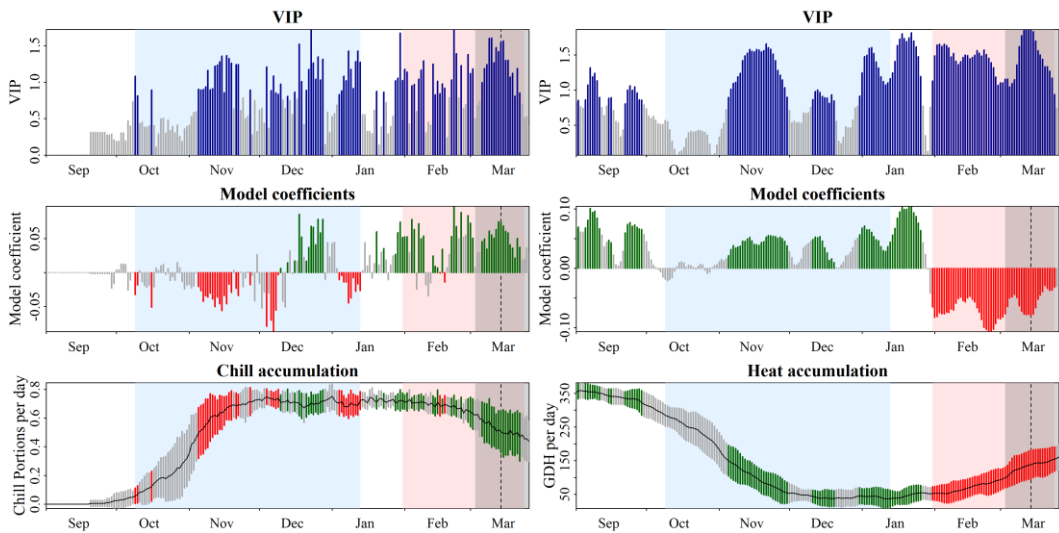
Stark Early Orange



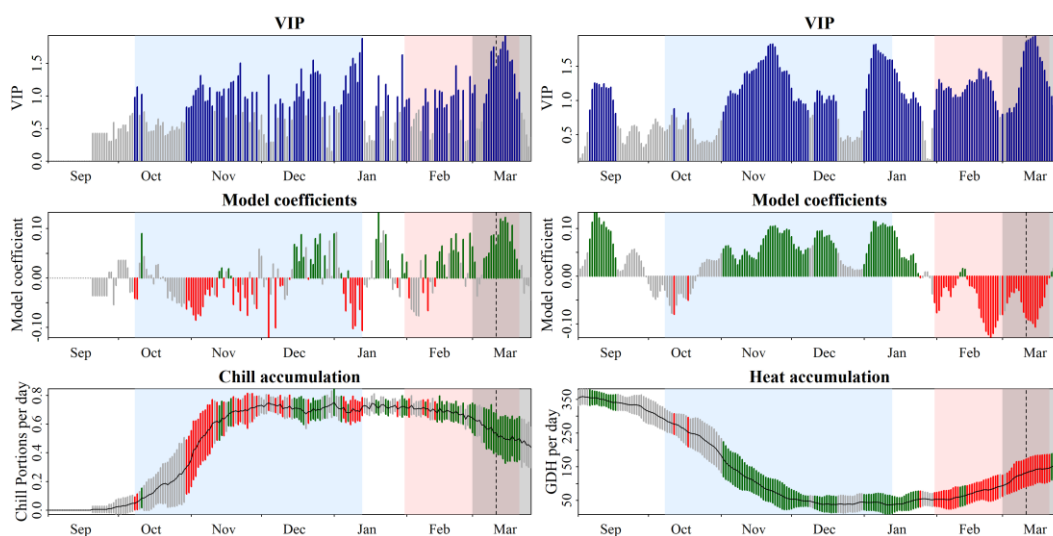
Stella



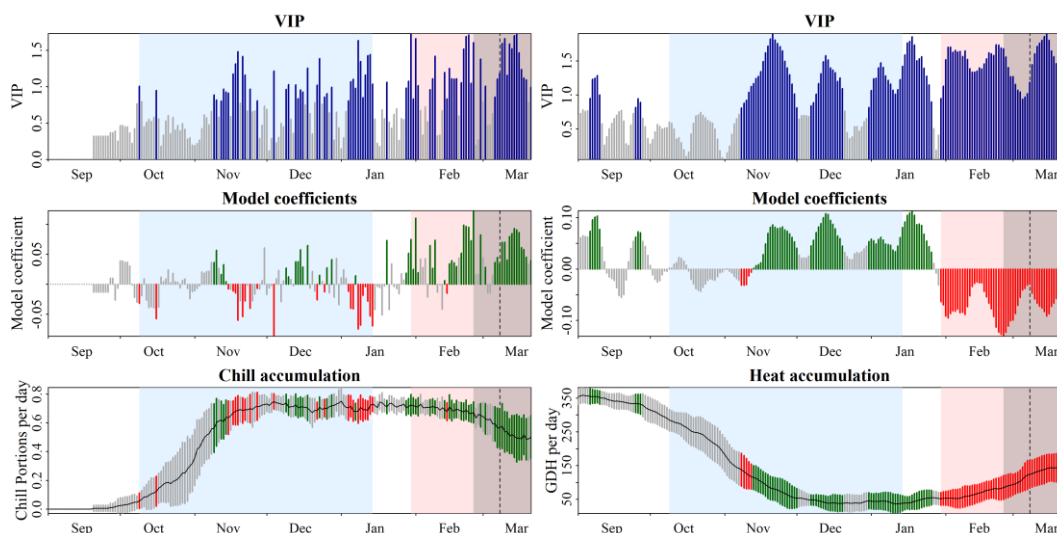
Sun Glo



Tadeo



Veecot



Supplementary Figure 2. Model coefficients of Partial Least Squares regression between accumulation rates of agroclimatic metrics (Chill Portions according to the Dynamic Model and Growing Degree Hours) and bloom dates of 20 apricot cultivars. Top panels show the Variable importance in the projection (VIP), with the blue bars values above 0.8 indicating the threshold for variable importance. Middle panels show the model coefficients of the centered and scaled data. Chilling period is colored in blue and heating period is colored in red. Bottom panels show mean temperatures (black line) and their standard deviation (grey areas). In middle and bottom panels, red and green scales show negative and positive coefficients, respectively.

Supplementary Table 1. Chilling and heat requirements of 20 apricot cultivars determined by the Forcing test. Dates of endodormancy and full flowering, yearly values of chill accumulation expressed in chill portions, chill units, and chill hours below 7 °C, yearly values of heat accumulation expressed in Growing Degree Hours (GDH), mean values, standard deviation (SD), and coefficient of variation (cv).

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Berdejo	2017	02 January	49.1	47.2	4.5	9.6	1093	953.8	175.4	18.4	873	826.5	49.0	5.9	03 March	5096	5277.8	706.0	13.4
	2018	05 January	42.1				757				775				17 March	6057			
	2020	12 January	50.6				1012				832				27 February	4681			
Canino	2017	25 December	44.2	40.2	6.1	15.1	973	815.3	200.1	24.5	684	635.3	43.1	6.8	26 February	4417	5025.5	905.6	18.0
	2018	22 December	33.2				590				601				08 March	6066			
	2020	02 January	43.2				883				622				25 February	4594			
Corbato	2017	02 January	49.1	47.5	1.5	3.1	1093	959.7	117.6	12.3	873	826.5	104.4	12.6	02 March	4944	4813.9	262.8	5.5
	2018	12 January	47.1				871				900				11 March	4987			
	2020	06 January	46.2				915				707				25 February	4512			
Goldrich	2017	29 December	46.7	42.0	7.7	18.2	1057	854.0	239.3	28.0	777	694.7	88.7	12.8	24 February	4203	4749.1	1146.2	24.1
	2018	22 December	33.2				590				601				08 March	6066			
	2020	06 January	46.2				915				707				20 February	3978			
Gönci Magyar	2017	25 January	65.6	60.2	5.1	8.4	1397	1200.8	171.3	14.3	1211	1092.8	122.5	11.2	07 March	4343	4287.8	77.6	1.8
	2018	30 January	59.5				1123				1102				21 March	4233			
	2020	19 January	55.5				1082				966				-	-			
Harcot	2017	29 December	46.7	45.0	2.5	5.6	1057	909.6	150.1	16.5	777	752.8	39.7	5.3	27 February	4480	4766.8	470.0	9.9
	2018	05 January	42.1				757				775				11 March	5309			
	2020	06 January	46.2				915				707				25 February	4512			
Henderson	2017	29 January	69.3	69.1	5.2	7.6	1457	1397.5	105.0	7.5	1262	1252.3	152.5	12.2	03 March	3522	2866.1	666.3	23.2
	2018	19 February	74.3				1459				1400				20 March	2886			
	2020	30 January	63.8				1276				1096				15 March	2190			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Luizet	2017	05 January	51.8	48.0	4.0	8.3	1127	967.4	158.6	16.4	935	847.5	79.4	9.4	03 March	5016	5075.6	407.0	8.0
	2018	08 January	43.9				810				829				14 March	5509			
	2020	09 January	48.3				966				779				26 February	4701			
Mitger	2017	02 January	49.1	47.5	1.5	3.1	1093	959.7	117.6	12.3	873	826.5	104.4	12.6	01 March	4810	4777.2	598.6	12.5
	2018	12 January	47.1				871				900				14 March	5359			
	2020	06 January	46.2				915				707				22 February	4163			
Moniqui 1006	2017	02 January	49.1	45.8	3.5	7.6	1093	921.7	168.2	18.3	873	784.8	83.2	10.6	02 March	4944	4921.5	399.3	8.1
	2018	05 January	42.1				757				775				11 March	5309			
	2020	06 January	46.2				915				707				25 February	4512			
Moniqui 2113	2017	05 January	51.8	49.8	2.4	4.9	1127	1003.1	128.3	12.8	935	888.8	52.2	5.9	03 March	5016	5143.7	538.3	10.5
	2018	12 January	47.1				871				900				17 March	5734			
	2020	12 January	50.6				1012				832				27 February	4681			
Muñoz	2017	02 January	49.1	46.5	3.8	8.2	1093	938.4	169.8	18.1	873	808.8	55.2	6.8	02 March	4944	5169.5	798.8	15.5
	2018	05 January	42.1				757				775				17 March	6057			
	2020	09 January	48.3				966				779				25 February	4508			
Pandora	2017	02 January	49.1	45.8	3.5	7.6	1093	921.7	168.2	18.3	873	784.8	83.2	10.6	24 February	4203	4923.9	993.3	20.2
	2018	05 January	42.1				757				775				08 March	6057			
	2020	06 January	46.2				915				707				25 February	4512			
Paviot	2017	02 January	49.1	47.2	4.5	9.6	1093	953.8	175.4	18.4	873	826.5	49.0	5.9	03 March	5096	5119.2	550.8	10.8
	2018	05 January	42.1				757				775				14 March	5681			
	2020	12 January	50.6				1012				832				26 February	4580			
Pepito del Rubio	2017	25 December	44.2	41.2	4.1	10.0	973	888.5	180.3	20.3	684	698.0	20.5	2.9	01 March	4839	5295.9	646.3	12.2
	2018	29 December	38.3				682				713				08 March	5753			
	2020	12 January	50.6				1012				832				25 February	4387			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Stark Early Orange	2017	15 January	58.5	60.4	2.6	4.3	1268	1229.4	53.9	4.4	1049	1109.5	85.6	7.7	02 March	4098	4151.6	75.9	1.8
	2018	03 February	62.2				1191				1170				23 March	4205			
Stella	2017	05 February	74.1	69.9	6.7	9.5	1567	1398.7	190.8	13.6	1319	1234.2	76.6	6.2	20 March	5801	5936.1	190.5	3.2
	2018	03 February	62.2				1191				1170				03 April	6071			
	2020	15 February	73.4				1438				1214				-	-			
Sun Glo	2017	11 January	56.3	53.9	3.5	6.4	1202	1076.5	128.1	11.9	1012	983.8	24.7	2.5	03 March	4645	4915.5	733.0	14.9
	2018	16 January	50.0				946				974				20 March	5745			
	2020	19 January	55.5				1082				966				26 February	4357			
Tadeo	2017	05 January	51.8	49.1	2.4	5.0	1127	987.8	129.6	13.1	935	871.2	81.7	9.4	01 March	4731	4741.7	239.5	5.1
	2018	12 January	47.1				871				900				11 March	4986			
	2020	09 January	48.3				966				779				25 February	4508			
Veecot	2017	02 January	49.1	48.9	1.7	3.5	1093	991.8	112.4	11.3	873	868.2	34.2	3.9	25 February	4296	4564.1	699.9	15.3
	2018	12 January	47.1				871				900				14 March	5359			
	2020	12 January	50.6				1012				832				22 February	4038			

Supplementary Table 2. Chilling and heat requirements of 20 apricot cultivars determined by the Correlation method. Dates of endodormancy and full flowering, values of chill accumulation expressed in chill portions, chill units, and hours below 7°C, values of heat accumulation expressed in Growing Degree Hours (GDH), standard deviation (SD), and coefficient of variation (cv).

Cultivar	Breaking dormancy	Chill portions (Dynamic model)			Chill units (Utah model)			Hours below 7 °C			Flowering (F50)	GDH (Richardson method)
		Value	S.D.	cv	Value	S.D.	cv	Value	S.D.	cv		
Berdejo	18 January	57.0	4.9	8.7	1090	152.1	14.0	974	142.1	14.6	10 March	4917
Canino	18 January	57.0	4.9	8.7	1090	152.1	14.0	974	142.1	14.6	03 March	4023
Corbato	16 January	55.7	4.8	8.7	1057	153.5	14.5	943	139.2	14.8	06 March	4520
Goldrich	17 January	56.4	4.8	8.6	1073	154.1	14.4	957	140.5	14.7	03 March	4086
Gönci Magyar	04 February	69.1	5.0	7.2	1346	163.0	12.1	1185	162.8	13.7	19 March	4861
Henderson	04 February	69.1	5.0	7.2	1346	163.0	12.1	1185	162.8	13.7	16 March	4441
Luizet	19 January	58.0	4.9	8.5	1104	151.1	13.7	988	143.5	14.5	09 March	4732
Mitger	16 January	55.7	4.8	8.7	1057	153.5	14.5	943	139.2	14.8	07 March	4638
Moniqui 1006	15 January	54.8	4.9	8.9	1041	155.2	14.9	928	140.5	15.1	08 March	4821
Moniqui 2113	16 January	55.7	4.8	8.7	1057	153.5	14.5	943	139.2	14.8	09 March	4902
Muñoz	18 January	57.0	4.9	8.7	1090	152.1	14.0	974	142.1	14.6	08 March	4650
Pandora	18 January	57.0	4.9	8.7	1090	152.1	14.0	974	142.1	14.6	04 March	4141
Paviot	18 January	57.0	4.9	8.7	1090	152.1	14.0	974	142.1	14.6	09 March	4781
Pepito del Rubio	16 January	55.7	4.8	8.7	1057	153.5	14.5	943	139.2	14.8	07 March	4638
Stark E Orange	06 February	70.7	5.0	7.1	1377	158.0	11.5	1210	161.8	13.4	15 March	4295
Stella	22 February	81.7	5.3	6.4	1595	162.0	10.2	1406	165.5	11.8	27 March	4511
Sunglo	20 January	58.6	4.9	8.4	1121	151.8	13.5	1003	145.6	14.5	11 March	4953
Tadeo	10 February	73.5	5.0	6.8	1440	157.1	10.9	1265	163.1	12.9	09 March	3000
Veecot	19 January	58.0	4.9	8.5	1104	151.1	13.7	988	143.5	14.5	04 March	4091

Supplementary Table 3. Chilling and heating requirements of 20 apricot cultivars determined by the PLS regression method. Chilling and forcing periods are established with Partial Least Squares regression based on the Dynamic Model and the Growing Degree Hours Model. Chill accumulation is expressed in chill portions, chill units, and hours below 7°C, values of heat accumulation are expressed in Growing Degree Hours (GDH), standard deviation (SD), and coefficient of variation (cv).

Cultivar	Chilling period		Chill portions (Dynamic model)			Chill units (Utah model)			Hours below 7°C			Forcing period		GDH (Richardson method)		
	Start Date	Final Date	Value	S.D.	cv	Value	S.D.	cv	Value	S.D.	cv	Start Date	Final Date	Value	S.D.	cv
Berdejo ²	08 October	14 January	54.2	4.6	8.5	850	104.2	12.3	963	136.2	14.1	30 January	17 March	3905	948	24.3
Canino ²	08 October	15 January	54.8	4.7	8.6	864	103.5	12.0	979	137.5	14.1	30 January	15 March	3634	905	24.9
Corbato ³	09 October	15 January	54.9	4.5	8.3	853	107.9	12.7	991	135.2	13.6	30 January	15 March	3640	870	23.9
Goldrich ⁵	09 October	13 January	53.6	3.9	7.3	832	96.6	11.6	968	137.4	14.2	24 January	13 March	4004	973	24.3
Gönci Magyar ¹	29 September	12 February	74.8	5.1	6.8	1222	116.6	9.5	1301	156.3	12.0	19 February	21 March	3448	779	22.6
Harcot ⁵	09 October	13 January	53.3	4.3	8.1	825	106.1	12.9	959	133.8	14.0	29 January	19 March	4209	937	22.3
Henderson ⁵	09 October	13 January	53.6	3.9	7.3	832	96.6	11.6	968	137.4	14.2	31 January	20 March	4604	1083	23.5
Luizet ¹	06 October	28 January	64.0	4.5	7.1	1029	123.1	12.0	1115	133.4	12.0	31 January	17 March	4004	906	22.6
Mitger ⁵	06 October	29 December	43.2	3.9	9.1	638	95.7	15.0	735	147.1	20.0	05 January	17 March	5326	951	17.8
Moniqui 1006 ¹	06 October	17 December	33.9	4.6	13.6	490	100.8	20.6	534	139.6	26.1	06 January	14 March	4811	921	19.1
Moniqui 2113 ³	09 October	14 January	55.0	4.5	8.2	856	107.3	12.5	986	136.6	13.9	30 January	27 March	5305	953	18.0
Muñoz ¹	06 October	21 January	58.6	4.7	8.0	949	118.9	12.5	1029	130.0	12.6	30 January	16 March	3934	881	22.4
Pandora ⁵	08 October	13 January	53.6	3.9	7.3	832	96.6	11.6	961	136.1	14.2	24 January	11 March	3723	926	24.9
Paviot ³	09 October	14 January	54.3	4.4	8.1	839	108.0	12.9	976	134.2	13.8	31 January	16 March	3721	886	23.8
Pepito del Rubio ³	07 October	13 January	53.3	4.3	8.1	826	105.8	12.8	944	132.3	14.0	26 January	15 March	3852	894	23.2
Stark Early Orange ⁵	09 October	14 February	76.7	4.2	5.5	1226	100.2	8.2	1440	156.6	10.9	17 February	20 March	3607	819	22.7
Stella ⁴	09 October	09 February	72.9	4.5	6.1	1163	107.7	9.3	1364	140.6	10.3	27 January	06 April	5468	847	15.5
Sun Glo ⁵	09 October	13 January	53.6	3.9	7.3	832	96.6	11.6	968	137.4	14.2	31 January	23 March	5054	1174	23.2
Tadeo ⁵	08 October	13 January	53.6	3.9	7.3	832	96.6	11.6	961	136.1	14.2	31 January	20 March	4604	1083	23.5
Veecot ⁵	09 October	14 January	54.6	4.0	7.3	846	98.5	11.6	985	138.2	14.0	30 January	20 March	4660	1093	23.5

¹: 11 years; ²: 13 years; ³: 14 years; ⁴: 15 years; ⁵: 18 years

Supplementary Table 4. Chilling and heating requirements of 20 apricot cultivars determined by the Male meiosis method. Dates of endodormancy and full flowering, yearly values of chill accumulation expressed in chill portions, chill units, and hours below 7°C, yearly values of heat accumulation expressed in Growing Degree Hours (GDH), mean values, standard deviation (SD), and coefficient of variation (cv).

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Berdejo	2010	27 January	65.4	61.9	5.1	8.3	1158	1218.5	121.9	10.0	1109	1024.8	53.1	5.2	20 March	3780	4170.5	497.7	11.9
	2012	02 February	60.9				1156				1001				15 March	3790			
	2013	30 January	69.0				1396				1041				13 March	4243			
	2016	06 February	66.2				1313				992				14 March	3730			
	2017	16 January	59.5				1286				1049				03 March	4141			
	2018	19 January	52.2				992				1000				17 March	5265			
	2019	22 January	60.0				1221				939				04 March	4339			
	2020	26 January	61.8				1227				1070				27 February	4075			
Canino	2010	11 January	53.5	53.2	6.2	11.6	996	1040.5	150.1	14.4	903	875.4	88.4	10.1	08 March	3954	4211.9	765.2	18.2
	2012	24 January	54.8				1002				851				09 March	3254			
	2013	25 January	65.3				1321				976				09 March	4047			
	2016	9 January	46.8				913				712				07 March	5768			
	2017	5 January	51.8				1127				935				26 February	4308			
	2018	9 January	44.8				818				853				08 March	4662			
	2019	13 January	53.3				1067				810				24 February	3539			
	2020	19 January	55.5				1082				966				25 February	4163			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Corbato	2010	25 January	63.8	59.7	4.6	7.6	1219	1180.1	112.6	9.5	1071	986.8	71.3	7.2	15 March	3179	3899.9	439.5	11.3
	2012	28 January	57.6				1059				921				11 March	3363			
	2013	27 January	67.1				1354				999				10 March	4050			
	2016	28 January	59.6				1189				909				07 March	4059			
	2017	14 January	57.9				1244				1032				02 March	4135			
	2018	19 January	52.2				992				1000				11 March	4518			
	2019	19 January	57.4				1157				893				02 March	4113			
	2020	26 January	61.8				1227				1070				25 February	3782			
Goldrich	2010	18 January	59.1	58.3	5.5	9.4	1102	1147.2	140.2	12.2	1000	965.4	53.0	5.5	11 March	3571	3712.3	342.9	9.2
	2012	26 January	55.9				1019				884				09 March	3145			
	2013	30 January	69.0				1396				1041				10 March	3839			
	2016	29 January	60.6				1209				909				05 March	3830			
	2017	11 January	56.3				1202				1012				24 February	3752			
	2018	16 January	49.9				946				974				08 March	4362			
	2019	22 January	60.0				1221				939				28 February	3570			
	2020	19 January	55.5				1082				966				20 February	3630			
Goldrich	2010	18 January	59.1	58.3	5.5	9.4	1102	1147.2	140.2	12.2	1000	965.4	53.0	5.5	11 March	3571	3712.3	342.9	9.2
	2012	26 January	55.9				1019				884				09 March	3145			
	2013	30 January	69.0				1396				1041				10 March	3839			
	2016	29 January	60.6				1209				909				05 March	3830			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Corbato	2010	25 January	63.8	59.7	4.6	7.6	1219	1180.1	112.6	9.5	1071	986.8	71.3	7.2	15 March	3179	3899.9	439.5	11.3
	2012	28 January	57.6				1059				921				11 March	3363			
	2013	27 January	67.1				1354				999				10 March	4050			
	2016	28 January	59.6				1189				909				07 March	4059			
	2017	14 January	57.9				1244				1032				02 March	4135			
	2018	19 January	52.2				992				1000				11 March	4518			
	2019	19 January	57.4				1157				893				02 March	4113			
	2020	26 January	61.8				1227				1070				25 February	3782			
Goldrich	2010	18 January	59.1	58.3	5.5	9.4	1102	1147.2	140.2	12.2	1000	965.4	53.0	5.5	11 March	3571	3712.3	342.9	9.2
	2012	26 January	55.9				1019				884				09 March	3145			
	2013	30 January	69.0				1396				1041				10 March	3839			
	2016	29 January	60.6				1209				909				05 March	3830			
	2017	11 January	56.3				1202				1012				24 February	3752			
	2018	16 January	49.9				946				974				08 March	4362			
	2019	22 January	60.0				1221				939				28 February	3570			
	2020	19 January	55.5				1082				966				20 February	3630			
Gönci Magyar	2010	-	-	66.8	5.2	7.7	-	1335.7	139.2	10.4	-	1110.5	64.6	5.8	20 March	-	4027.3	512.9	12.7
	2012	10 February	66.8				1257				1174				14 March	3474			
	2013	07 February	75.0				1525				1123				14 March	3439			
	2016	12 February	69.2				1375				1023				28 March	4743			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Harcot	2017	25 January	65.6				1397				1211				07 March	4343			
	2018	30 January	59.5				1123				1102				21 March	4233			
	2019	03 February	69.4				1445				1072				08 March	3932			
	2020	26 January	61.8				1227				1070				-	-			
	2010	25 January	63.8	59.8	5.2	8.7	1219	1184.1	131.5	11.1	1071	996.4	64.3	6.5	17 March	3382	3997.7	402.4	10.1
	2012	28 January	57.6				1059				921				14 March	3844			
	2013	30 January	69.0				1396				1041				11 March	3985			
	2016	23 January	56.0				1105				887				07 March	4591			
	2017	14 January	57.9				1244				1032				27 February	3671			
	2018	19 January	52.2				992				1000				11 March	4518			
Henderson	2019	26 January	62.8				1294				979				04 March	3967			
	2020	23 January	59.0				1165				1042				25 February	4024			
	2010	18 February	81.8	70.9	10.4	14.6	1597	1412.1	208.8	14.8	1485	1186.8	161.8	13.6	25 March	3949	4159.4	967.7	23.3
	2012	20 February	73.8				1400				1346				16 March	3369			
	2013	11 February	79.0				1606				1185				23 March	4188			
	2016	02 March	82.9				1645				1230				28 March	2923			
	2017	18 January	60.7				1310				1081				03 March	4032			
	2018	23 January	54.2				1017				1007				20 March	4699			
	2019	02 February	68.7				1421				1053				08 March	3953			
	2020	02 February	65.8				1302				1109				15 March	6161			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Luizet	2010	25 January	63.8	61.3	4.8	7.9	1219	1223.0	121.5	9.9	1071	1016.3	41.1	4.0	18 March	3511	4120.1	437.5	10.6
	2012	02 February	60.9				1156				1001				14 March	3584			
	2013	30 January	69.0				1396				1041				14 March	4255			
	2016	03 February	63.4				1278				949				16 March	4018			
	2017	16 January	59.5				1286				1049				03 March	4141			
	2018	19 January	52.2				992				1000				14 March	4890			
	2019	26 January	62.8				1294				979				06 March	4345			
	2020	23 January	59.0				1165				1042				26 February	4217			
Mitger	2010	18 January	59.1	58.0	5.4	9.3	1102	1143.0	124.7	10.9	1000	960.9	63.0	6.6	18 March	4109	4084.0	467.2	11.4
	2012	26 January	55.9				1019				884				13 March	3732			
	2013	30 January	69.0				1396				1041				10 March	3839			
	2016	29 January	60.6				1209				909				06 March	3901			
	2017	05 January	51.8				1127				935				01 March	4731			
	2018	19 January	52.2				992				1000				14 March	4890			
	2019	19 January	57.4				1157				893				28 February	3735			
	2020	22 January	58.0				1142				1028				22 February	3736			
Moniqui 1006	2010	-		59.6	5.3	8.9	-	1188.4	144.7	12.2	-	983.6	64.3	6.5	25 March	-	4044.6	240.9	6.0
	2012	26 January	55.9				1019				884				13 March	3732			
	2013	30 January	69.0				1396				1041				11 March	3985			
	2016	29 January	60.6				1209				909				07 March	3952			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Moniqui 2113	2017	14 January	57.9				1244				1032				02 March	4135			
	2018	19 January	52.2				992				1000				11 March	4518			
	2019	26 January	62.8				1294				979				04 March	3967			
	2020	23 January	59.0				1165				1042				25 February	4024			
	2010	20 January	60.1	61.7	5.9	9.6	1130	1221.4	132.5	10.8	1002	1036.4	93.0	9.0	20 March	4165	4196.5	541.5	12.9
	2012	13 February	68.8				1284				1240				14 March	3440			
	2013	30 January	69.0				1396				1041				13 March	4243			
	2016	28 January	59.6				1189				909				09 March	4215			
	2017	14 January	57.9				1244				1032				03 March	4288			
	2018	19 January	52.2				992				1000				17 March	5265			
Muñoz	2019	30 January	66.7				1372				1027				04 March	3638			
	2020	23 January	59.0				1165				1042				27 February	4317			
	2010	20 January	60.1	59.1	4.8	8.1	1130	1164.1	129.7	11.1	1003	987.6	66.4	6.8	15 March	3515	4184.6	527.2	12.6
	2012	25 January	55.9				1011				866				13 March	3771			
	2013	30 January	69.0				1396				1041				11 March	3985			
	2016	26 January	58.6				1154				909				09 March	4442			
	2017	14 January	57.9				1244				1032				02 March	4135			
	2018	19 January	52.2				992				1000				17 March	5265			
	2019	22 January	60.0				1221				939				04 March	4339			
	2020	23 January	59.0				1165				1042				25 February	4024			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Pandora	2010	19 January	60.1	60.7	9.6	15.8	1114	1135.7	134.8	11.9	1000	965.3	70.3	7.3	11 March	3430	3863.0	498.1	12.9
	2012	26 January	55.9				1019				884				09 March	3145			
	2013	30 January	69.0				1397				1041				11 March	3985			
	2016	22 January	54.9				1083				877				07 March	4661			
	2017	12 January	56.3				1206				1012				24 February	3562			
	2018	16 January	49.9				946				974				08 March	4362			
	2019	19 January	80.1				1157				893				28 February	3735			
	2020	23 January	59.0				1165				1042				25 February	4024			
Paviot	2010	25 January	63.8	61.6	5.0	8.1	1219	1223.3	112.0	9.2	1071	1023.7	51.1	5.0	20 March	3829	4134.6	410.8	9.9
	2012	07 February	65.2				1223				1109				14 March	3507			
	2013	28 January	68.1				1373				1009				13 March	4362			
	2016	03 February	63.4				1278				949				16 March	4018			
	2017	14 January	57.9				1244				1032				03 March	4288			
	2018	19 January	52.2				992				1000				14 March	4890			
	2019	26 January	62.8				1294				979				04 March	3967			
	2020	23 January	59.0				1165				1042				26 February	4217			
Pepito del Rubio	2010	25 January	63.8	58.9	5.1	8.7	1219	1162.2	129.9	11.1	1071	979.3	65.1	6.7	20 March	3829	4019.3	312.5	7.8
	2012	26 January	55.9				1019				884				11 March	3436			
	2013	27 January	67.1				1354				999				10 March	4050			
	2016	26 January	58.6				1154				909				09 March	4442			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Stark Early Orange	2017	14 January	57.9				1244				1032				01 March	4002			
	2018	16 January	49.9				946				974				08 March	4362			
	2019	22 January	60.0				1221				939				02 March	3948			
	2020	22 January	58.0				1142				1028				25 February	4085			
	2010	08 February	75.3	71.4	6.4	9.0	1457	1429.2	118.3	8.3	1276	1201.1	113.0	9.4	20 March	2990	4052.5	1220.1	30.1
	2012	17 February	71.5				1359				1302				16 March	3590			
	2013	07 February	75.0				1525				1123				26 March	4912			
	2016	02 March	82.9				1654				1230				28 March	2923			
	2017	21 January	63.5				1337				1153				02 March	3873			
	2018	14 February	70.7				1404				1378				23 March	3773			
Stella	2019	02 February	68.7				1421				1053				06 March	3694			
	2020	30 January	63.8				1276				1096				15 March	6665			
	2010	25 February	86.9	84.8	7.5	8.8	1695	1680.9	187.4	11.2	1549	1436.9	95.0	6.6	25 March	3276	3878.9	745.8	19.2
	2012	01 March	79.0				1458				1467				21 March	3044			
	2013	07 March	96.0				1936				1446				04 April	4446			
	2016	08 March	86.8				1741				1293				06 April	3909			
	2017	28 February	89.1				1881				1563				20 March	3442			
	2018	17 February	72.3				1436				1392				03 April	5206			
	2019	12 March	88.8				1762				1454				06 April	3829			
	2020	25 February	79.3				1538				1334				-	-			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)			
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv					
Sun Glo	2010	09 February	75.3	66.3	7.0	10.6	1481	1323.6	157.7	11.9	1292	1104.0	91.9	8.3	20 March	2932	3789.1	472.3	12.5
	2012	10 February	66.8				1257				1174				15 March	3681			
	2013	07 February	75.0				1525				1123				14 March	3439			
	2016	08 February	67.2				1335				1009				19 March	3898			
	2017	18 January	60.7				1310				1081				03 March	4032			
	2018	26 January	56.7				1071				1040				20 March	4441			
	2019	03 February	69.4				1445				1072				06 March	3673			
	2020	23 January	59.0				1165				1042				26 February	4217			
Tadeo	2010	25 January	63.8	60.5	5.3	8.7	1219	1197.0	132.5	11.1	1071	998.9	64.5	6.5	15 March	3179	4126.2	576.0	14.0
	2012	28 January	57.6				1059				921				13 March	3659			
	2013	30 January	69.0				1396				1041				13 March	4243			
	2016	05 February	65.3				1303				976				25 March	5028			
	2017	16 January	59.5				1286				1049				01 March	3855			
	2018	19 January	52.2				992				1000				11 March	4518			
	2019	19 January	57.4				1157				893				04 March	4504			
	2020	23 January	59.0				1165				1042				25 February	4024			
Veecot	2010	13 January	55.1	54.5	5.4	9.8	1005	1065.2	137.0	12.9	950	904.9	105.1	11.6	11 March	3982	4283.8	897.8	21.0
	2012	23 January	54.0				988				842				09 March	3380			
	2013	25 January	65.3				1321				976				10 March	4211			
	2016	09 January	46.8				913				712				11 March	6073			

Cultivar	Year	Breaking dormancy	Chill portions (Dynamic model)				Chill units (Utah model)				Hours below 7 °C				Flowering (F50)	GDH (Richardson method)
			Value	Mean	S.D.	cv	Value	Mean	S.D.	cv	Value	Mean	S.D.	cv		
	2017	11 January	56.3				1202				1012				25 February	3844
	2018	16 January	49.9				946				974				14 March	5202
	2019	13 January	53.3				1067				810				26 February	3764
	2020	19 January	55.5				1082				966				22 February	3814

CAPÍTULO 6. Molecular Characterization of Genetic Diversity in Apricot Cultivars: Current Situation and Future Perspectives

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CAPÍTULO 6

Molecular Characterization of Genetic Diversity in Apricot Cultivars: Current Situation and Future Perspectives

Caracterización molecular de la diversidad genética en variedades de albaricoquero: Situación actual y perspectivas de futuro

RESUMEN

En los últimos años se está produciendo una importante renovación varietal en el albaricoquero en todo el mundo, con la introducción de un gran número de nuevas variedades que están reemplazando a las variedades tradicionales y locales en muchas situaciones. Para estudiar la diversidad genética actual, un grupo de 202 accesiones, que incluyen variedades locales y obtenciones de programas de mejora de diversos países, se ha caracterizado usando 13 marcadores microsatélites. Los parámetros de diversidad mostraron una mayor diversidad en las variedades procedentes de los programas de mejora que en las variedades locales, pero también sugirió una pérdida de diversidad asociada con la mejora más reciente. En el análisis filogenético basado en la distancia genética de Nei se diferenciaron claramente dos grupos principales según el origen genealógico de las accesiones. El primer grupo lo formaron en su mayoría variedades tradicionales europeas y norteamericanas, y el segundo grupo incluyó la mayoría de las variedades comerciales y las selecciones avanzadas más recientes de los programas de mejora. Los análisis poblacionales adicionales mostraron la misma tendencia de agrupamiento en la distribución de individuos y grupos, confirmando los resultados obtenidos en el análisis filogenético. Estos resultados muestran la erosión y disminución de la diversidad genética en el albaricoquero cultivado actualmente, y resaltan la importancia de preservar las variedades tradicionales y el germoplasma local para asegurar los recursos genéticos para la mejora genética.

Article

Molecular Characterization of Genetic Diversity in Apricot Cultivars: Current Situation and Future Perspectives

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Abstract: In the recent years, an important renewal of apricot cultivars is taking place worldwide with the introduction of a large number of new releases, which are replacing traditional and local cultivars in many situations. To study the current genetic diversity, a group of 202 apricot accessions, including landraces and releases from breeding programs in several countries, has been characterized using 13 microsatellite markers. The diversity parameters showed higher diversity in modern releases than in landraces, but also suggested a loss of diversity associated with recent breeding. Two main clusters according to the pedigree origin of the accessions were clearly differentiated in the phylogenetic analysis based on Nei's genetic distance. The first group comprised mostly European and North American traditional cultivars, and the second group included the majority of recent and commercial releases from breeding programs. Further population analyses showed the same clustering trend on the distribution of individuals and clusters, confirming the results obtained in the molecular phylogenetic analysis. These results provide a sight of the erosion and the decrease of the genetic diversity in the currently grown apricot and highlight the importance of preserve traditional cultivars and local germplasm to assure genetic resources for further breeding.

Keywords: apricot; genetic diversity; germplasm; microsatellites; population structure; *Prunus armeniaca* L.; SSR markers



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1. Introduction

Apricot (*Prunus armeniaca* L.) is a diploid fruit tree species of the Rosaceae family. It was originated in Central Asia, where the first pieces of evidence of apricot cultivation date from 406–250 BC [1,2]. The crop was spread worldwide throughout three diffusion routes: Eastern Asia to Japan, the Irano-Caucasian region, and Continental Europe [3]. From the Irano-Caucasian region, it reached the Mediterranean countries by two secondary routes, Southern Europe and North Africa, originating three major apricot gene pools throughout the Mediterranean Basin: the “Irano-Caucasian”, the “North-Mediterranean Basin” and the “South-Mediterranean Basin” [4]. A recent study of single nucleotide polymorphisms (SNPs) in apricot revealed that the cultivated apricot resulted as a consequence of two different domestication events. The European cultivated apricots diverged from the wild populations of Northern Central Asia and resulted in four differentiated groups: Mediterranean countries, Continental Europe, North-America and North-Africa. On the other hand, Chinese cultivated apricots were domesticated from Southern Central Asian wild populations [5].

Nowadays, apricots are cultivated in temperate regions around the world, constituting the third stone fruit tree in economic importance worldwide. Near 50% of the world

production is concentrated in Mediterranean countries. Turkey was the first world producer in 2019 with 846,606 t (20.7% of world production), followed by Uzbekistan (536,544 t, 13.1%), Iran (329,638 t, 8.1%), Italy (272,990 t, 6.7%) and Algeria (209,224 t, 5.1%) [6].

Apricot cultivars have been traditionally classified into eco-geographical groups according to their geographical origin: Central Asian, East Chinese, North Chinese, Dzhungar-Zailij, Irano-Caucasian, and European [7]. Cultivars from the Central Asian group, which is the oldest and most diverse, are mainly self-incompatible, show high chilling requirements and produce small-medium fruits. The Dzhungar-Zailij group includes mostly self-incompatible and small-fruited cultivars. Asian cultivars have been recently differentiated in two main gene pools: Central Asia and Eastern Asia, which includes Japanese apricots [3]. Most cultivars from the Irano-Caucasian group are characterized by self-incompatibility and low chilling requirements. The European group includes most of the commercial cultivars of Europe, North-America, South Africa and Australia. Most of them are self-compatible [8] and have low chilling requirements [9] and a short ripening time [10,11]. Two main gene pools have been recently differentiated in the European group: Mediterranean Europe and Continental Europe [3].

In the last years, an important renewal of apricot cultivars is taking place worldwide with the introduction of a large number of new releases in response to productive and industrial changes in the crop. Breeding programs from several countries have developed a number of new commercial cultivars focused on common objectives: self-compatibility [12,13], resistance to Plum Pox Virus (PPV), fruit quality, and extension of the ripening period [14]. The release of these new cultivars has led to the displacement of local cultivars in many countries resulting in genetic erosion of apricot diversity [15].

Microsatellites, or simple sequence repeats (SSRs), are highly polymorphic molecular markers uniformly distributed throughout the genome. Their Mendelian inheritance, and the cross-species transportability make them suitable for phylogenetic and population diversity studies [16,17]. Microsatellites have been used to characterize numerous *Prunus* species [18]. In apricot, they have been used to study the evolutionary history of the crop [3,5,19–21], and to characterize traditional [22] and local cultivars from Spain [15,23], Turkey [24], Tunisia [25,26] and Iran [27].

However, information is lacking on the diversity relationships of most of recent apricot releases. In order to fill this gap, a group of 202 apricot accessions, including landraces and releases from breeding programs in several countries were characterized using SSRs to (i) evaluate the current genetic diversity, (ii) establish the similarity relationships between cultivars, and (iii) estimate the levels of population structure in the main cultivars currently grown.

2. Materials and Methods

2.1. Plant Material

A group of 202 apricot accessions was analyzed, including 30 landraces and 171 releases from breeding programs, and one aprium, an interspecific hybrid between apricot (*P. armeniaca*) and plum (*Prunus salicina* Lindl.) (Table 1). Plant material was collected from germplasm collections and commercial orchards of Aragón, Cataluña, and Extremadura (Spain). Landraces are originating from six countries and releases are of 33 private and public breeding programs from ten countries. Two groups have been considered within the bred accessions: commercial cultivars registered in the Community Plant Variety Office (N = 132) [28] and recent unregistered releases (N = 39). The aprium hybrid was considered a commercial release for the analysis (Table 1).

Table 1. Origin of the 201 apricot accessions and one interspecific hybrid (aprium) analyzed in this study.

Accession	Country	Accession	Country	Accession	Country	Accession	Country
Beliana	France ^a	F003	Spain ^{b,9}	Faralia	France ^{c,18}	Mikado 01	Spain ^{c,23}
Bergeron	France ^a	F004	Spain ^{b,9}	Farbaly	France ^{c,18}	Mikado 02	Spain ^{c,28}
Búlida	Spain ^a	F005	Spain ^{b,9}	Farbela	France ^{c,18}	Milord	Spain ^{c,23}
Canino	Spain ^a	F006	Spain ^{b,9}	Farclo	France ^{c,18}	Mirlo Blanco	Spain ^{c,4}
Castelbrite	Italy ^a	M001	France ^{b,21}	Fardao	France ^{c,18}	Mirlo Rojo	Spain ^{c,4}
Corbató	Spain ^a	M002	France ^{b,21}	Farely	France ^{c,18}	Mogador	Spain ^{c,23}
Cristalí	Spain ^a	T001	Spain ^{b,28}	Farfia	France ^{c,18}	Monster Cot	USA ^{c,27}
Currot	Spain ^a	T002	Spain ^{b,28}	Farhial	France ^{c,18}	Murciana	Spain ^{c,4}
Fabara	Spain ^a	T003	Spain ^{b,28}	Farius	France ^{c,18}	Ninfa	Italy ^{c,6}
Ginesta	Spain ^a	T004	Spain ^{b,28}	Farlis	France ^{c,18}	Ninja	France ^{c,7}
Henderson	USA ^a	T005	Spain ^{b,28}	Fartoli	France ^{c,18}	Orange Rubis	France ^{c,8}
Lito	Greece ^a	T006	Spain ^{b,28}	Feria Cot	France ^{c,5}	Orangered	USA ^{c,20}
Mitger	Spain ^a	T007	Spain ^{b,28}	Flash Cot	USA ^{c,27}	Oscar	Spain ^{c,23}
Moniquí (1006)	Spain ^a	T008	Spain ^{b,28}	Flavor Cot	USA ^{c,31}	Ouardi	Tunisia ^{c,15}
Moniquí (2113)	Spain ^a	T009	Spain ^{b,28}	Flodea	Spain ^{c,23}	Pacha	Spain ^{c,23}
Muñoz	Spain ^a	T010	Spain ^{b,28}	Flopria	Spain ^{c,23}	Palsteyn	South Africa ^{c,2}
Palabras	Spain ^a	T011	Spain ^{b,28}	Fuego	Spain ^{c,23}	Perle Cot	USA ^{c,26}
Pandora	Greece ^a	T012	Spain ^{b,28}	Goldbar	USA ^{c,31}	Pinkcot	France ^{c,7}
Paviot	France ^a	A159	Unknown ^{c,33}	Goldensweet	USA ^{c,17}	Playa Cot	France ^{c,5}
Peñaflor 01	Spain ^a	A161	Unknown ^{c,33}	Goldrich 01	USA ^{c,29}	Priabel	France ^{c,18}
Peñaflor 02	Spain ^a	A252	Unknown ^{c,33}	Goldrich 02	USA ^{c,29}	Pricia	France ^{c,18}
Pepito del Rubio	Spain ^a	AC1	USA ^{c,26}	Goldstrike	USA ^{c,31}	Primaya	France ^{c,18}
P. de Tiryntos	Greece ^a	Alba	Spain ^{c,33}	Gönci magyar	Hungary ^{c,3}	Primidi	France ^{c,21}
Rojo de Carlet	Spain ^a	Alb-Rojo	Spain ^{c,28}	Harcot	Canada ^{c,12}	Primorosa	Spain ^{c,4}
Stark E. Orange	USA ^a	Almabar	Spain ^{c,9}	Hargrand	Canada ^{c,12}	Rambo	Spain ^{c,23}
Stella	USA ^a	Almadulce	Spain ^{c,9}	Harval	Canada ^{c,12}	Robada	USA ^{c,30}
Sun Glo	USA ^a	Almater	Spain ^{c,9}	Holly Cot	France ^{c,5}	Rojo Pasión	Spain ^{c,4}
Tadeo	Spain ^a	Apribang	France ^{c,1}	IPS16121	France ^{c,18}	Rouge Cot	France ^{c,5}
Tilton	USA ^a	Apriqueen	France ^{c,1}	IPS20390	France ^{c,21}	Rubely	France ^{c,21}
Veecot	Canada ^a	Aprisweet	France ^{c,1}	IPS21239	France ^{c,21}	Rubilis	France ^{c,21}
S001	France ^{b,1}	Aprium	Spain ^{c,28}	IPS21512	France ^{c,21}	Rubissia	France ^{c,21}
S002	France ^{b,1}	Aprix 116	Spain ^{c,24}	IPS23214	France ^{c,21}	Rubista	France ^{c,18}
C001	France ^{b,5}	Aprix 20	Spain ^{c,24}	JNP	Unknown ^{c,33}	Samourai	France ^{c,7}
C002	France ^{b,5}	Aprix 33	Spain ^{c,24}	Justo Cot	France ^{c,5}	Sandy cot	France ^{c,5}
C003	France ^{b,5}	Aprix 9	Spain ^{c,24}	Kalao	Spain ^{c,23}	Sherpa	Spain ^{c,23}
C004	France ^{b,5}	Bergarouge	France ^{c,13}	Katy	USA ^{c,32}	Soledane	France ^{c,14}
C005	France ^{b,5}	Bergcot	France ^{c,22}	Kioto	France ^{c,7}	Spring Blush	France ^{c,7}
C006	France ^{b,5}	Big Red	France ^{c,7}	Kosmos	Spain ^{c,23}	Sunny Cot	USA ^{c,26}
C007	France ^{b,5}	Cebas 57 01	Spain ^{c,4}	Lady Cot	France ^{c,19}	Sweet Cot	USA ^{c,31}
C008	France ^{b,5}	Cebas 57 02	Spain ^{c,4}	Lido	Spain ^{c,23}	Swired	Switzerland ^{c,25}
C009	France ^{b,5}	Cebas Red	Spain ^{c,4}	Lilly Cot	USA ^{c,26}	Tardorange	Spain ^{c,4}
C010	France ^{b,5}	Charisma	South Africa ^{c,2}	Lorna	USA ^{c,30}	Tom Cot 01	USA ^{c,31}
C011	France ^{b,5}	Cheyenne	Spain ^{c,23}	Luizet	France ^{c,11}	Tom Cot 02	USA ^{c,31}
C012	France ^{b,5}	Cocot	France ^{c,5}	Magic Cot	USA ^{c,26}	Tornado	France ^{c,7}
C013	France ^{b,5}	Colorado 01	Spain ^{c,23}	Maya Cot	France ^{c,5}	Tsunami	France ^{c,7}
C014	France ^{b,5}	Colorado 02	Spain ^{c,23}	Medaga	France ^{c,18}	Valorange	Spain ^{c,4}
B001	Spain ^{b,4}	Cooper Cot	France ^{c,5}	Medflo	France ^{c,21}	Vanilla Cot	USA ^{c,26}
B002	Spain ^{b,4}	Dama rosa	Spain ^{c,16}	Mediabel	France ^{c,18}	Vitillo	Italy ^{c,10}
B003	Spain ^{b,4}	Dama taronja	Spain ^{c,16}	Mediva	France ^{c,18}	Wonder Cot	USA ^{c,26}
F001	Spain ^{b,9}	Delicot	France ^{c,19}	Memphis	Spain ^{c,23}		
F002	Spain ^{b,9}	Dorada	Spain ^{c,4}	Micaelo	Spain ^{c,4}		

^a Landrace; ^b Recent release from breeding program; ^c Commercial cultivar from breeding program; ¹ Agro Selection Fruits; ² ARC Infruitec-Nietvoorbij; ³ Brózik Sándor and Korponai Gyula; ⁴ CEBAS-CSIC; ⁵ COT International; ⁶ Daniele Bassi (Università di Bologna); ⁷ Escande; ⁸ Européinières; ⁹ Frutaria; ¹⁰ G. Pugliano (Università degli Studi di Napoli); ¹¹ Gabriel Luizet; ¹² Harrow Research Center; ¹³ INRAE; ¹⁴ INRAE/Centrex; ¹⁵ INRAE; ¹⁶ IVIA; ¹⁷ L. Bradford and N. Bradford (Le Grand, CA); ¹⁸ Marie-France BOIS (International Plant Selection); ¹⁹ Marie-Laure ETEVE (COT International); ²⁰ New Jersey Agricultural Experiment Station; ²¹ Newcot S.A.S. (International Plant Selection); ²² Norbert and Pierre-Gilles Servien (COT International); ²³ PBS Producción Vegetal SL; ²⁴ Proseplan; ²⁵ Regibus (COT International); ²⁶ SDR FRUIT LLC; ²⁷ SMS UNLIMITED (COT International); ²⁸ Tulare; ²⁹ USDA ARS (COT International); ³⁰ USDA-Fruit Genetics and Breeding Research Unit of Fresno; ³¹ Washington State University (COT International); ³² Zaiger Genetics; ³³ Unknown.

2.2. DNA Extraction and Amplification

Young leaves were collected and placed in a plastic bag with silica gel for preservation until DNA extraction [29]. Genomic DNA of each cultivar was extracted from the preserved leaves following the protocol described by Hormaza [22] and using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA was quantified using a NanoDrop™ND-1000 spec-

trophotometer (Bio-Science, Budapest, Hungary). Thirteen selected primer combinations previously developed by different research groups in apricot and peach (*Prunus persica* L. Batsch) were used for the amplification of SSR loci (Table 2). Amplification reactions were carried out in 15 µL volumes, containing 10x NH₄ Reaction Buffer (160 mM (NH₄)₂SO₄, 670 mM Tris-HCl (pH 8.8 at 25 °C) and stabilizer), 25 mM MgCl₂, 2.5 mM of each dNTP, 10 µM of each primer, 100 ng of genomic DNA and 0.5 U of BioTaq™ DNA polymerase (Bioline, London, UK). The temperature profile used was an initial step of 1 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 47, 51, 56, 57 or 60 °C depending on each primer pair (Table 2), 1 min at 72 °C, and a final step of 5 min at 72 °C. Fluorescently labeled PCR products were analyzed in a CEQ™ 8000 capillary electrophoresis DNA analysis system (Beckman Coulter, Fullerton, CA, USA). Samples were denaturalized at 90 °C for 120 s, injected at 2.0 kV for 30 s, and separated at 6.0 kV for 35 min [30]. Each reaction was repeated at least twice to ensure the reproducibility of the results. The amplified fragments were sized using the Beckman Coulter CEQ™ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA).

Table 2. List of the 13 apricot SSR loci from different *Prunus* species, primer sequences, linkage group, SSR motive, predicted length, and annealing temperature (T_a) used in this study.

Locus	Sequence (5' → 3')	Linkage Group	SSR Motive	Predicted Length (bp)	T _a (°C)	Species
pchgms3 ¹	ACGGTATGTCCGTACACTCTCCATG CAACCTGTGATTGCTCCTATTAAAC	1	(CT) ₁₉	179	57	Peach
UDP96-001 ²	AGTTTGATTTTCTGATGCATCC TGCCATAAGGACCGGTATGT	Unknown	(CA) ₁₇	120	57	Peach
ssrPaCITA7 ³	CTTTTGTGCCTCAGCTTCCCAACAC CCTGGCCTGACCCTAAGCAATTCG	1	(AG) ₂₂	211	51	Apricot
ssrPaCITA10 ³	GGTGAGGTCTGTGCTGAATATGCCA CGATTAAAGAAATAAGAAAAAGAGC	3	(CT) ₂₆	175	47	Apricot
ssrPaCITA12 ³	GAGACACCCCAACCACCCATCATGT GGTGTGGAAATGTGGAAAGAAATG	6	(TC) ₁₆	151	47	Apricot
ssrPaCITA19 ³	GACAAATACAATCAAGAAGTGTCGC GAACAGCTAGCCCCCTTGTGCATAC	2	(TC) ₁₆	114	51	Apricot
ssrPaCITA23 ³	GTGAATACAAAATTTTACTACATTG CGGTCTCTGACTCTCTGACTTGCGG	3	(AC) ₂ (AG) ₁₈	146	51	Apricot
ssrPaCITA27 ³	GATCCCTCAACTGAATCTCTC CGTCACAACAATAGATGCGAAGG	Unknown	(TC) ₈ (TA) ₆ (TG) ₁₇	262	47	Apricot
UDAp-410 ⁴	TTGTTGACAAGAAGAAAACAAAGC CAACGGGTTGGTTTCAGAAG	1	(AG) ₂₄	155	56	Apricot
UDAp-415 ⁴	AACTGATGAGAAGGGGCTTG ACTCCCGACATTGTGCTTC	1	(GA) ₂₁	156	56	Apricot
UDAp-420 ⁴	TTCCTTGCTTCCCTTCATTG CCCAGAACTTGATTCTGACCA	6	(CT) ₂₀	175	56	Apricot
UDP98-409 ²	GCTGATGGGTTTTATGGTTTTTC CGGACTCTTATCCTCTATCAACA	8	(AG) ₁₉	129	60	Peach
UDP98-412 ⁵	AGGGAAAGTTTCTGCTGCAC GCTGAAGACGACGATGATGA	6	(AG) ₂₈	129	60	Peach

¹: [31]; ²: [32]; ³: [33]; ⁴: [34]; ⁵: [35].

2.3. Data Analysis

In order to analyze the genetic variability and the population structure, different statistical analyses were performed using the R programming environment ([36], version 4.1.0). The genetic profiles were stored in a csv file in which each allele was coded by a character string. In order to process the SSR dataset, the file was converted into a matrix of allelic frequencies stored in a genind class with the “df2genind” function using the R package “adegenet” v. 2.1.3 [37]. Missing data (<0.1%) were replaced with the mean

frequency of the corresponding allele, which avoids adding artefactual between-group differentiation [38].

Number of alleles per locus (N_a), allelic richness (A_r), private alleles (P_a), observed heterozygosity (H_o), expected heterozygosity (H_e), tests for deviations from Hardy-Weinberg expectations (HWE) and inbreeding coefficients (F_{IS}) were calculated on the landraces and bred cultivars using the “adegenet” v. 2.1.3 [37], “hierfstat” v. 0.5–7 [39], “pegas” v. 1.0–1 [40] and “PopGenReport” v. 3.0.4 [41] packages. The levels of genetic differentiation between all pairs of populations for pre-defined and inferred groups were estimated using Nei’s pairwise F_{ST} values using the “hierfstat” v. 0.5–7 R package [39]. In order to validate the F_{ST} pairwise values, a bootstrap with 1000 replicates was carried out using the function “boot.ppfst”. Results were plotted as a correlation plot with the R package “corrplot” v. 0.90 [42]. Additionally, the distribution of genetic diversity across the population structure was evaluated with an Analysis of Molecular Variance (AMOVA) using the “poppr” R package v. 2.9.2 [43]. A three-level hierarchical analysis was designed to show the variations within/among the source of origin, the classification (landrace, commercial and recent releases), and the breeding program. All analyses were performed in the “ade4” v. 1.7–16 package [44] using 1000 permutations to assess the significance of variance components.

A R script was developed to detect homonymies and synonymies in the data. Homonymies were identified by the comparison of all accession names by the “duplicated” function, and the allele data were compared using the function “duplicated” in order to detect identical genetic profiles considered as synonymies.

Genetic relatedness among genotypes was analyzed by UPGMA (Unweighted Pair Group Method with Arithmetic averages) cluster analysis based on the Nei and Li similarity index. A dendrogram was generated using the “poppr” package v. 2.9.2 [43] with 1000 bootstrap replicates and plotted with “ape” package v. 5.3 [45].

The genetic structure of the set of accessions was analyzed by a Principal Components Analysis (PCA) and a Discriminant Analysis of Principal Components (DAPC). PCA was performed using “stats” package v. 3.6.0 and was plotted using “ggplot2” v. 3.3.4 package [46]. DAPC was carried out using the “adegenet” package v. 2.1.3 [37]. First, genetic data were transformed using PCA, and then a Discriminant Analysis was performed on the principal components (PC) retained using a cross-validation method. The optimal number of clusters (k) was determined according to the lowest Bayesian Information Criterion (BIC) value obtained with the “find.clusters” function. A cross-validation function (“XvalDapc”) was used to confirm the appropriate number of PCA to be retained.

3. Results and discussion

3.1. Microsatellite Polymorphism and Genetic Diversity

Ten of the 13 microsatellite markers resulted polymorphic in the analysis of the 202 accessions (Table 1), but no amplification patterns were obtained with three loci (UDP96-001, *ssrPaCITA12* and *ssrPaCITA19*) (Table 2). The alleles obtained for each accession and locus can be found in Supplementary Table S1.

In order to evaluate the genetic diversity, different parameters were compared between the landraces and the releases from breeding programs. Additionally, the diversity indexes were studied for each group of landraces, commercial cultivars and recent releases (Table 3). The mean number of alleles found in landraces (6.50) was higher than those obtained in previous reports for traditional cultivars in Spain (4.00 [23]; 4.27 [15]) or Iran (4.62 [47]; 3.01 [27]) resulting presumably from the larger and diverse number of accessions analyzed in this work. However, the number of alleles was lower than those obtained in reports that include wild apricots (23.00 [48]; 16.75 [20]), probably due to not having included cultivars from China, the center of origin [20], as reported by Bourguiba et al. [3]. The highest number of alleles for all of the studied SSR loci was detected in the group of releases from breeding programs, ranging from 7 to 12, in which the average number of alleles (9.40) was higher than that of landraces although without significant differences ($p < 0.05$) (Supplementary Table S2). Traditional cultivars from different countries have

been widely used as material for breeding [49,50]. In Europe, traditional cultivars were susceptible to Sharka (PPV) and most of them were replaced in the late 20th century. Then, most breeding programs focused on developing new cultivars by crossing traditional cultivars from each country, to conserve interesting traits, with PPV-resistant cultivars developed in North America in order to transmit the source of PPV-resistance to the new releases. This admixture in new cultivars could explain the higher number of alleles observed in bred releases due to the incorporation of genotypes developed in North America with Asian ancestors. In commercial cultivars, the average number of alleles (9.30) was higher but not significant ($p < 0.05$) than in recent releases (6.30), indicating a gradient of decreasing genetic diversity, which suggests that controlled selection in breeding programs may be causing a reduction in the diversity of the crop in recent years. In spite of the higher allelic diversity found in the releases from breeding programs, the allelic richness calculated to measure genetic diversity (6.97) did not showed significant differences ($p < 0.05$) with respect to landraces (6.20), which can be related to the diversity of origins in the bred accessions.

Table 3. Genetic parameters of apricot landraces and cultivars released from breeding programs (commercial and recent). Mean number of alleles (N_a), mean allelic richness (A_r), number of private alleles (P_a), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e), and mean inbreeding coefficient (F_{IS}).

Group	Statistic	N_a	A_r	P_a	H_o	H_e	F_{IS}
Landraces		6.50	6.20	1	0.68	0.73	0.08
Bred cultivars		9.40 *	6.97	30	0.77	0.74	−0.04
	<i>t</i> -test	ns	ns		ns	ns	
	<i>p</i> -value	1.000	0.999		0.994	0.866	
Recent releases		6.30	5.84	1	0.77	0.70	−0.10
Commercial releases		9.30 **	7.17	22	0.77	0.74	−0.03
	<i>t</i> -test	ns	ns		ns	ns	
	<i>p</i> -value	1.000	1.000		0.447	0.989	

* Percentage of missing data 0.06%; ** Percentage of missing data 0.08%; ns: non-significant values at $p < 0.05$ significance level.

Only one private allele was exclusively found in the landraces, while 30 were found in releases from breeding programs. The presence of rare alleles in bred cultivars is related to the fact that these cultivars have been enriched with germplasm of landraces from different origins. However, the number of private alleles was lower when two separate groups were considered into the group of bred releases: commercial cultivars (22) and recent releases (1), showing a loss of variability in the next generation of apricot cultivars. In general, most of the private alleles were also unique, as they were exclusive to only one genotype; for example, the one found in the group of landraces is only present in the cultivar “Stella” (Tables 1 and 3).

The mean observed heterozygosity was lower in the landraces (0.68) compared to the recent releases (0.77) and commercial cultivars (0.77) although without significant differences ($p < 0.05$). These values are higher than those in previous studies of diversity in apricot (0.51 [22]; 0.32 [51]; 0.52 [52]; 0.63 [19]; 0.58 [53]; 0.68 [23]; 0.65 [54]; 0.39 [15]; 0.52 [27]; 0.63 [55]; 0.36 [26]; 0.72 [20]; 0.65, 0.66 [21]). In the landraces, the expected heterozygosity ranged from 0.57 to 0.84, with a mean value of 0.73. Similar mean values of expected heterozygosity were obtained for the releases from breeding programs (0.74), with slight differences between recent releases (0.70) and commercial cultivars (0.74), probably because the individuals in both groups are related.

The mean inbreeding coefficient (F_{IS}) had a positive value in the landraces (0.08) whereas in both recent and commercial releases was lower (−0.04), indicating a certain degree of inbreeding (Table 3). In all accessions, seven out of ten loci showed no deviation from Hardy-Weinberg Equilibrium (HWE), and three (ssrPaCITA23, ssrPaCITA27 and UDP98-412) showed significant departures from HWE ($p < 0.05$) (Supplementary Table S2). A loss of apricot genetic diversity during domestication and diffusion from the center

of origin to areas of more recent cultivation has been reported in previous works [3,4]. Our results showed an excess of heterozygosity, which is an indicator of recent bottleneck, suggesting a loss of diversity associated to breeding. This can be due to the use of the same or very related parental genotypes in different breeding programs, thus reducing the diversity found in the new cultivars. On the other hand, we found a deficit of heterozygotes in the group of landraces as a result of inbreeding, which is a sign of expansion since traditional cultivars are a source of genetically interesting traits.

To analyze the genetic distance between all pairs of populations, we calculated the Nei's pairwise F_{ST} matrix (Supplementary Table S3). The value of pairwise F_{ST} among the pre-defined groups showed a moderate and significative genetic difference ranging from 0.09 to 0.12 ($p < 0.001$), although lower than that observed in previous studies in which wild apricots (0.14 [48]) or cultivars from different eco-geographic groups were analyzed (0.58 [19]; 0.14 [26]; 0.32 [51]; 0.38 [15]).

The hierarchical population structure examined by an Analysis of Molecular Variance (AMOVA) (Table 4) showed that the highest variation (93.55%) occurred within the cultivars, although no significant differences were found. The distribution of genetic diversity suggested a significant population structure considering two origin levels, landraces and breeding releases, and a moderate (6.18%) and significant differentiation among the breeding programs. The level of variation among populations is lower than that obtained when apricot cultivars are grouped by geographical criteria [3,4] or reported in other *Prunus* [56,57]. However, these values together with those of fixation index seem to support the hypothesis that there is a certain level of variation among landraces and new releases.

Table 4. Analysis of Molecular Variance (AMOVA) calculated using three population levels for 202 apricot accessions.

Source of Variation	df	Sum of Squares	Estimated Variance	% of Variation	p-Value
Among Source	1	87.34	0.520	6.55	0.001
Among Classification within Source	1	18.21	0.018	0.22	0.241
Among Breeding Program within Classification	37	386.14	0.490	6.18	0.001
Among Cultivars within Breeding Program	162	1034.95	−0.516	−6.50	1.000
Within Cultivars	202	1498.83	7.420	93.55	0.214
Total	403	3025.47	7.931	100	

df: degree of freedom.

3.2. Identification of the Different Genotypes

The R script developed to detect synonymies and homonymies allowed to distinguish 192 different apricot genotypes from the 202 accessions analyzed based on their unique genetic profiles using ten microsatellite loci. The 23 accessions identified as synonymies were probably samples of the same cultivars with different labeling in each collection. Five synonymies corresponded to the traditional Spanish cultivars “Moniquí (1006)”, “Moniquí (2113)” and the local accessions “Fabara”, “Muñoz”, and “Peñaflor 01”. A high rate of synonymies and homonymies has also been previously found in local Spanish accessions [15], probably due to the predominance of a few traditionally cultivars grown in Spain. Accessions “A159”, “A161”, “A252”, and “JNP” were identified as “Goldstrike”, “Sweet Cot”, “Soledane”, and “Almabar”, respectively. Two different recent releases, “T002” and “T011”, had the same genotype profile. Finally, the identity of accessions with the same name collected from different collections was confirmed (“Cebas 57 01” and “Cebas 57 02;” “Colorado 01” and “Colorado 02;” “Goldrich 01” and “Goldrich 02;” “Mikado 01” and “Mikado 02”) and two pairs of homonymous accessions collected from different orchards were detected (“Tom Cot 01” and “Tom Cot 02;” “Peñaflor 01” and “Peñaflor 02”).

3.3. Genetic Relationships and Similarities among Genotypes

The dendrogram generated from the UPGMA cluster analysis based on the Nei and Li similarity index revealed two main clusters supported by a strong bootstrap value (100) (Figure 1A), which clearly differentiated landraces (I) from breeding releases (II). The first

cluster (I), containing mostly European and North American traditional cultivars, was subdivided into two sub-clusters according to the geographical origin. One of them (I.A) was composed of traditional cultivars from Spain (e.g., “Currot”, “Moniqui”, “Búlida”), France (“Beliana”) and Greece (“Lito”, “Précoce de Tiryntos”). Additionally, recent cultivars from Spanish public breeding programs as “Dama rosa” (IVIA), “Mirlo rojo”, “Mirlo blanco” and “Murciana” (CEBAS) were grouped closely to the Spanish traditional cultivars, which is expected since they were developed from local Spanish germplasm. The other sub-cluster (I.B) included three North American cultivars: “Stella” and “Veecot” from USA, and “Harval” from Canada. This grouping supports that North American cultivars originated from European cultivars as suggested by Faust et al. [1] and later shown in different studies [22,58,59]. Our results are also in agreement with the recent breeding activity, since a great varietal renewal has taken place in the last 15 years, with the introduction of 322 new cultivars in Europe [28] from breeding programs worldwide [14]. The fact that breeding programs share some objectives, including resistance to sharka (PPV), has led to the use of a few PPV-resistant North American cultivars as parental lines to introduce this trait into European germplasm to generate new resistant cultivars, since all European cultivars are susceptible to PPV [49,50].

The second cluster (II) comprises the aprium hybrid and two sub-clusters formed mainly of breeding accessions, including recent and commercial releases. Sub-cluster II.A included cultivars from France and Hungary. The Hungarian cultivar “Gönci magyar” was clustered close to the French cultivars “Luizet”, “Paviot”, “Bergeron”, “Bergecot”, which is a mutation of “Bergeron”, and “Bergarouge”, which is a descendent of “Bergeron” [60]. This and previous studies [22,51] support the hypothesis of the presence of Hungarian apricots in the pedigree of some French cultivars [1].

Most recently developed selections were included in sub-cluster II.B and allocated in five sub-groups. In general, recently released cultivars showed only a partial tendency to cluster by their breeding program. One of the sub-groups (II.B.i) comprised a mixture of cultivars from Cot International breeding programs such as “Justo Cot”, “Goldrich”, “Sweet Cot”, “Magic Cot”, or “Goldbar”. Sub-group II.B.ii included mainly some recent releases from France (“Tsunami”, “Tornado”, “Spring Blush”, “Samourai”) and Spain (“Colorado”, “Lido”, “Flodea”, “Rambo”, “Kosmos”). Thirteen accessions from the IPS breeding program (“Medaga”, “Priabel”, “Farbela”, “IPS16121”, “Fardao”, “Farclo”, “Fartoli”, “Farlis”, “Farfia”, “Farhial”, “Farbaly”, “Farius”, and “Mediva”) were clustered highly related in sub-group II.B.iii. In previous studies, commercial cultivars have been grouped according to their geographic origins [3,19,20,22,51,52,55,59]. However, our results showed a tendency to cluster by breeding program rather than by country of origin, probably due to the fact that genotypes developed in the same breeding program would share the same parents, although this information is unknown or has not been made public for most new apricot cultivars.

3.4. Population Structure

To study the genetic structure of the set of apricot accessions, a Principal Components Analysis (PCA) was carried, and a Discriminant Analysis of Principal Components (DAPC) to further analyze the population structure. This approach identifies and describes clusters of genetically related individuals, which provide a visual assessment of between-population genetic structures.

The two first components of PCA were represented in Figure 2. The first axis (PC-1, 12.85%) reflected population differentiation corresponding to the breeding origin, forming two main clusters. Landraces were located on the left along the x-axis, and breeding releases on the right, without a clear division between recent releases and commercial cultivars. In the second axis (PC-2, 9.05%), no clear differentiated groups were observed.

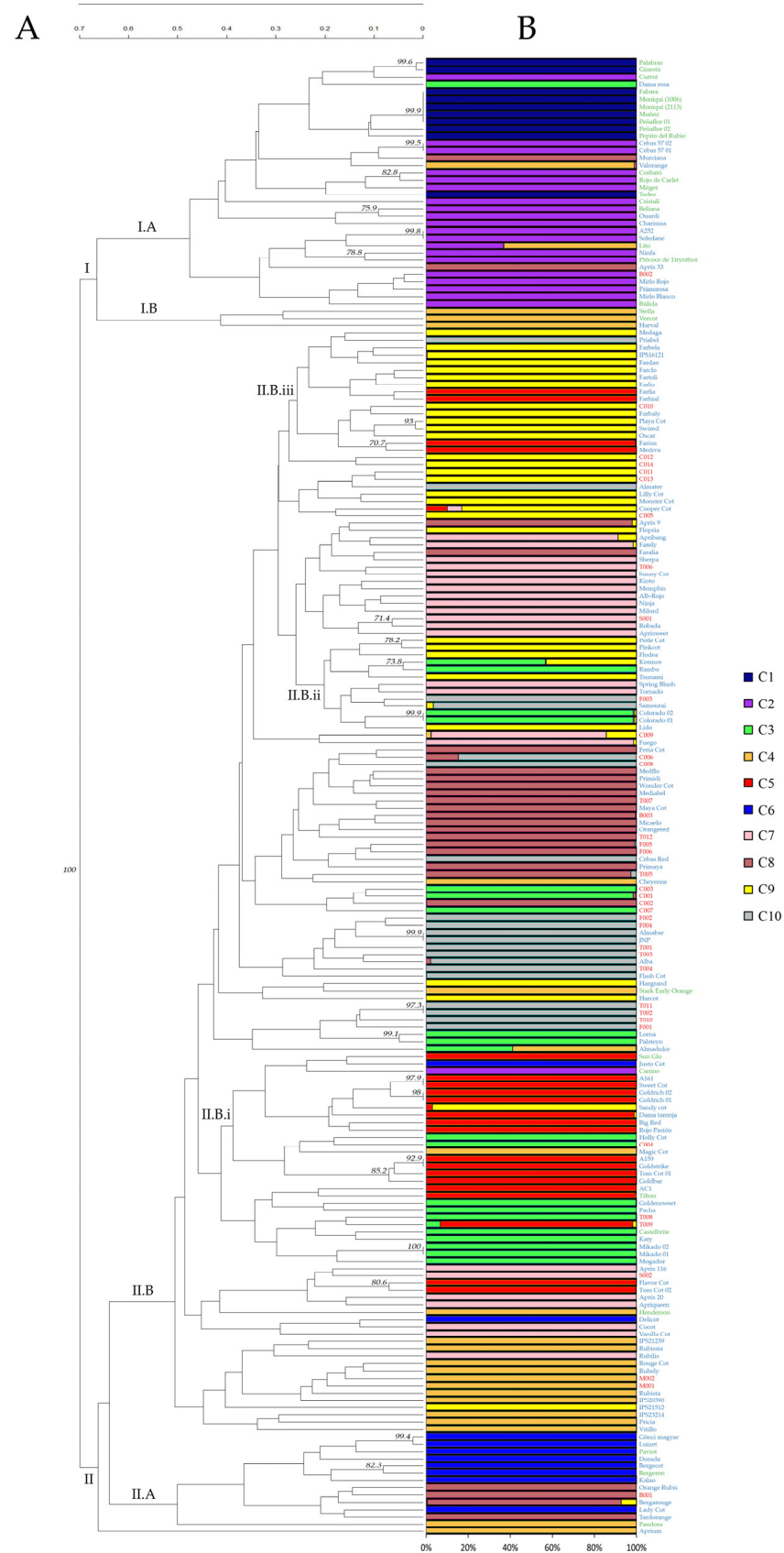


Figure 1. Clustering of the 201 apricot cultivars and one interspecific hybrid (aprium) included in this study. (A) The dendrogram was constructed based on UPGMA analysis using the similarity matrix generated by the Nei and Li coefficient. The numbers at specific nodes represent the bootstrap

support >70% that results from 1000 bootstrap replicates. Cultivars are color-coded by breeding origin: landrace (green), recent releases from breeding programs (red) and commercial releases from breeding programs (blue). **(B)** Representative estimate of population structure. The bar-plot colors indicate the individual's estimated membership fractions of each apricot cultivar to the population ($k = 10$). The clusters are depicted in dark blue (C1), in purple (C2), in green (C3), in orange (C4), in red (C5), in blue (C6), in pink (C7), in brown (C8), in yellow (C9), and in grey (C10).

In the DAPC, BIC values were used to determine the most appropriate number of clusters ($k = 10$) (Supplementary Figures S1 and S2). The first two principal components of DAPC were plotted in Figure 3. The distribution of individuals and clusters showed the same clustering trend that we detected in the molecular phylogenetic analysis (Figure 1A,B) with a hierarchical structure where three groups could be identified. Spanish landraces were included in only two clusters (Supplementary Table S4). Clusters 1 ($N = 10$) and 2 ($N = 20$) were markedly separated from the others on the first principal component (Figure 3, horizontal axis), suggesting genetic structure differentiation. Although the clusters are partially overlapped, these results corroborate the hypotheses of the existence of two main genetic pools in Spain [23]: Cluster 1 encompassed “Moniqui” and synonymies, and Cluster 2 comprised the cultivars originating in Valencia and Murcia.

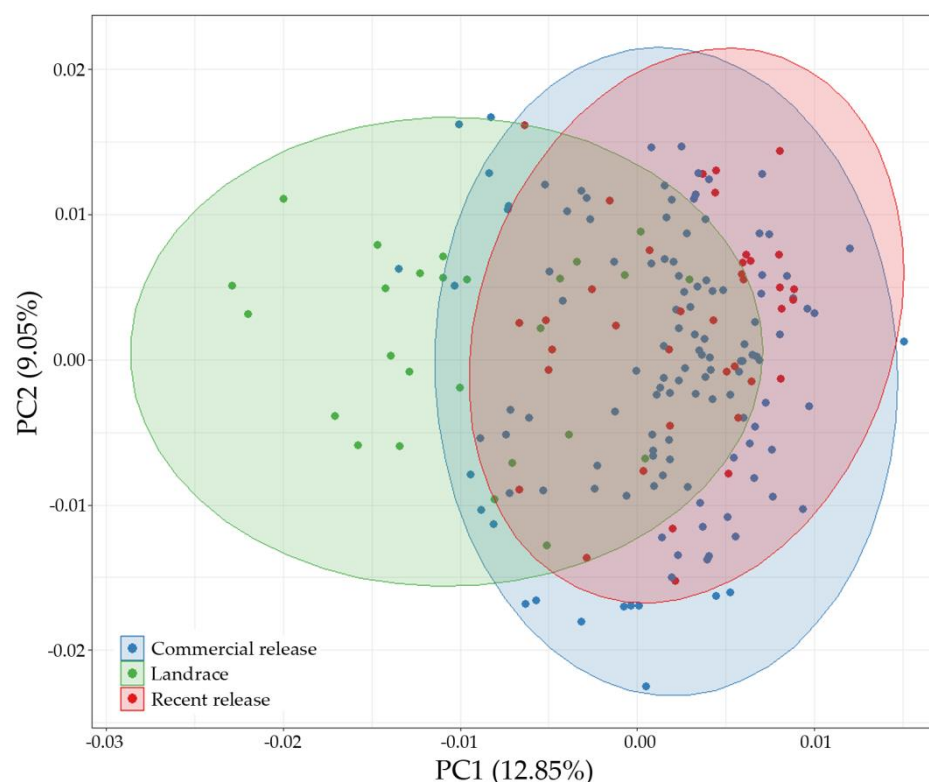


Figure 2. Principal Component Analysis based on ten microsatellite allele frequency data. Cultivars are color-coded by breeding origin: landraces (green), recent releases from breeding programs (red) and commercial releases from breeding programs (blue).

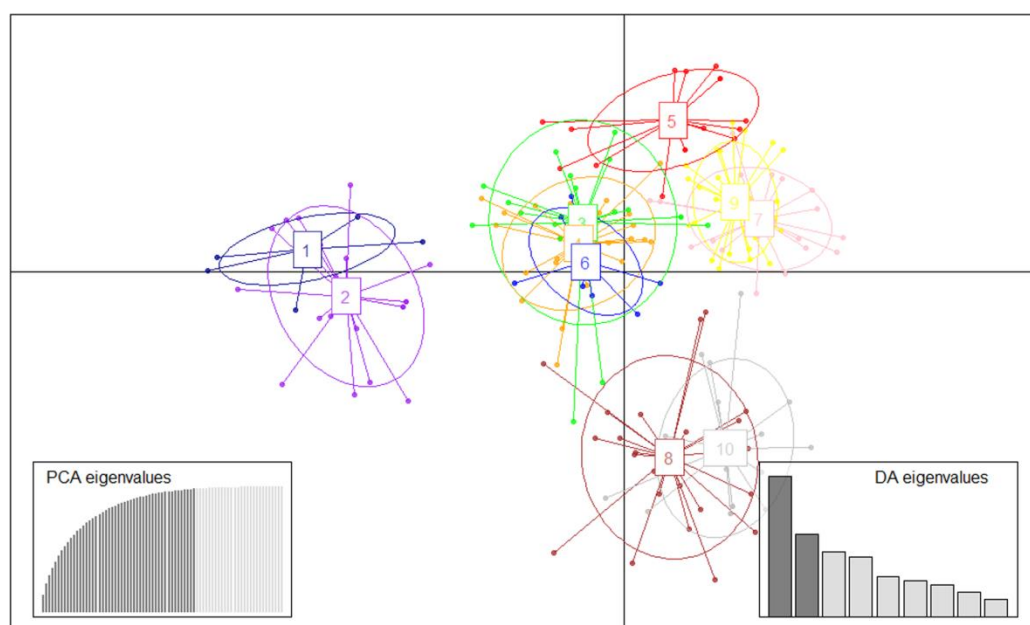


Figure 3. Scatterplot of DAPC of 202 apricot accessions, showing the first two principal linear discriminants of the DAPC according to the optimal K value ($K = 10$). Each circle represents a group and each dot represents an accession. The groups are depicted in dark blue (G1), in purple (G2), in green (G3), in orange (G4), in red (G5), in blue (G6), in pink (G7), in brown (G8), in yellow (G9), and in grey (G10). The insets represent the eigenvalues of the Principal Component Analysis (PCA) and Discriminant Analysis (DA).

Interestingly, North American cultivars (“Harval”, “Henderson”, “Stark Early Orange”, “Stella”, and “Veecot”) and most of the commercial cultivars were included in the other clusters (Supplementary Table S4). This is in agreement with the use of American genotypes as a genetic source for Sharka resistance in apricot breeding. The majority of the recent releases from Spanish breeding programs and a high number of commercial Spanish cultivars, were plotted overlapped and separately from the rest of the bred cultivars in clusters 8 ($N = 24$) and 10 ($N = 20$). Finally, the other six clusters were overlapped, indicating a tendency of the relatedness between them within the breeding program. This repeated use of some common genotypes as parental lines in most of the breeding programs may result in loss of genetic diversity and, as consequence, there is a risk of a bottleneck in future generations of this crop.

F_{ST} values between each cluster were calculated in order to study the differentiation between the populations obtained by DAPC (Figure 4; Supplementary Table S5). All the correlations were highly significant ($p < 0.001$), showing moderate or high genetic differentiation among the defined clusters. The values were higher among cluster 1 and the rest of the clusters (0.20–0.39) reflecting a great genetic differentiation. Therefore, these results provide very interesting information for breeding programs and conservation of germplasm collections. The choice of landraces from cluster 1 in breeding programs would increase the diversity of the new cultivars. Furthermore, the use of this traditional germplasm would provide a source of interesting traits that could be used to respond to new market demands or agroclimatic conditions.

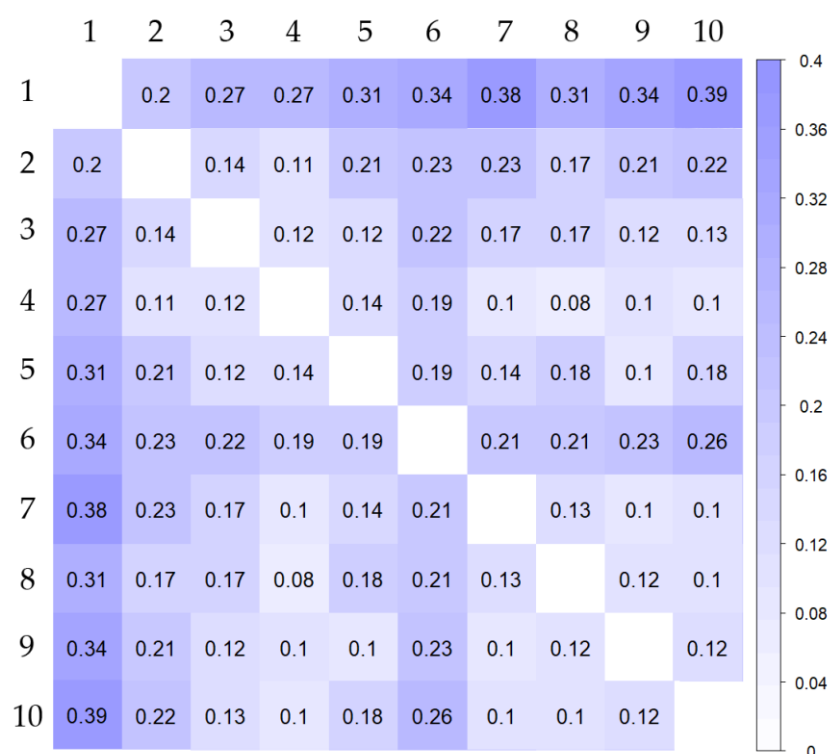


Figure 4. Corplot of pairwise F_{ST} values calculated among populations identified by DAPC using “hierfstat” v. 0.5–7 R package.

4. Conclusions

Results reveal a clear differentiation between apricot landraces, commercial cultivars and recent releases developed from breeding programs. The results showed higher diversity in bred cultivars than in landraces. This could seem a paradox but this situation could be explained by the introduction in most breeding programs of North American genotypes with alleles from Asian genotypes not present in the European landraces. As a consequence, although the introduction of new releases is increasing allelic diversity in cultivated apricot germplasm, our results suggest that the use of common parents in breeding programs can lead to a genetic bottleneck. Thus, the replacement of local landraces and traditional cultivars by genetically related bred genotypes is resulting in an erosion and decrease of the genetic diversity in grown apricot worldwide. The preservation of traditional cultivars and local germplasm is important in order to reduce the genetic erosion and conserve valuable genetic resources for further breeding.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11091714/s1>, Figure S1: Cross-validation procedure to choose the optimal number of Principal Components for the DAPC analysis. (A) A general analysis with 30 replicates of cross-validation each PC. (B) Specifying a number interval of PC around 50 with 1000 replicates. Figure S2: Graph of BIC values shows the optimum number of clusters ($k = 10$). Table S1: Genotypes of 201 diploid apricot accessions and one interspecific hybrid (aprium) revealed by 10 microsatellite markers. Table S2: Genetic parameters of apricot landraces and cultivars released from breeding programs (commercial and recent) for the 10 studied microsatellite loci. Table S3: Pairwise F_{ST} calculated among apricot accessions using “hierfstat” v. 0.5–7 R package. (A) Landraces (LN) and cultivars released from breeding programs (BP). (B) Upper limit (above the diagonal) and lower limit (below the diagonal) of the 99% confidence interval based on 1000 bootstrap replicates between LN and BP. (C) Landraces (LN), commercial (CR) and recent released selections (RR) from breeding programs. (D) Upper limit (above the diagonal) and lower limit (below the diagonal) of the 99% confidence interval based on 1000 bootstrap replicates among LN, CR, and RR. Table S4: List of accessions including group assignment from DAPC analysis ($k = 10$). Table S5: Upper limit

(above the diagonal) and lower limit (below the diagonal) of the 99% confidence interval based on 1000 bootstrap replicates among populations identified by DAPC using “hierfstat” v. 0.5–7 R package.

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MATERIAL SUPLEMENTARIO. Molecular Characterization of Genetic Diversity in Apricot Cultivars: Current Situation and Future Perspectives

El material suplementario puede ser encontrado y descargado en:

<https://www.mdpi.com/article/10.3390/agronomy11091714/s1>

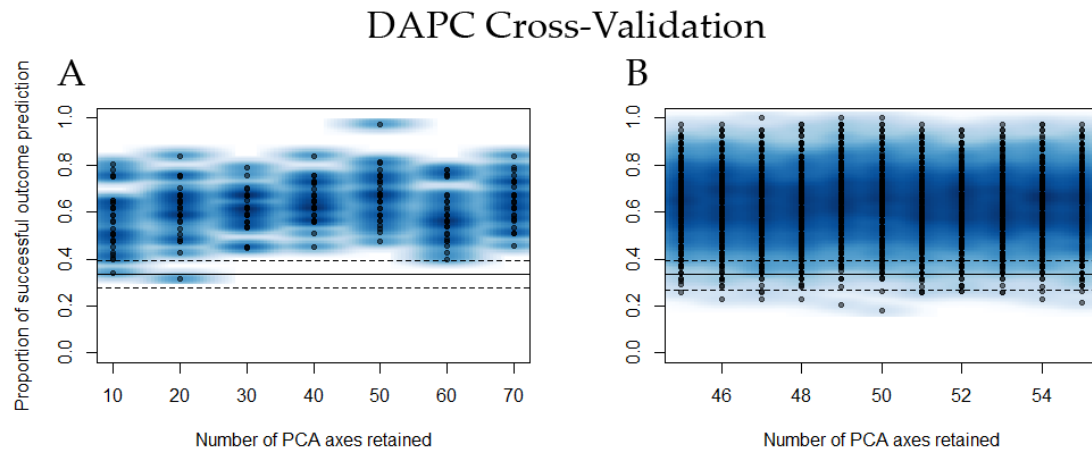


Figure S1. Cross-validation procedure to choose the optimal number of Principal Components for the DAPC analysis. A) A general analysis with 30 replicates of cross-validation each PC. B) Specifying a number interval of PC around 50 with 1000 replicates.

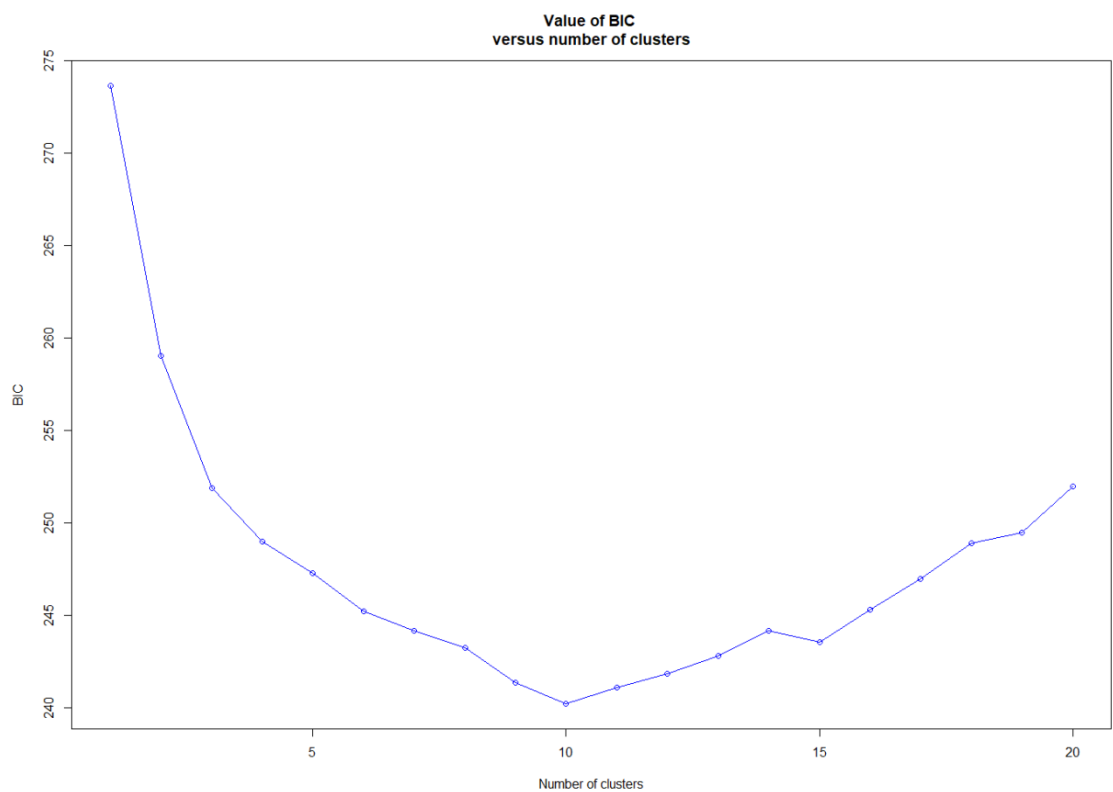


Figure S2. Graph of BIC values showing the optimum number of clusters (k=10).

Table S1. Genotypes of 201 diploid apricot accessions and one interspecific hybrid (aprium) revealed by 10 microsatellite markers.

Cultivars	pchgms3	ssrPa CITA7	ssrPa CITA10	ssrPa CITA23	ssrPa CITA27	UDAp-410	UDAp-415	UDAp-420	UDP98-409	UDP98-412
A159	190/197	212/212	168/178	142/150	253/253	125/127	159/171	166/173	133/155	108/108
A161	197/197	206/212	178/180	142/150	256/265	125/125	161/171	173/181	133/141	108/114
A252	197/197	206/212	159/164	144/150	251/265	127/129	161/161	173/179	133/137	87/87
AC1	190/195	194/212	168/178	140/142	253/265	125/127	155/159	166/171	133/133	108/114
Alba	187/197	196/214	159/176	147/156	253/253	125/127	149/161	173/181	133/161	87/108
Alb-Rojo	190/197	212/212	176/178	147/147	253/265	125/129	155/171	158/173	133/149	106/108
Almabar	187/190	194/214	176/180	147/156	253/253	129/141	161/161	158/181	133/161	108/108
Almadulce	190/197	191/212	172/178	144/144	253/256	125/129	161/173	173/173	133/141	106/108
Almater	187/197	212/212	176/180	147/156	253/265	125/127	161/161	173/173	133/161	108/108
Apribang	197/197	191/212	176/178	140/147	253/265	125/141	149/161	158/181	133/161	106/108
Apriqueen	190/197	191/194	155/178	146/147	228/253	125/129	159/171	158/173	149/155	106/108
Aprisweet	197/197	191/212	176/178	147/150	253/265	141/147	161/171	158/181	149/163	108/118
Aprium	184/197	189/191	155/159	144/152	253/269	129/131	155/161	173/187	143/161	108/125
Aprix 116	190/197	191/194	155/176	140/147	253/253	125/125	149/159	158/181	155/161	106/106
Aprix 20	190/197	194/212	176/178	147/147	228/253	125/129	159/161	158/158	149/161	106/108
Aprix 33	187/197	206/214	159/176	140/150	251/265	121/129	161/161	179/181	137/163	87/106
Aprix 9	197/197	191/206	176/180	147/150	253/265	121/125	161/161	158/173	155/161	106/108
B001	190/197	191/214	176/178	140/147	253/267	121/129	161/161	158/171	141/161	106/106
B002	187/197	194/214	170/176	144/144	253/265	129/147	149/161	173/181	153/161	87/87
B003	187/197	194/214	176/178	140/144	253/265	125/127	155/161	171/173	133/161	106/108
Beliana	197/200	194/196	159/178	140/156	253/265	127/147	149/149	158/181	133/153	87/87
Bergarouge	190/197	191/212	176/178	140/147	265/265	129/147	161/161	158/181	141/161	106/118
Bergecot	190/190	212/212	178/178	140/140	265/267	121/147	155/161	167/181	161/163	114/118
Bergeron	190/190	212/212	178/178	140/140	267/267	121/121	155/155	166/181	161/163	118/118
Big Red	190/197	194/206	178/180	140/142	256/265	125/125	155/171	166/181	133/133	108/114
Búlida	197/200	194/194	159/172	144/144	265/265	127/147	149/155	181/181	133/161	87/87
C001	187/195	212/214	172/176	142/156	265/265	127/141	155/161	173/173	133/141	87/106
C002	187/197	214/224	172/176	142/147	253/265	127/141	155/161	158/173	133/161	87/106
C003	187/197	214/224	172/180	142/156	265/265	125/141	155/161	173/173	133/161	87/106
C004	190/195	212/212	172/176	142/150	253/265	127/141	155/159	173/173	133/137	87/114
C005	197/197	191/224	176/180	156/156	265/265	125/141	161/161	158/181	141/161	108/108
C006	187/197	212/214	176/180	142/147	256/265	125/141	161/161	166/173	133/141	106/108
C007	197/197	224/224	172/176	142/156	265/265	141/141	155/171	173/181	133/161	87/87
C008	187/190	212/214	168/176	146/147	253/265	125/125	159/161	158/173	133/141	106/108
C009	197/197	191/224	168/176	147/147	253/265	125/129	161/167	173/181	137/161	108/114
C010	190/197	212/212	172/176	142/150	253/265	125/141	155/155	173/182	133/161	108/108
C011	197/197	212/224	172/176	140/147	228/265	125/125	161/161	173/181	133/141	108/108
C012	197/197	206/224	172/176	140/142	265/265	125/141	155/161	166/166	133/161	108/114
C013	190/197	212/212	176/178	147/150	228/265	125/125	161/161	173/181	133/161	108/108
C014	190/197	212/224	172/176	140/147	265/265	125/141	161/161	166/173	133/141	108/114
Canino	197/200	194/212	178/178	140/150	228/265	125/147	149/161	173/181	137/153	87/114
Castelbrite	195/197	206/212	178/180	142/142	265/265	141/141	161/171	171/173	133/133	87/87
Cebas 57 01	195/197	191/194	159/176	147/150	228/265	125/129	149/161	173/173	153/161	87/87
Cebas 57 02	195/197	191/194	159/176	147/150	228/265	125/129	149/161	173/173	153/161	87/87
Cebas Red	187/195	212/214	176/176	144/147	253/265	129/141	161/161	158/173	133/161	87/108
Charisma	190/200	194/212	159/178	142/156	253/256	125/147	149/149	158/166	133/133	87/87
Cheyenne	195/197	194/224	174/176	144/147	265/298	125/129	155/161	171/173	141/161	106/108
Cocot	190/197	191/212	153/176	140/148	253/253	121/141	155/161	158/181	149/163	106/108
Colorado 01	195/197	191/212	172/176	142/147	253/265	125/141	155/161	158/173	133/149	87/108
Colorado 02	195/197	191/212	172/176	142/147	253/265	125/141	155/161	158/173	133/149	87/108
Cooper Cot	190/197	191/194	176/180	142/156	228/265	125/125	161/171	158/181	133/161	87/108
Corbató	195/197	194/212	159/178	140/156	228/265	121/125	149/155	171/173	137/153	87/87
Cristalí	195/200	194/194	172/172	140/144	253/253	121/127	149/155	173/181	155/161	87/106
Currot	195/195	194/194	159/172	150/156	228/265	121/147	149/149	173/173	153/161	87/87
Dama rosa	195/197	194/212	172/180	142/150	253/265	125/147	149/161	173/173	133/137	87/108
Dama taronja	190/197	212/212	178/180	146/150	256/265	125/125	155/171	171/181	133/133	108/108

Cultivars	pchgms3	ssrPaCITA7	ssrPaCITA10	ssrPaCITA23	ssrPaCITA27	UDAp-410	UDAp-415	UDAp-420	UDP98-409	UDP98-412
Delicot	190/197	191/212	176/180	140/147	253/267	121/141	155/161	181/181	149/163	106/114
Dorada	190/195	194/212	178/178	140/150	265/267	125/147	155/161	166/171	137/163	106/118
F001	187/197	214/224	174/176	144/156	256/265	125/141	161/171	158/173	133/161	108/114
F002	187/195	212/214	176/180	142/147	253/253	125/141	161/161	158/173	133/133	108/108
F003	187/197	212/214	176/176	147/147	253/253	125/141	155/161	158/173	133/161	87/108
F004	187/195	212/214	172/176	142/147	253/253	129/141	155/161	158/158	133/133	108/108
F005	187/197	191/214	176/180	147/147	265/265	129/141	161/161	158/173	133/161	87/108
F006	187/197	191/214	176/176	142/147	265/265	125/129	161/161	158/181	149/161	87/108
Fabara	195/195	194/194	172/178	140/150	265/265	125/129	149/155	171/173	133/137	106/114
Faralia	187/197	206/214	176/178	147/150	265/265	125/129	161/171	158/181	133/161	106/108
Farbaly	197/197	212/212	172/176	142/150	253/265	125/153	155/155	166/181	133/161	106/108
Farbela	197/197	206/212	176/178	144/150	253/265	125/129	155/161	158/158	133/141	108/108
Farclo	197/197	212/212	172/176	144/150	265/265	125/153	155/161	158/181	133/141	106/108
Fardao	197/197	206/212	176/178	140/144	253/265	125/153	155/155	158/166	133/141	106/108
Farely	197/197	191/206	176/178	140/147	253/253	125/125	155/161	158/181	133/161	108/108
Farfia	197/197	206/212	168/178	142/150	265/265	125/153	161/171	158/181	133/141	106/108
Farhial	197/197	212/212	168/178	142/150	265/265	125/153	155/171	158/181	133/133	106/108
Farius	190/197	206/212	176/178	142/144	256/265	125/125	155/155	166/173	133/133	106/108
Farlis	197/197	191/212	172/176	147/150	265/265	129/153	155/161	158/181	141/161	106/108
Fartoli	197/197	212/212	172/176	147/150	265/265	125/147	161/161	158/181	133/141	106/108
Feria Cot	187/190	212/214	176/180	142/147	265/265	125/127	161/161	158/166	133/141	106/108
Flash Cot	187/197	212/214	155/180	147/147	253/256	125/141	161/161	171/181	133/149	106/108
Flavor Cot	190/197	194/206	150/178	146/147	253/265	125/125	159/161	166/181	133/155	106/108
Flodea	197/197	191/212	174/176	147/147	256/265	125/125	161/167	158/173	133/133	106/108
Flopria	197/197	191/206	176/178	146/147	253/265	125/141	155/161	166/173	133/155	106/108
Fuego	197/197	191/191	174/176	144/147	256/298	125/125	167/171	158/181	161/161	108/114
Ginesta	195/195	194/194	172/172	150/150	265/265	147/147	149/149	171/173	137/153	87/87
Goldbar	190/197	212/212	168/178	142/150	253/253	125/127	159/171	166/173	141/161	106/108
Goldensw.	190/190	194/212	150/172	144/150	256/265	125/127	155/173	166/173	133/133	87/106
Goldrich 01	197/197	206/212	178/180	142/146	253/256	125/125	161/171	173/181	133/137	108/114
Goldrich 02	197/197	206/212	178/180	142/146	253/256	125/125	161/171	173/181	133/137	108/114
Goldstrike	190/197	212/212	168/178	142/150	253/253	125/127	159/171	166/173	133/155	108/108
Gönci magyar	190/195	189/212	178/178	140/148	265/267	125/147	155/161	181/181	161/163	87/118
Harcot	197/197	191/212	159/168	138/142	228/265	141/153	155/159	166/181	133/141	108/110
Hargrand	197/197	189/212	176/180	142/144	265/265	127/141	159/161	173/181	141/163	108/114
Harval	180/195	194/218	176/176	146/147	251/251	129/129	149/155	173/173	133/163	108/114
Henderson	187/197	191/194	159/168	144/147	228/228	121/121	159/161	158/173	133/155	106/108
Holly Cot	190/197	212/224	172/176	142/147	253/253	125/141	159/171	171/173	133/137	87/114
IPS16121	197/197	206/206	176/176	144/147	253/265	125/129	155/161	158/166	141/161	108/108
IPS20390	195/197	210/212	150/174	144/147	253/265	129/153	149/171	158/173	161/161	87/106
IPS21239	195/197	206/210	174/180	142/144	253/253	141/153	155/161	173/173	141/155	108/108
IPS21512	195/197	210/212	174/176	142/150	253/265	125/153	149/155	166/166	161/161	108/108
IPS23214	197/197	206/212	174/180	142/144	251/251	129/153	161/161	158/166	133/155	87/108
JNP	187/190	194/214	176/180	147/156	253/253	129/141	161/161	158/181	133/161	108/108
Justo Cot	190/197	212/212	155/178	140/146	253/267	125/129	161/161	173/181	137/161	114/118
Kalao	190/197	212/212	176/178	140/147	265/267	121/141	155/155	159/181	133/161	106/114
Katy	190/195	212/212	180/180	142/150	265/265	125/141	155/171	171/173	133/133	87/108
Kioto	197/197	212/212	176/178	142/147	253/265	141/141	155/171	158/181	133/161	106/108
Kosmos	197/197	191/224	176/180	147/148	265/265	125/141	161/171	158/173	133/133	87/108
Lady Cot	190/197	191/212	155/178	140/147	253/267	121/129	155/161	158/181	161/161	106/118
Lido	197/197	191/212	176/176	142/147	265/265	125/141	155/161	158/173	133/161	106/108
Lilly Cot	190/197	191/212	174/176	142/147	256/265	125/127	155/161	173/181	141/161	106/108
Lito	197/197	194/212	168/172	144/147	265/265	127/129	161/161	173/179	133/141	87/106
Lorna	197/197	212/224	174/174	144/156	256/256	141/141	161/171	173/173	133/133	114/114
Luizet	190/195	189/212	178/178	140/147	265/267	125/147	155/161	181/181	161/163	87/118
M001	190/197	206/212	174/176	144/147	228/265	125/127	149/161	166/173	133/161	106/108
M002	197/197	206/212	174/176	144/147	228/253	127/129	161/161	158/158	155/161	106/106
Magic Cot	190/197	212/224	168/176	147/150	253/267	125/127	159/161	171/173	137/141	108/114
Maya Cot	187/197	191/194	159/176	147/156	265/265	125/127	149/161	158/173	133/141	87/106
Medaga	197/197	191/212	176/176	144/144	265/265	125/153	161/161	158/166	133/141	108/108

Cultivars	pchgms3	ssrPaCITA7	ssrPaCITA10	ssrPaCITA23	ssrPaCITA27	UDAp-410	UDAp-415	UDAp-420	UDP98-409	UDP98-412
Medflo	187/197	206/214	176/180	147/147	253/265	125/125	161/161	158/173	141/161	106/106
Mediabel	187/197	206/214	176/178	146/147	253/265	125/129	161/161	173/173	141/155	106/106
Mediva	190/197	206/212	168/176	142/142	265/265	125/125	155/155	173/181	133/133	106/108
Memphis	197/197	212/212	172/176	140/147	253/253	125/141	155/171	158/166	133/161	108/108
Micaelo	187/190	214/214	176/178	140/147	253/265	125/127	161/161	171/173	133/161	106/108
Mikado 01	190/195	212/212	172/178	142/147	253/256	141/141	155/161	158/181	133/133	87/87
Mikado 02	190/195	212/212	172/178	142/147	253/256	141/141	155/161	158/181	133/133	87/87
Milord	190/197	191/212	176/178	147/150	253/253	125/129	155/171	158/181	133/149	106/108
Mirlo Blanco	187/200	194/214	170/176	144/156	253/265	127/129	155/161	173/181	133/153	87/87
Mirlo Rojo	187/197	194/214	170/176	144/144	265/265	129/147	149/161	173/181	161/161	87/87
Mitger	195/197	194/212	159/159	156/156	228/265	121/121	155/155	171/173	161/161	87/87
Mogador	190/195	212/212	176/180	147/148	253/256	125/141	155/161	173/173	133/149	87/87
Moniquí (1006)	195/195	194/194	172/178	140/150	265/265	125/129	149/155	171/173	133/137	106/114
Moniquí (2113)	195/195	194/194	172/178	140/150	265/265	125/129	149/155	171/173	133/137	106/114
Monster Cot	190/197	191/212	176/180	142/147	265/265	127/129	161/167	173/181	141/161	108/108
Muñoz	195/195	194/194	172/178	140/150	265/265	125/129	149/155	171/173	133/137	106/114
Murciana	195/197	191/194	159/176	147/150	265/265	125/147	149/161	173/173	141/161	87/106
Ninfa	197/200	196/206	159/172	144/156	265/265	121/129	149/167	158/179	133/133	87/87
Ninja	190/197	212/212	176/176	147/147	253/265	125/141	155/171	166/181	133/149	106/108
Orange Rubis	187/190	212/214	176/178	140/147	265/267	121/125	161/161	158/181	161/163	106/106
Orangered	187/197	191/214	159/178	144/147	253/265	125/129	161/161	158/173	NA/NA	106/108
Oscar	197/197	206/212	172/176	147/150	265/265	125/141	155/155	159/166	133/133	108/108
Ouardi	197/200	194/196	159/178	140/156	228/265	121/147	149/149	158/173	133/137	87/87
Pacha	190/195	212/224	155/172	142/147	265/265	125/129	155/171	166/173	133/133	87/106
Palabras	195/195	194/194	172/172	150/150	265/265	125/147	149/149	171/173	137/153	87/87
Palsteyn	195/197	212/224	172/174	144/156	256/256	141/141	161/171	166/173	133/133	114/114
Pandora	195/197	208/212	150/176	147/148	251/267	121/125	161/161	166/173	137/153	87/106
Paviot	195/197	189/212	178/178	140/150	265/267	125/127	161/161	181/181	133/161	114/118
Peñaflor 01	195/195	194/194	172/178	140/150	265/265	125/129	149/155	171/173	133/137	106/114
Peñaflor 02	195/195	189/194	172/178	140/150	265/265	125/125	149/161	171/181	133/137	114/118
Pepito del Rubio	195/197	194/194	172/178	150/150	265/265	125/127	149/155	171/181	133/137	87/114
Perle Cot	190/197	191/212	174/176	142/147	256/265	125/141	155/161	173/181	133/133	108/108
Pinkcot	190/197	191/212	174/176	142/147	256/265	125/141	155/167	166/173	133/133	108/108
Playa Cot	197/197	212/224	176/176	147/150	256/265	125/125	155/155	166/181	133/133	108/108
Précoce de Tiryntos	197/200	194/206	164/172	144/144	265/265	129/129	161/167	179/181	133/133	87/87
Priabel	187/197	212/214	176/176	144/147	253/265	125/141	159/161	158/181	141/141	108/108
Pricia	197/197	206/224	174/180	156/156	228/253	127/129	161/161	158/181	149/161	87/87
Primaya	187/197	206/214	176/180	142/147	253/265	129/141	155/161	173/181	149/161	87/106
Primidi	187/197	206/214	176/180	147/156	265/265	125/129	140/161	158/173	141/141	106/106
Primorosa	187/200	194/214	170/176	144/144	265/265	127/129	149/161	173/181	153/161	87/87
Rambo	197/197	191/224	174/180	147/148	253/265	125/141	161/171	158/173	133/133	87/108
Robada	197/197	191/212	155/176	147/147	253/253	129/141	161/171	158/181	149/161	106/108
Rojo de Carlet	195/197	194/212	159/178	140/156	228/265	121/125	149/155	171/173	133/161	87/87
Rojo Pasión	195/197	194/206	172/178	142/150	256/265	125/125	155/171	173/181	133/137	87/108
Rouge Cot	195/197	206/212	174/176	144/147	228/253	127/141	149/161	173/181	161/161	106/106
Rubely	195/197	190/210	174/176	144/147	228/253	127/129	149/161	158/173	161/161	106/108
Rubilis	197/197	191/206	155/174	144/147	253/298	141/153	149/171	158/181	149/155	87/108
Rubissia	195/197	210/212	174/176	144/147	253/298	127/141	149/161	173/181	149/161	108/108
Rubista	195/197	206/210	174/178	144/156	228/228	127/153	149/161	158/173	155/161	87/106
S001	197/197	191/212	155/176	147/147	253/265	129/141	161/171	158/181	141/149	106/118
S002	190/197	206/212	150/176	147/147	253/253	125/141	159/161	173/181	149/155	106/106
Samourai	190/197	212/212	176/176	147/147	253/256	125/141	155/161	158/173	133/133	87/108
Sandy cot	197/197	191/212	176/180	142/146	253/265	125/125	161/161	181/181	133/161	108/114

Cultivars	pchgms3	ssrPa CITA7	ssrPa CITA10	ssrPa CITA23	ssrPa CITA27	UDAp-410	UDAp-415	UDAp-420	UDP98-409	UDP98-412
Sherpa	197/197	191/206	176/178	147/150	253/265	125/129	161/171	158/181	133/149	106/108
Soledane	197/197	206/212	159/164	144/150	251/265	127/129	161/161	173/179	133/137	87/87
Spring Blush	197/197	212/224	155/176	147/147	253/265	125/129	161/161	158/158	133/149	87/108
Stark Early Orange	197/197	191/212	168/176	144/147	251/265	121/127	159/161	173/173	137/141	106/108
Stella	195/195	189/189	172/176	146/154	251/251	127/127	155/161	158/158	133/137	106/106
Sun Glo	197/197	212/212	178/178	146/150	253/265	125/127	161/161	173/181	133/137	106/114
Sunny Cot	197/197	206/212	150/176	146/147	253/253	125/129	161/171	158/166	155/161	106/108
Sweet Cot	197/197	206/212	178/180	142/150	256/265	125/125	161/171	173/181	133/141	108/114
Swired	197/197	212/224	176/176	147/150	256/265	125/125	155/155	166/181	133/161	108/108
T001	187/197	191/214	176/176	142/156	253/253	141/141	155/161	158/173	133/161	87/108
T002	187/197	212/214	174/176	147/156	253/256	125/141	161/161	158/173	133/161	106/114
T003	187/197	212/214	174/176	147/156	253/253	127/129	159/161	173/181	133/161	108/108
T004	190/197	191/194	174/176	147/156	253/253	121/129	161/161	173/173	133/141	108/108
T005	187/195	194/214	172/176	144/150	265/265	125/125	155/161	158/173	133/141	106/108
T006	197/197	212/214	168/176	147/150	253/253	125/127	161/171	158/173	155/161	106/108
T007	187/197	196/214	159/176	147/156	265/265	121/125	149/161	158/158	133/141	87/106
T008	190/197	212/212	168/172	140/142	265/265	141/147	161/171	171/173	133/133	108/114
T009	190/197	212/212	178/180	140/142	253/265	125/147	161/161	166/173	133/133	106/108
T010	187/197	212/214	174/176	144/144	253/256	125/141	161/171	158/173	133/141	106/114
T011	187/197	212/214	174/176	147/156	253/256	125/141	161/161	158/173	133/161	106/114
T012	187/197	191/214	178/178	147/150	265/265	125/127	155/161	158/171	137/161	106/106
Tadeo	195/195	194/194	159/172	156/156	228/265	121/129	155/161	171/171	133/161	87/106
Tardorange	197/197	191/214	159/176	147/150	267/267	121/129	155/161	158/181	161/161	106/106
Tilton	190/197	189/194	178/178	140/150	228/265	125/127	155/161	166/181	133/133	87/114
Tom Cot 01	190/197	212/212	168/178	142/150	253/253	125/125	159/171	166/173	155/155	106/108
Tom Cot 02	190/197	194/206	150/178	146/147	228/253	125/125	159/161	166/173	155/155	106/108
Tornado	197/197	212/224	176/176	147/147	253/253	125/141	161/161	158/181	133/149	108/108
Tsunami	197/197	191/224	176/176	147/147	265/265	127/141	161/167	158/173	133/133	87/108
Valorange	195/197	191/194	159/176	147/150	228/253	125/147	149/161	158/173	141/159	87/106
Vanilla Cot	195/197	191/208	153/176	147/148	253/253	125/141	140/161	156/158	149/149	108/114
Veecot	190/195	189/194	176/180	144/146	251/265	153/153	155/161	158/173	133/163	106/114
Vitillo	197/197	189/206	178/180	142/150	228/228	127/143	161/161	173/181	133/141	106/108
Wonder Cot	187/197	191/214	176/180	147/156	265/265	125/127	161/161	158/173	141/161	106/108

Table S2. Genetic parameters of apricot landraces and cultivars released from breeding programs (commercial and recent) for 10 microsatellite loci.

Landrace (N=30)							
Loci	N_a	A_r	P_a	H_o	H_e	F_{IS}	P-value
UDAp_415	6	5.4	0	0.67	0.72	0.07	0.06
ssrPaCITA7	7	6.4	0	0.50	0.61	0.18	0.46
UDAp_420	6	5.9	0	0.80	0.76	-0.06	0.12
ssrPaCITA10	8	7.4	0	0.70	0.76	0.08	0.29
ssrPaCITA23	9	8.4	1	0.70	0.82	0.15	0.00
ssrPaCITA27	5	5.0	0	0.43	0.57	0.24	0.04
pchgms3	5	4.7	0	0.50	0.63	0.21	0.07
UDAp_410	7	6.9	0	0.70	0.83	0.15	0.07
UDP98_409	7	6.8	0	0.87	0.76	-0.14	0.13
UDP98_412	5	4.9	0	0.93	0.84	-0.12	0.00
Total	65	62	1				
Mean	6.50	6.20	0.10	0.68	0.73	0.08	

Breeding program (N=172)							
Loci	N_a	A_r	P_a	H_o	H_e	F_{IS}	P-value
UDAp_415	8	6.3	2	0.70	0.68	-0.03	0.55
ssrPaCITA7	12	8.3	5	0.82	0.78	-0.05	0.10
UDAp_420	11	6.4	5	0.84	0.76	-0.12	0.21
ssrPaCITA10	12	9.5	4	0.87	0.79	-0.10	0.12
ssrPaCITA23	10	7.9	2	0.85	0.80	-0.06	0.01
ssrPaCITA27	8	6.3	3	0.59	0.68	0.13	0.01
pchgms3	7	4.9	2	0.67	0.61	-0.11	0.08
UDAp_410	9	7.1	2	0.81	0.76	-0.07	0.21
UDP98_409	10	7.7	3	0.76	0.76	-0.01	0.16
UDP98_412	7	5.1	2	0.75	0.73	-0.02	0.00
Total	94	70	30				
Mean	9.40*	6.97	3.00	0.77	0.74	-0.04	

* Percentage of missing data 0.06%

Recent release (N=39)

Loci	N_a	A_r	P_a	H_o	H_e	F_{IS}	P-value
UDAp_415	6	5.6	0	0.62	0.55	-0.11	1.00
ssrPaCITA7	7	6.6	0	0.85	0.77	-0.10	0.07
UDAp_420	6	5.6	1	0.79	0.71	-0.13	0.44
ssrPaCITA10	10	8.5	0	0.90	0.72	-0.25	0.47
ssrPaCITA23	7	6.6	0	0.79	0.79	-0.01	0.56
ssrPaCITA27	5	4.6	0	0.49	0.62	0.21	0.35
pchgms3	4	4.0	0	0.79	0.63	-0.26	0.00
UDAp_410	6	5.9	0	0.85	0.74	-0.14	0.49
UDP98_409	7	6.5	0	0.90	0.71	-0.26	0.01
UDP98_412	5	4.6	0	0.69	0.73	0.05	0.77
Total	63	58	1				
Mean	6.30	5.84	0.10	0.77	0.70	-0.10	

Commercial release (N=133)

Loci	N_a	A_r	P_a	H_o	H_e	F_{IS}	P-value
UDAp_415	8	6.5	2	0.73	0.71	-0.02	0.49
ssrPaCITA7	12	8.6	3	0.81	0.78	-0.05	0.12
UDAp_420	10	6.5	4	0.86	0.76	-0.12	0.10
ssrPaCITA10	12	9.8	1	0.86	0.80	-0.08	0.38
ssrPaCITA23	10	8.1	2	0.86	0.81	-0.07	0.03
ssrPaCITA27	8	6.6	2	0.62	0.70	0.11	0.05
pchgms3	7	5.1	2	0.64	0.59	-0.07	0.23
UDAp_410	9	7.3	2	0.80	0.76	-0.05	0.36
UDP98_409	10	7.9	2	0.72	0.77	0.06	0.10
UDP98_412	7	5.3	2	0.77	0.74	-0.04	0.00
Total	93	72	22				
Mean	9.30*	7.17	2.20	0.77	0.74	-0.03	

* Percentage of missing data 0.08%

N_a: Number of alleles; A_r: Allelic richness ; P_a: Number of private alleles; H_o: Observed heterozygosity; H_e: Expected heterozygosity; F_{IS}: inbreeding coefficient

Table S3. Pairwise FST calculated among apricot accessions using “hierfstat” v. 0.5–7 R package. (A) Landraces (LN) and cultivars released from breeding programs (BP). (B) Upper limit (above the diagonal) and lower limit (below the diagonal) of the 99% confidence interval based on 1000 bootstrap replicates between LN and BP. (C) Landraces (LN), commercial (CR) and recent released selections (RR) from breeding programs. (D) Upper limit (above the diagonal) and lower limit (below the diagonal) of the 99% confidence interval based on 1000 bootstrap replicates among LN, CR, and RR.

A)			
	LN	BP	
LN		0.09	
BP	0.09		

B)			
	LN	BP	
LN		0.14	
BP	0.06		

C)			
	LN	CR	
CR	0.09		
RR	0.12	0.01	

D)				
	LN	CR	RR	
LN		0.12	0.17	
CR	0.06		0.03	
RR	0.08	0.03		

Table S4. List of accessions including group assignment from DAPC analysis (k = 10).

Accession	Number of cluster	Accession	Number of cluster	Accession	Number of cluster
Fabara	1	Katy	3	Flavor Cot	5
Ginesta	1	Lorna	3	Goldbar	5
Moniquí (1006)	1	Mikado 01	3	Goldrich 01	5
Moniquí (2113)	1	Mikado 02	3	Goldrich 02	5
Muñoz	1	Mogador	3	Goldstrike	5
Palabras	1	Pacha	3	Mediva	5
Peñaflor 01	1	Palsteyn	3	Rojo Pasión	5
Peñaflor 02	1	Rambo	3	Sun Glo	5
Pepito del Rubio	1	T008	3	Sweet Cot	5
Tadeo	1	Almadulce	4	T009	5
A252	2	Aprium	4	Tilton	5
B002	2	Cheyenne	4	Tom Cot 01	5
Beliana	2	Harval	4	Tom Cot 02	5
Búlida	2	Henderson	4	Bergecot	6
Canino	2	IPS20390	4	Bergeron	6
Cebas 57 01	2	IPS21239	4	Delicot	6
Cebas 57 02	2	IPS23214	4	Dorada	6
Charisma	2	Lito	4	Gönci magyar	6
Corbató	2	M001	4	Justo Cot	6
Cristalí	2	M002	4	Kalao	6
Currot	2	Magic Cot	4	Lady Cot	6
Mirlo Blanco	2	Pandora	4	Luizet	6
Mirlo Rojo	2	Pricia	4	Paviot	6
Mitger	2	Rouge Cot	4	Alb-Rojo	7
Ninfa	2	Rubely	4	Apribang	7
Ouardi	2	Rubissia	4	Apriqueen	7
Précoce de Tiryntos	2	Rubista	4	Aprisweet	7
Primorosa	2	Stark Early Orange	4	Aprix 116	7
Rojo de Carlet	2	Stella	4	Aprix 20	7
Soledane	2	Valorange	4	C009	7
C001	3	Veecot	4	Cocot	7
C003	3	Vitillo	4	Farely	7
C004	3	A159	5	Fuego	7
C007	3	A161	5	Kioto	7
Castelbrite	3	AC1	5	Memphis	7
Colorado 01	3	Big Red	5	Milord	7
Colorado 02	3	Dama taronja	5	Ninja	7
Dama rosa	3	Farfia	5	Robada	7
Goldensweet	3	Farhial	5	Rubilis	7
Holly Cot	3	Farius	5	S001	7

Accession	Number of cluster	Accession	Number of cluster
S002	7	Flodea	9
Sherpa	7	Flopria	9
Spring Blush	7	Harcot	9
Sunny Cot	7	Hargrand	9
T006	7	IPS16121	9
Tornado	7	IPS21512	9
Vanilla Cot	7	Kosmos	9
Aprix 33	8	Lido	9
Aprix 9	8	Lilly Cot	9
B001	8	Medaga	9
B003	8	Monster Cot	9
Bergarouge	8	Oscar	9
C002	8	Perle Cot	9
F005	8	Pinkcot	9
F006	8	Playa Cot	9
Faralia	8	Sandy cot	9
Feria Cot	8	Swired	9
Maya Cot	8	Tsunami	9
Medflo	8	Alba	10
Mediabel	8	Almabar	10
Micaelo	8	Almater	10
Murciana	8	C006	10
Orange Rubis	8	C008	10
Orangered	8	Cebas Red	10
Primaya	8	F001	10
Primidi	8	F002	10
T005	8	F003	10
T007	8	F004	10
T012	8	Flash Cot	10
Tardorange	8	JNP	10
Wonder Cot	8	Priabel	10
C005	9	Samourai	10
C010	9	T001	10
C011	9	T002	10
C012	9	T003	10
C013	9	T004	10
C014	9	T010	10
Cooper Cot	9	T011	10
Farbaly	9		
Farbela	9		
Farclo	9		
Fardao	9		
Farlis	9		
Fartoli	9		

Table S5. Upper limit (above the diagonal) and lower limit (below the diagonal) of the 99% confidence interval based on 1000 bootstrap replicates among populations identified by DAPC using “hierfstat” v. 0.5–7 R package.

	1	2	3	4	5	6	7	8	9	10
1		0.323	0.422	0.350	0.460	0.523	0.543	0.431	0.5474	0.516
2	0.087		0.198	0.265	0.369	0.380	0.393	0.338	0.411	0.391
3	0.143	0.081		0.186	0.215	0.308	0.248	0.232	0.229	0.190
4	0.156	0.041	0.061		0.201	0.141	0.246	0.161	0.173	0.141
5	0.185	0.099	0.034	0.082		0.266	0.232	0.230	0.201	0.254
6	0.183	0.114	0.115	0.099	0.121		0.309	0.277	0.350	0.339
7	0.201	0.137	0.095	0.052	0.053	0.135		0.252	0.235	0.187
8	0.151	0.061	0.103	0.020	0.137	0.111	0.052		0.224	0.240
9	0.158	0.087	0.046	0.062	0.045	0.118	0.026	0.035		0.272
10	0.251	0.117	0.080	0.042	0.087	0.177	0.031	0.015	0.017	

DISCUSIÓN GENERAL



DISCUSIÓN GENERAL

A pesar de la importancia del albaricoquero a nivel mundial, el cultivo presenta grandes limitaciones (Zhebentyayeva et al., 2012). Programas de mejora en todo el mundo tienen como objetivo obtener nuevas variedades resistentes al Plum Pox Virus (PPV), pero también autocompatibles, con fruta de alta calidad y con amplia adaptabilidad a las condiciones climáticas (Karayiannis, 2006; Martínez-Calvo et al., 2009; Krška and Vachůn, 2016; Fremondiere et al., 2018; Ruiz et al., 2018). La enfermedad de la Sharka causada por el PPV es el factor más limitante para la producción del albaricoquero en los países europeos (García and Cambra, 2007). Los genes de resistencia al virus de la Sharka probablemente derivan del germoplasma asiático (Badenes et al., 1996), ya que se han encontrado principalmente en variedades de América del Norte (por ejemplo, ‘Goldrich’, ‘Henderson’, ‘Stark Early Orange’ y ‘Stella’) que fueron obtenidas a través de cruzamientos con especies asiáticas como *P. mandshurica* (Bassi and Audergon, 2006). Los programas de mejora de albaricoquero encuentran importantes limitaciones en relación con el desarrollo de nuevas variedades resistentes a PPV, ya que los parentales norteamericanos resistentes a PPV tienen altas necesidades de frío, son autoincompatibles y tienen un período de maduración de medio a tardío. Esto ha provocado que se desconozcan las necesidades agroclimáticas y el carácter de auto(in)compatibilidad de muchas de las nuevas variedades obtenidas.

Para dar una respuesta a la falta de información disponible sobre las características de las nuevas variedades comerciales de albaricoquero y evaluar la situación actual de la estructura varietal, en esta tesis se ha profundizado en el estudio de las necesidades de polinización y agroclimáticas de variedades tradicionales y nuevas obtenciones, así como en el análisis de la diversidad genética del material vegetal disponible en la actualidad.

La polinización como condicionante del cultivo

Variedades autocompatibles y variedades autoincompatibles

La importancia de la auto(in)compatibilidad en el cultivo del albaricoquero se ha incrementado en los últimos años. A diferencia del almendro, cerezo o ciruelo japonés, las variedades de albaricoquero tradicionalmente cultivadas en Europa eran autocompatibles, por lo que no era necesaria la presencia de árboles de variedades polinizadoras en las plantaciones (Herrera et al., 2021b). Sin embargo, a finales de los 90 del siglo XX se detectaron algunas variedades autoincompatibles originadas por cruces con variedades autoincompatibles procedentes de América del Norte (Burgos et al., 1997). La presencia de variedades autoincompatibles se ha incrementado posteriormente, debido al desarrollo y utilización de nuevas variedades autoincompatibles como parentales en los programas de mejora por ser resistentes a PPV (Milatović et al., 2010, 2013a, 2013b). Como consecuencia, el conocimiento de la auto(in)compatibilidad de las nuevas variedades y de las selecciones de los programas de mejora es de gran importancia para mejoradores, viveristas y productores. Los resultados de esta tesis han permitido la identificación de 87 variedades autocompatibles. En 50 de ellas, la autocompatibilidad ha sido determinada mediante la observación al microscopio del crecimiento de los tubos polínicos en flores autopolinizadas, y en 63 variedades la caracterización del genotipo *S* ha permitido identificar el alelo *S_c* [Capítulo 1 (Herrera et al., 2020a), capítulo 2 (Herrera et al., 2018a), capítulo 3 (Herrera et al., 2018b), capítulo 4 (Herrera et al., 2022c)]. En albaricoquero, el principal marcador de autocompatibilidad es el alelo *S_c* (Burgos et al., 1998; Halász et al., 2007). Los resultados del análisis de diversidad del locus *S* han mostrado la alta frecuencia del alelo *S_c* en las variedades procedentes de los programas de mejora [Capítulo 4 (Herrera et al., 2022c)].

En los últimos años está aumentando el número de nuevas variedades comerciales autocompatibles [Capítulo 2 (Herrera et al., 2018a) y capítulo 3 (Herrera et al., 2018b)]. Sin embargo, el número de variedades autoincompatibles que hay en el mercado sigue siendo importante, por lo que es necesario considerar las necesidades de polinización en el diseño de las plantaciones. Para el cultivo de variedades autoincompatibles es necesaria la presencia de árboles de variedades polinizadoras en las plantaciones que coincidan en floración y aseguren la presencia de polen compatible.

En esta tesis se han caracterizado 49 variedades autoincompatibles [Capítulo 1 (Herrera et al., 2020a), capítulo 2 (Herrera et al., 2018a), capítulo 3 (Herrera et al., 2018b) y capítulo 4 (Herrera et al., 2022c)] que, junto a 35 variedades analizadas en estudios previos (Egea and Burgos, 1996; Burgos et al., 1998; Halász et al., 2005, 2010; Donoso et al., 2009; Lachkar et al., 2013; Muñoz-Sanz et al., 2017; Herrera et al., 2022b), han permitido la clasificación de las variedades comerciales más importantes cultivadas actualmente en sus correspondientes grupos de incompatibilidad, lo que facilita la selección de variedades polinizadoras para cada variedad autoincompatible. Sin embargo, la intensa renovación varietal que se sigue produciendo en este cultivo hace necesario continuar con el análisis de las necesidades de polinización de nuevas variedades.

Avances en la identificación de la auto(in)compatibilidad

En los últimos años, el desarrollo de marcadores moleculares para el genotipado *S* ha provocado cambios en la metodología utilizada para identificar la auto(in)compatibilidad en albaricoquero. Los métodos moleculares están desplazando a los ensayos de polinizaciones controladas en campo y seguimiento del cuajado, así como a los de polinizaciones en laboratorio y posterior observación al microscopio del crecimiento de los tubos polínicos en flores autopolinizadas, métodos que han sido ampliamente usados para determinar la auto(in)compatibilidad (Rodrigo and Herrero, 1996; Burgos et al., 1997; Andres and Duran, 1998; Milatović et al., 2010, 2013a, 2013b, 2018). El uso de cebadores específicos permite la detección de genotipos autoincompatibles a través de PCR en las primeras etapas del desarrollo de la planta, a diferencia de los métodos tradicionales que necesitan árboles adultos con flores y pueden ser influenciados por las condiciones ambientales y el manejo del cultivo (Yamane and Tao, 2009).

Sin embargo, en algunos casos el análisis de los alelos *S* mediante la identificación por genotipado molecular puede proporcionar una identificación inexacta sobre la auto(in)compatibilidad. La imprecisión del método basado en PCR podría reflejarse en la amplificación de un solo alelo en algunas accesiones. En esta tesis, solo se ha identificado un único alelo *S* en 29 de los genotipos analizados después de la amplificación por PCR [Capítulo 2 (Herrera et al., 2018a) y capítulo 3 (Herrera et al., 2018b)]. Esto podría ser debido a homocigosis en el caso del alelo *S_c* o a problemas de

amplificación, como puede ser el caso de 11 variedades en las que, posteriormente, se pudo identificar un segundo alelo con el desarrollo de cebadores alelo-específicos [Capítulo 4 (Herrera et al., 2022c)]. En albaricoquero, incluyendo los resultados de esta tesis, se han identificado 33 haplotipos S [Capítulo 1 (Herrera et al., 2020a), capítulo 2 (Herrera et al., 2018a), capítulo 3 (Herrera et al., 2018b) y capítulo 4 (Herrera et al., 2022c)] (Halász et al., 2005; Vilanova et al., 2005; Zhang et al., 2008; Muñoz-Sanz et al., 2017; Murathan et al., 2017). Recientemente, se han encontrado discrepancias y anotaciones dobles en asignaciones de algunos alelos S, como en el caso de S_9 , S_{17} y S_{44} (Muñoz-Sanz et al., 2017). En esta tesis se han detectado similitudes entre los alelos S_{52} (Murathan et al., 2017) y S_6 [Capítulo 2 (Herrera et al., 2018a)], entre los alelos S_{20} (Zhang et al., 2008) y S_{55} , y entre los alelos S_7 , S_{13} y S_{46} [Capítulo 3 (Herrera et al., 2018b)]. Además, se espera que el número de alelos S aumente a medida que se estudien más variedades (Yamane and Tao, 2009; Herrera et al., 2021b). Esta situación podría ocasionar errores en la detección de la auto(in)compatibilidad en el futuro, pudiendo provocar fallos en la caracterización del genotipo S y una errónea clasificación en los grupos de incompatibilidad, por lo que es necesario la estandarización de criterios para la identificación de alelos S a través del uso de los mismos pares de cebadores y la secuenciación completa de los alelos S.

Los estudios moleculares y genéticos han revelado que la autocompatibilidad en albaricoquero, así como en otras Rosáceas como peral asiático, albaricoquero japonés y cerezo, está causada principalmente por mutaciones en los genes del locus S (Ushijima et al., 2004; Vilanova et al., 2006; Marchese et al., 2007; Yamane et al., 2009). Sin embargo, existen otras fuentes de autocompatibilidad no relacionadas con el locus S, como las que se han encontrado en cerezo (Wünsch and Hormaza, 2004; Wünsch et al., 2010) o almendro (Fernández i Martí et al., 2014). En albaricoquero, se ha encontrado una mutación adicional en el locus *M* no ligada al locus S, que es capaz de superar las barreras del sistema de incompatibilidad (Vilanova et al., 2006; Zuriaga et al., 2012, 2013; Muñoz-Sanz et al., 2017). En esta tesis se han determinado 5 variedades como autocompatibles mediante las observaciones al microscopio que, sin embargo, no presentan en su genotipo el alelo S_c , lo que podría estar relacionado con el locus *M*. Por ello, la identificación de los alelos S puede no ser suficiente para caracterizar la auto(in)compatibilidad en algunas accesiones. Los resultados de esta tesis han puesto de manifiesto la importancia de combinar varias técnicas en la determinación de la

autocompatibilidad, utilizando marcadores moleculares de forma complementaria a la observación al microscopio del crecimiento de los tubos polínicos en flores autopolinizadas, para proveer de mayor información sobre la auto(in)compatibilidad de cada variedad.

Necesidades agroclimáticas y cambio climático

La meiosis como biomarcador de la salida del reposo

Al igual que el conocimiento sobre las necesidades de polinización, la caracterización de las necesidades agroclimáticas de cada variedad es crucial para el diseño y manejo del cultivo del albaricoquero, ya que es necesario que se cubran adecuadamente las necesidades de frío y calor para que se produzcan la floración y la fructificación. Además, las necesidades agroclimáticas determinan la fecha de floración, que es fundamental para el cultivo de variedades autoincompatibles al ser necesario un solapamiento de floración con las variedades polinizadoras para asegurar la polinización cruzada (Herrera et al., 2021b). La disminución del frío invernal provocada por el cambio climático está aumentando la importancia de conocer las necesidades agroclimáticas, que son un rasgo clave a tener en cuenta no solo en el cultivo, sino también en la mejora genética, ya que determinan la adaptación al medio (Guo et al., 2014). Sin embargo, la falta de una comprensión completa de los mecanismos biológicos que regulan el proceso de reposo y la ausencia de cambios visibles en la planta hacen que sea difícil establecer cuándo se produce la salida de la endodormancia, que es necesario para poder estimar adecuadamente las necesidades de frío de cada variedad (Rohde and Bhalerao, 2007; Lloret et al., 2018).

Se han desarrollado diversos métodos, tanto experimentales como estadísticos, para determinar el fin de la endodormancia (Fadón et al., 2020b). La determinación experimental consiste en evaluar cuándo las yemas recuperan la capacidad de crecer en varetas recogidas a lo largo del invierno y trasladadas a condiciones artificiales en cámaras de cultivo. Esta metodología ha sido ampliamente utilizada para determinar la salida de endodormancia en albaricoquero (Tabuenca, 1964; Andres and Duran, 1998; Valentini et al., 2004; Guerriero et al., 2006; Ruiz et al., 2007), y otros frutales de hueso (Fadón et al., 2020b). Por otro lado, existen métodos estadísticos, como el desarrollado en almendro para estimar la fecha de salida de endodormancia mediante la correlación de las fechas de floración de variedades con registros de temperatura de largas series de

años (Alonso et al., 2005). Los resultados de esta tesis han permitido validar este método en albaricoquero [Capítulo 5 (Herrera et al., 2022a)]. Recientemente, se ha desarrollado otro método estadístico, la regresión de mínimos cuadrados parciales (PLS), basado en el análisis de largos registros fenológicos y series de temperatura para la estimación de las necesidades agroclimáticas. Esta metodología fue desarrollada en cerezo (Luedeling et al., 2013) y ha sido aplicada en diferentes frutales (Benmoussa et al., 2017; Díez-Palet et al., 2019; Fadón et al., 2021), incluyendo el albaricoquero (Guo et al., 2015). Sin embargo, ambos tipos de metodologías tienen limitaciones. En los métodos experimentales podría existir cierta variabilidad causada por los factores que regulan las condiciones de las cámaras de cultivo y por los criterios para considerar cuándo se han cubierto las necesidades de frío, mientras que en los métodos estadísticos son necesarias largas series de años que solo están disponibles para unas pocas variedades que excluyen a las nuevas obtenciones comerciales (Dennis, 2003; Luedeling and Gassner, 2012; Fadón and Rodrigo, 2018).

Otro factor limitante de las estimaciones de necesidades agroclimáticas es la aplicación de los modelos de cuantificación de frío en zonas climáticas diferentes a las que fueron desarrollados debido a que las estimaciones obtenidas no son extrapolables con garantía a otras zonas (Dennis, 2003; Campoy et al., 2011). En esta tesis, la cuantificación del frío se ha llevado a cabo utilizando tanto el “Modelo Dinámico”, debido a su idoneidad en nuestras condiciones climáticas (Ruiz et al., 2007; Luedeling and Brown, 2011; Campoy et al., 2012b, 2019; Luedeling and Gassner, 2012), como los modelos “Weinberger” y “Utah”, por ser ampliamente utilizados en estudios previos (Tabuenca, 1964, 1968, 1979; Garcia et al., 1999; Guerriero et al., 2006; Viti et al., 2010; Campoy et al., 2012a; Ruml et al., 2018).

La meiosis de las células madre del polen emergió como un posible indicador de que las necesidades de frío ya se han cubierto y se ha salido de la fase de endodormancia en albaricoquero, ya que se produce con posterioridad al establecimiento de conexiones vasculares (Julian et al., 2011). Este proceso tiene lugar una semana después de la salida de endodormancia, como ocurre en melocotonero (Proebsting, 1963; Reinoso et al., 2002). En esta tesis se han confirmado los resultados de estudios previos (Julian et al., 2011) en varias variedades y se han determinado las necesidades agroclimáticas mediante la meiosis del polen como indicador de la salida de endodormancia, comparándolas con las obtenidas mediante el método experimental

tradicional y dos métodos estadísticos; correlaciones (Alonso et al., 2005) y PLS (Luedeling et al., 2013). Los resultados obtenidos han permitido validar la meiosis del polen como un biomarcador ligado a la salida de endodormancia, lo que, junto a la estandarización de las diferentes aproximaciones metodológicas, permitirá identificar de forma fiable la salida de la endodormancia y obtener una estimación más precisa de las necesidades agroclimáticas de otras variedades de albaricoquero.

El proceso del reposo es complejo, con varios procesos fisiológicos influenciados por la acumulación de frío invernal (Fadón et al., 2020a). La acumulación de las reservas de almidón en los primordios florales en cerezo (Fadón et al., 2018) así como la acumulación de ácido ascórbico y prunasina en almendro (Guillamón et al., 2020) han sido propuestos como indicadores de la salida de endodormancia. En los últimos años, se han realizado estudios transcriptómicos en diferentes estados de reposo en yemas de *Prunus* para profundizar en los conocimientos sobre la genética del reposo (Vimont et al., 2019; Yu et al., 2020; Canton et al., 2021; Prudencio et al., 2021). Además, se han caracterizado algunos genes candidatos, como los *DORMANCY-ASSOCIATED MAD-box (DAM)*, cuya expresión varía durante el reposo en albaricoquero (Balogh et al., 2019), almendro (Prudencio et al., 2018), cerezo (Vimont et al., 2019), ciruelo europeo (Quesada-Traver et al., 2020) y melocotonero (Bielenberg et al., 2004). La caracterización de la meiosis del polen como un biomarcador de la salida del reposo podría ayudar a complementar estos estudios además de facilitar su interpretación.

Adaptación del cultivo a las nuevas condiciones climáticas

La obtención de nuevas variedades adaptadas a las condiciones climáticas de cada zona es uno de principales objetivos de los programas de mejora de albaricoquero de todo el mundo (Bassi and Audergon, 2006). Debido a la situación de cambio climático, en las regiones de clima mediterráneo se estima una disminución de frío invernal de hasta 40 porciones de frío (PF) a finales del siglo XXI (Luedeling et al., 2011). Este escenario afectará principalmente a las zonas donde se cultivan frutales de hueso, volviéndose un factor limitante a la hora de elegir las variedades de las nuevas plantaciones. Otro de los aspectos a considerar en la adaptación de las variedades es la sensibilidad a las heladas primaverales. Las variedades con menos necesidades de frío pueden florecer antes en zonas y/o años menos fríos, haciéndolas más vulnerables a heladas durante la fase de ecodormancia y el periodo de floración

(Scorza and Okie, 1991). Por otro lado, la tendencia a buscar genotipos de floración tardía puede conducir a la introducción de variedades con altas necesidades de frío que son difíciles de satisfacer en regiones con inviernos suaves, pudiendo ocasionar floraciones irregulares, flores estériles y bajos niveles de cuajado (Dennis, 2000). Una alternativa a esta problemática sería la obtención de nuevas variedades con altas necesidades de calor que provocan una floración tardía sin efectos negativos (Zhebentyayeva et al., 2012).

En el diseño de nuevas plantaciones es importante conocer las necesidades agroclimáticas de las nuevas variedades, así como de las variedades ya establecidas para prever problemas de producción en los años de inviernos más cálidos. Los resultados de esta tesis han permitido determinar las necesidades agroclimáticas de 20 variedades de albaricoquero y clasificarlas en 3 grupos, mostrando gran variabilidad. Sin embargo, el número de variedades actualmente en el mercado es elevado, y en la mayoría de ellas se desconocen sus necesidades agroclimáticas. Para decidir las variedades de albaricoquero más adecuadas en cada zona de cultivo, es necesario realizar más investigaciones sobre la estimación de las necesidades de frío y de calor que permita conocer su posible adaptación a áreas en las que no se han cultivado de forma tradicional. Además, el conocimiento de las necesidades agroclimáticas puede proporcionar información valiosa para mejorar y simplificar las decisiones de gestión del cultivo. El uso de la meiosis del polen como biomarcador de la salida de endodormancia puede facilitar el estudio de las necesidades agroclimáticas de otras variedades.

Diversidad genética

Mayor diversidad en las nuevas variedades

El estudio de diversidad genética llevado a cabo en esta tesis muestra la evolución de la estructura varietal del cultivo del albaricoquero. El número de variedades comerciales se ha incrementado en los últimos años como consecuencia de la intensa actividad de los programas de mejora como respuesta a los factores limitantes del cultivo (Hormaza et al., 2007). Los dos grupos claramente diferenciados en el análisis filogenético [Capítulo 6 (Herrera et al., 2021a)] están relacionados con el hecho de que muchas de las nuevas variedades son resultado de cruzar variedades locales con variedades procedentes de América del Norte (Ledbetter, 2008). Las variedades tradicionales están adaptadas a cada zona de cultivo y aportan rasgos muy interesantes

como la autocompatibilidad [Capítulo 1 (Herrera et al., 2020a), capítulo 2 (Herrera et al., 2018a), capítulo 3 (Herrera et al., 2018b), capítulo 4 (Herrera et al., 2022c)] y las bajas y medias necesidades de frío [Capítulo 5 (Herrera et al., 2022a)]. Debido a sus antecesores asiáticos, las variedades norteamericanas usadas como parentales se caracterizan por la autoincompatibilidad [Capítulo 1 (Herrera et al., 2020a), capítulo 2 (Herrera et al., 2018a), capítulo 3 (Herrera et al., 2018b), capítulo 4 (Herrera et al., 2022c)], además de tener necesidades de frío elevadas [Capítulo 5 (Herrera et al., 2022a)]. Estos cruzamientos han generado gran variabilidad en las descendencias, con mayores índices de diversidad genética en variedades comerciales procedentes de programas de mejora que las variedades tradicionales [Capítulo 6 (Herrera et al., 2021a)].

Como resultado del proceso de domesticación del albaricoquero, se ha observado una disminución en la diversidad genética del Este (área irano-caucásica) al Sudoeste (cuenca mediterránea norte y cuenca sur) (Bourguiba et al., 2012). Se ha detectado una pérdida de variabilidad al comparar la diversidad genética entre acervos genéticos regionales en varias áreas mediterráneas, lo que puede estar asociado con la selección y difusión del cultivo en la cuenca del Mediterráneo (Bourguiba et al., 2012). Sin embargo, también existen en Europa una multitud de variedades autóctonas, como resultado de siglos de selección natural y humana (Faust et al., 1998; Hormaza et al., 2007). Los resultados de esta tesis destacan la importancia de la variedades locales como reservorio de diversidad genética, en especial las variedades tradicionales españolas, que mostraron una gran diferenciación genética [Capítulo 6 (Herrera et al., 2021a)]. El uso de este germoplasma tradicional en los programas de mejora proporcionaría una fuente de rasgos de interés que podrían utilizarse para responder a nuevas demandas del mercado o a nuevas condiciones agroclimáticas.

Una estructura varietal en proceso de cambio

Los programas de mejora en la actualidad muestran varios objetivos comunes, siendo uno de los principales la obtención de variedades autocompatibles [Capítulo 1 (Herrera et al., 2020a), capítulo 2 (Herrera et al., 2018a), capítulo 3 (Herrera et al., 2018b), capítulo 4 (Herrera et al., 2022c)] (Bassi and Audergon, 2006). Sin embargo, la endogamia producida por los retrocruzamientos durante el proceso de mejora puede dar lugar a pérdida de diversidad genética (Badenes et al., 2016). Al analizar por separado

las selecciones avanzadas más recientes de los programas de mejora y las variedades comerciales que existen ya en el mercado, se ha observado una tendencia a la reducción en la diversidad genética [Capítulo 6 (Herrera et al., 2021a)]. Esta situación puede estar relacionada con el uso reiterado de un grupo reducido de parentales. Sin embargo, esta hipótesis no puede ser confirmada por la falta de información sobre los parentales utilizados en la mayoría de los programas de mejora, principalmente los de financiación privada.

Una situación similar se ha observado en los híbridos interespecíficos tipo plumcot. Los plumcots se obtienen por el cruzamiento de una variedad de *P. armeniaca*, que actúa como parental masculino, y una variedad de *P. salicina*, que actúa como parental femenino (Faust et al., 1998), aunque se ha observado mayor cercanía genética al ciruelo que al albaricoquero debido probablemente a que la mayoría de variedades se obtienen tras varios retrocruzamientos con ciruelo (Ahmad et al., 2004; Guerrero et al., 2022). A pesar de la gran variabilidad en su genealogía, en un estudio llevado a cabo con 115 accesiones de híbridos interespecíficos ciruelo x albaricoquero y 27 genotipos de referencia se ha encontrado poca diversidad genética, lo que podría estar relacionado con el uso repetido de un pequeño grupo de progenitores y la consanguinidad producida por los retrocruzamientos durante el proceso de mejora (Guerrero et al., 2022), al igual que lo observado en albaricoquero en esta tesis [Capítulo 6 (Herrera et al., 2021a)].

El estudio y caracterización del germoplasma de albaricoquero local es de gran importancia para seleccionar los genotipos adecuados para la mejora de rasgos de interés específicos que, además, permitiría aumentar la variabilidad genética del cultivo.

CONCLUSIONES



CONCLUSIONES

1. Se han identificado 92 variedades de albaricoquero como autocompatibles y 49 como autoincompatibles, que engloban tanto variedades tradicionales como nuevas variedades comerciales.
2. Las 49 variedades autoincompatibles se han clasificado en 15 grupos de incompatibilidad, 9 de ellos descritos por primera vez (del XVIII al XXVI).
3. Para la identificación de los alelos S_1 y S_7 , se han diseñado 2 pares de cebadores específicos, SHLM1/SHLM2 y SHLM3/SHLM4, respectivamente, para amplificar el segundo intrón de la *S-RNasa*.
4. La secuenciación de fragmentos genómicos ha permitido encontrar homologías entre los alelos S_6 y S_{52} , los alelos S_{20} y S_{55} , y los alelos S_7 , S_{13} y S_{46} .
5. El estudio de diversidad genética del locus *S* ha mostrado que en los últimos años está aumentando el número de nuevas variedades autocompatibles. Sin embargo, una proporción significativa de las nuevas variedades son autoincompatibles, por lo que es necesario continuar el estudio de sus necesidades de polinización.
6. Se han identificado 5 variedades como autocompatibles por polinizaciones controladas que no presentan el alelo S_c , confirmando la existencia de otras fuentes de autocompatibilidad en albaricoquero.
7. Se ha puesto de manifiesto la importancia de utilizar varias técnicas complementarias en la determinación de la autocompatibilidad de algunas variedades, combinando polinizaciones controladas y análisis con marcadores moleculares.
8. La meiosis de polen ha sido validada como un biomarcador de la salida de la endodormancia en albaricoquero, facilitando la estimación de las necesidades agroclimáticas de variedades de forma fiable en yemas muestreadas en campo.
9. Se han determinado las necesidades agroclimáticas de 20 variedades, lo que suministra información sobre su adaptación a cada zona de cultivo y a las nuevas condiciones causadas por el cambio climático.

10. Se ha probado la eficacia de tres métodos previamente descritos para la determinación de las necesidades agroclimáticas en albaricoquero.
11. El estudio de la diversidad genética actual ha mostrado mayor diversidad en las variedades procedentes de los programas de mejora que en las variedades tradicionales.
12. La utilización de un reducido número de parentales en los programas de mejora revela una posible disminución de la diversidad genética en las futuras variedades.

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ANEXOS



ANEXO I

Herrera, S., Lora, J., Hormaza, J. I., and Rodrigo, J. (2021b). “Pollination Management in Stone Fruit Crops,” in *Production Technology of Stone Fruits*, eds. M.M. Mir, M. Iqbal, S.A. Mir (Singapore: Springer), 75–102. doi:10.1007/978-981-15-8920-1_3.



Pollination Management in Stone Fruit Crops

3

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Abstract

Pollination and subsequent fertilization are needed to ensure fruit set in stone fruit crops. Knowing the pollination requirements for particular cultivars is a necessity for an appropriate orchard design. Pollination management is receiving increasing importance due to the release of a number of new cultivars of unknown pollination requirements in most *Prunus* species. In addition, variable environmental conditions due to climate change and the interest in expanding stone fruit crops to new production areas with different climatic conditions are leading to a lack of coincidence in flowering time between pollinating and pollinated cultivars in many situations. In this work, the available information on pollination requirements of cultivars of the most cultivated stone fruit crops (almonds, apricots, cherries, peaches, and plums) is reviewed, paying special attention to the reproductive process, the self-(in)compatibility of each cultivar, the inter-incompatibility *S*-alleles and incompatibility groups, and the external factors affecting flowering and pollination. Results obtained in the last decades have allowed establishing the pollination requirements of most commercial cultivars in the main stone fruit crops. However, for the coming years, it will be necessary to continue determining the self-(in)compatibility and the inter-incompatibility relationships of the new releases.

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Keywords

Almond · Apricot · Cherry · Fertilization · Flowering · Inter-incompatibility · Peach · Plum · Pollination · Self-(in)compatibility

3.1 Introduction

Stone fruit crops include different plant species that produce a type of indehiscent fruit called drupe that contains a single large seed covered by an outer shell that constitutes the endocarp. The endocarp together with the fleshy mesocarp (pulp) and exocarp (skin) constitute the pericarp. The pericarp and the seed coat are of maternal origin. Most stone fruit tree crops belong to the genus *Prunus*, and examples include peach (*P. persica* L. Batsch), European (*P. armeniaca* L.) and Japanese (*P. mume* Siebold & Zucc.) apricots, sweet (*P. avium* L.) and sour (*P. cerasus* L.) cherries, almond (*P. dulcis* (Mill.) D.A. Webb), or different species of plums such as the European (*P. domestica* L.) and the Japanese (*P. salicina* Lindl.) plums (Byrne 2005; Badenes and Byrne 2012).

One of the main factors that limit production in crops in which the main product is fruits is the reproductive phase. During this phase, a critical stage is the days following flower opening, since pollination and subsequent fertilization are needed to ensure fruit set because *Prunus* species are unable to set fruit parthenocarpically (Sedgley and Griffin 1989). Indeed, some efforts to induce artificial parthenocarpy using gibberellins have been unsuccessful (Bukovac 1963; Webster and Goldwin 1978). This period between pollination and fertilization is the progamic phase and takes place in a few days, but the development of the flower buds occurs for several months, and a number of external and internal factors during this period may condition the success of the reproductive process (Hedhly et al. 2009; Hedhly 2011). Knowing the pollination requirements for particular cultivars is a requirement for an appropriate orchard design and management.

Although the fruit crops considered as stone fruits belong to the same genus, *Prunus*, in the Rosaceae family, there is a high variability among species in flower characteristics, chilling and heat requirements, pollination behavior, and flowering times. However, the main biological processes are conserved although with different timing of the different processes among species.

In recent years, pollination management is emerging as a field with increasing importance, due to the release, in most stone fruit crops, of a number of new cultivars with unknown pollination requirements. In addition to understanding incompatibility relationships among cultivars, variable environmental conditions can lead to a lack of coincidence in flowering time between pollinating and pollinated cultivars (Atkinson et al. 2013). Thus, the increasing interest in expanding the marketing season in many crops is resulting in the cultivation of different fruit crop species in new production areas with different climatic conditions (Hedhly et al. 2009; Fadón and Rodrigo 2018). On the other hand, the decrease in winter chilling in many

regions caused by climate change is resulting in alterations in flowering time (Luedeling et al. 2011; Atkinson et al. 2013) and, as a consequence, in the advance of the flowering season in low-chilling cultivars and the delay in high-chilling cultivars (Luedeling 2012). In this work, the available information on pollination requirements of cultivars of the most cultivated stone fruits (almonds, apricots, cherries, peaches, and plums) is reviewed, paying special attention to the reproductive process and the factors affecting flowering and pollination.

3.2 Reproductive Biology in *Prunus*

Flower bud development in *Prunus* species requires several months from the summer of the previous year to flowering in late winter or early spring of the following season (Fadón et al. 2015). Flower induction occurs during the summer, and flower differentiation starts before dormancy in late summer and autumn (Fadón et al. 2018a, b). After breaking dormancy, flowers complete differentiation when temperatures rise.

In autumn, prior to the exposure to low temperatures, flower buds cease development and progressively enter in a dormant stage, endodormancy (Lang et al. 1987), during which they are completely closed and covered by brown scales, corresponding to phenological stage A in the Baggiolini code (Baggiolini 1952) or to 50/00 in the BBCH scale (Fadón et al. 2015). Each flower bud can contain one (almond, apricot, peach) (Herrero and Arbeloa 1989; Kester and Gradziel 1996; Julian et al. 2010) or several (sweet and sour cherry, plum) (Fadón et al. 2015; Guerra and Rodrigo 2015; Herrero et al. 2017) flower primordia. In each flower primordium, the different whorls (sepals, petals, stamens and carpels) start to differentiate before endodormancy (Fadón et al. 2018a, b). The carpel and stamens are surrounded by the petals, which, in turn, are surrounded by the sepals (Diaz et al. 1981; Lamp et al. 2001; Julian et al. 2010; Fadón et al. 2015).

Flowers of *Prunus* are hermaphroditic, with numerous stamens surrounding a single pistil, enclosed by five petals and five sepals (Sterling 1964). Flowering time depends on the cultivar and the weather conditions that may cause high year-to-year variations. Pollination, the transfer of pollen from the anthers to the stigma, is usually entomophilous, mainly by bees (Griggs 1953, Fig. 3.1). The sequence of events from pollination to fertilization is consistent in different species of the genus (Herrero 1992, 2000).

Pollination can also be hindered by a short period of stigmatic receptivity that reduces the effective pollination period (EPP) in which pollination is effective to produce a fruit. The length of the EPP in stone fruits varies depending on location, season, cultivar, and environmental conditions (mainly temperature and humidity), ranging from 2 days to more than 1 week (Sanzol and Herrero 2001). Usually, stigmas are receptive at anthesis, as it has been shown in apricot (Egea et al. 1991), peach (Martínez-Tellez and Crossa-Raynaud 1982), or sweet cherry (Guerrero-Prieto et al. 1985), although immature stigmas at anthesis have been observed in some almond (Yi et al. 2006) and peach cultivars (Sanzol and Herrero 2001).



Fig. 3.1 Honeybee pollinating apricot flowers

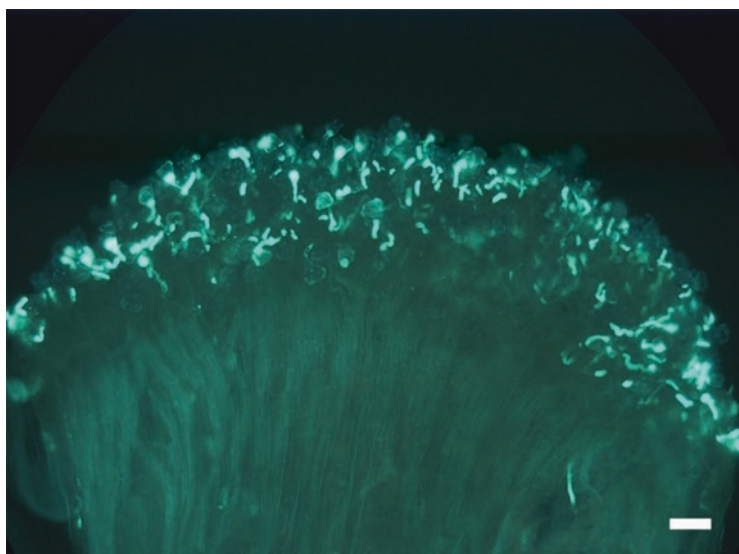


Fig. 3.2 Pollen grains germinating on the stigma surface of an apricot flower. Bar = 100 μm

The EPP can be shortened by warm temperatures that can accelerate both stigma and ovule degeneration (Ortega et al. 2004; Hedhly et al. 2005; Kodad and Socias i Company R 2013; Zhang et al. 2018).

The pollen grain germinates on the stigma within few hours after pollination (Fig. 3.2), producing a pollen tube that grows along the closed style and reaches the ovary in the following days, where it goes through one of the two ovules reaching

the embryo sac and achieving double fertilization. Intense competition among pollen tubes occurs in the style, reducing the number of more than 100 germinating pollen grains on the stigma to just one to three pollen tubes reaching the ovary (Hormaza and Herrero 1996). The duration of this progamic phase may vary between species, cultivars, and years (Williams 1970; Sedgley and Griffin 1989; Guerra and Rodrigo 2015). Thus, this phase can last for 4–8 days in sweet and sour cherry and Japanese plum (Cerovic and Micic 1999; Hedhly et al. 2007; Jia et al. 2008), 5–8 days in apricot and almond (Pimienta and Polito 1983; Rodrigo and Herrero 2002a), and more than 2 weeks in peach and European plum (Thompson and Liu 1973; Herrero and Arbeloa 1989).

Only a reduced number of the pollinated flowers develop into fruits. The percentage of fruit set varies among species and cultivars: 5–15% in Japanese plum, (Dorsey 1919; Beppu et al. 2005; Jia et al. 2008; Guerra et al. 2010), 18–33% in apricot and sweet cherry (Rodrigo and Herrero 2002a; Hedhly et al. 2007), 30–40% in almond and peach (Harrold 1935; Kester and Griggs 1959), and 25–50% in sour cherry (Bradbury 1929), although the final fruit set is highly dependent on pollination and weather conditions.

3.3 Pollen-Pistil Incompatibility

Different barrier mechanisms to avoid self-fertilization and promote outcrossing have arisen in seed plants. Some are physical barriers such as dichogamy, in which the female and male parts do not mature at the same time, or heterostyly in which populations are composed of two (distyly) or three (tristyly) different floral morphs with reciprocal arrangements of height of anthers and stigmas (reciprocal herkogamy). However, the most common mechanism in evolutionary-derived angiosperms, such as the Rosaceae, is a genetic self-incompatibility system based in cell-to-cell recognition. There are different systems of genetic self-incompatibility, and the most widespread in flowering plants is gametophytic self-incompatibility (GSI) in which the cell-to-cell recognition occurs in the style (Kao and McCubbin 1996) and is controlled by a multi-allelic locus called *S*-locus (Kao and Huang 1994) (Fig. 3.3). GSI has been found in Solanaceae, Plantaginaceae, and Rosaceae, including stone fruit species of *Prunus* such as apricot, peach, almond, European plum, Japanese plum, sweet cherry, and sour cherry. Since fertilization and seed formation are essential for fruit production, the knowledge of the genetic basis of GSI is essential for the development of breeding programs and efficient design of stone fruit orchards (Herrera et al. 2018a, b).

GSI has been extensively studied in Solanaceae. Indeed, the first protein associated with self-incompatibility, a stylar glycoprotein, was isolated in *Nicotiana glauca* (Anderson et al. 1986). A few years later, several *S*-glycoproteins were shown to be RNases (McClure et al. 1989, 1990). The *S*-glycoprotein is secreted in the extracellular matrix and enters the pollen tube, where the incompatibility reaction, as hypothesized in the inhibitor model, has been supported by immunocytochemical evidences in *Solanum chacoensis* (Luu et al. 2000). Similar evidences are still lacking in Rosaceae, but the inhibitor model has also been generally accepted in the

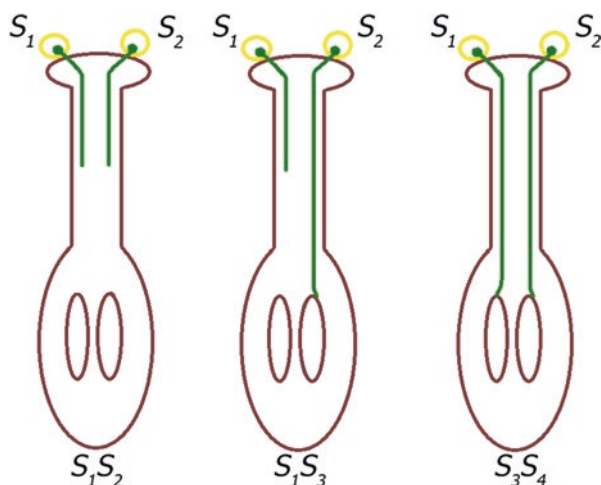


Fig. 3.3 Diagram of the gametophytic self-incompatibility system (GSI) present in stone fruit crops. Pollen tubes containing an S -allele present in the pistil will not reach the ovule, avoiding fertilization

cell-to-cell recognition in this family (Tao and Iezzoni 2010). Thus, the recognition between the female (RNase) and the male S -determinants occurs in the pollen tube, in which only the self-RNase shows cytotoxic activity. In Rosaceae, the first S -RNase DNA sequence was isolated from apple (*Malus × domestica*) (Broothaerts et al. 1995), although previously an S -RNase protein had been isolated from *Pyrus* (Sassa et al. 1993). Later on, S -RNases were also identified and isolated in almond (Tao et al. 1997), sweet cherry (Tao et al. 1999; Sonneveld et al. 2001), sour cherry (Yamane et al. 2001), Japanese plum (Yamane et al. 1999), apricot (Romero et al. 2004), and peach (Tao et al. 2006). In other cases, such as European plum, self-incompatibility is relatively rare probably due to its hexaploid ($2n = 6x = 48$) genome (Hegedűs and Halász 2006) although S -RNase alleles have been characterized in this species (Sutherland et al. 2004). This breakdown of SI in polyploid species has also been observed in tetraploids in Solanaceae and Plantaginaceae (Tao and Iezzoni 2010), but, interestingly, this is not the case in tetraploid sour cherry. This has been explained in terms of the disfunction of some S -haplotypes caused by polyploidization and gene duplication in sour cherry that has not been observed in diploid sweet cherry (Tsukamoto et al. 2006).

During the first years of the twenty-first century, several studies in *Antirrhinum* (Plantaginaceae) (Lai et al. 2002), *Prunus* (Rosaceae) (Entani et al. 2003; Ushijima et al. 2003, 2004), and *Petunia* (Solanaceae) (Sijacic et al. 2004) identified the pollen S -determinant as an F-box protein. In almond, the F -box gene named *SFB* (S haplotype-specific F-box protein) was linked to the S -RNase and showed a specific pollen expression (Ushijima et al. 2003). Studies of the S_1 and S_7 haplotypes of *Prunus mume* allowed the identification of an F -box in this species that was named *SLF* (Entani et al. 2003). The linkage between the F -box and the S -RNase genes was

also confirmed in *Prunus armeniaca* in which three *F*-box allelic variants (*SFB*₁, *SFB*₂, and *SFB*₄) were linked to the three *S*-RNase alleles *S*₁, *S*₂, and *S*₄, respectively (Romero et al. 2004).

The F-box protein is a component of the Skp1/Cullin or CDC53/F-box (SCF) complex that regulates protein degradation, expected to be involved in detoxification of non-self S-RNases by the ubiquitin-proteasome pathway in self-compatible interactions (del Pozo and Estelle 2000; Qiao et al. 2004). According to this model, self-compatible cultivars with mutations in the *F*-box gene have not been observed in Solanaceae and Plantaginaceae since it is believed that these mutations would result in self-incompatibility or lethality (Tao and Iezzoni 2010). However, mutations in the *F*-box gene result in self-compatibility in *Prunus*. That is the case for most traditional apricot cultivars in Western Europe (Vilanova et al. 2006) and Hungary (Halász et al. 2007b). *SFB* mutations have also been reported in self-compatible cultivars of peach, sweet cherry, and Japanese apricot (Tao et al. 2006; Abdallah et al. 2020). Thus, in *Prunus*, an S-RNase inhibitor that interacts with F-box has been proposed (Tao and Iezzoni 2010). Indeed, recent experimental evidence suggested an *S*-locus F-box like-2 protein as the “general inhibitor” that detoxifies S-RNase in a nonspecific manner if it is not affected by the F-box protein (Matsumoto and Tao 2016).

The disruption of the female *S*-determinant can also result in self-compatibility. Mutations in the *S*-RNase gene have been reported in several species of the Rosaceae, such as pear (Sassa et al. 1997), peach (Tao et al. 2006), or almond (Bošković et al. 2007). In addition to the disruptions in the male or female *S*-determinant genes, the study of self-compatible cultivars in the Rosaceae has revealed additional factors not linked to the *S*-locus involved in cell-cell recognition. Thus, the *M*-locus, localized in chromosome 3 (Zuriaga et al. 2012), appears to be involved in self-compatibility in the apricot cultivars Katy and Canino (Zuriaga et al. 2013; Muñoz-Sanz et al. 2017). In sweet cherry, in which natural self-compatibility is rare, self-compatibility was also found associated with a modifier gene in the Spanish cultivar Cristobalina (Wünsch and Hormaza 2004a; Cachi and Wünsch 2011).

3.4 Pollination Requirements

Although most of the species of the *Prunus* genus exhibit GSI, mutations in both the *F*-box and *SFB* genes have resulted in a variable number of self-compatible genotypes in different species (Tao et al. 2006; Vilanova et al. 2006; Bošković et al. 2007; Halász et al. 2007a). Both compatible and incompatible pollen grains are able to germinate on the stigma producing a pollen tube that grows along the style (Fig. 3.4). However, pollen tube growth is arrested in the style (Fig. 3.5), preventing fertilization in incompatible relationships, whereas compatible pollen tubes may reach the ovary and fertilize the ovules (Fig. 3.3). Self-(in)compatibility affects orchard management, since self-incompatible cultivars need compatible pollen from other cultivars, and, therefore, it is necessary to design the orchards with trees



Fig. 3.4 Pollen tubes growing along the style of an apricot flower. Bar = 100 μm

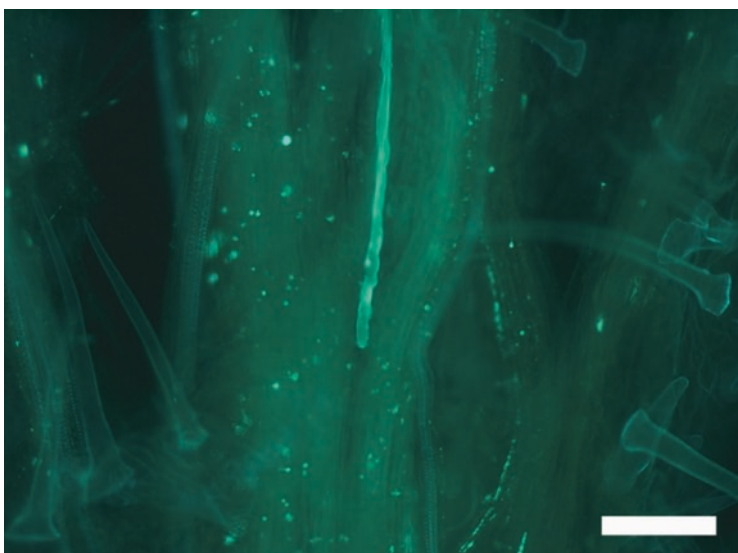


Fig. 3.5 Pollen tube arrested in the style of an apricot flower. Bar = 100 μm

of several inter-compatible cultivars. On the other hand, the ovules of self-compatible cultivars can be fertilized with their own pollen and, therefore, can be grown without the presence of trees of other cultivars.

Peach commercial cultivars are self-compatible (Abdallah et al. 2020), but in the other cultivated *Prunus* species, both self-incompatible and self-compatible

cultivars are usually found. In almond, sweet cherry, and Japanese and European plums, most commercial cultivars are self-incompatible and need cross-pollination, whereas most apricot cultivars are self-compatible. However, the release of new cultivars from breeding programs in which self-compatibility is one of their main objectives is resulting in an increasing number of self-compatible cultivars in almond (Table 3.1), sweet cherry (Table 3.2), and Japanese plum (Table 3.3). On the other hand, the use of self-incompatible cultivars as parents in apricot breeding programs, with the purpose of introducing a source of resistance to sharka, a common disease among stone fruit crops caused by the plum pox virus (PPV), is resulting in the release of an increasing number of self-incompatible cultivars (Table 3.4).

3.5 Inter-incompatibility S-Alleles and Incompatibility Groups

The sequences of the *S-RNase* gene reveal a high diversity that results in numerous *S*-alleles, although all showing five highly conserved regions (C1–C5). The conserved regions C1, C2, C3, and C5 are similar in Plantaginaceae, Solanaceae, and Rosaceae, but the fourth region differs in Rosaceae and is called RC4. In Rosaceae, one hypervariable region (RHV) is found, whereas two (HV_a and HV_b) are found in Plantaginaceae and Solanaceae (Fig. 3.6) (Tao and Iezzoni 2010). In addition to the high diversity of the encoding gene sequences, a higher diversity is found in the introns, and this diversity is essential for *S*-allele identification in addition to the general use of specific primers from the exon sequences (Herrera et al. 2018a, b) (Fig. 3.7).

Knowing the *S*-alleles of each cultivar is necessary to select compatible pollinizers in the design of new orchards and also to identify and solve situations of low yield related to lack of pollination. In different *Prunus* species, the identification of a variable number of *S-RNase* and *SFB* alleles has allowed allocating the self-incompatible cultivars in incompatibility groups: almond (Table 3.1), sweet cherry (Table 3.2), Japanese plum (Table 3.3), apricot (Table 3.4), European plum (Table 3.5), and sour cherry (Table 3.6). Those cultivars with the same *S*-alleles are genetically inter-incompatible and are included in the same incompatibility group. On the other hand, those cultivars with at least one different *S*-allele are inter-compatible and are assigned to different incompatibility groups. Those cultivars whose *S*-genotype has not been found in any other cultivar until now are included in group 0. Self-compatible cultivars are grouped in the last row of each table as group SC. Cultivars from both groups 0 and SC may be considered as universal pollinizers. Adequate pollinizers must be inter-compatible and coincident in flowering time.

Table 3.1 Incompatibility groups and *S*-genotype of 219 almond cultivars

I.G.	<i>S</i> -RNase genotype	Cultivars
I	S_7S_8	Galaxy, Golden state, Grace, IXL, Long IXL, Mckinlay's Magnificent, Nonpareil, Riedenhoure, Tardy Nonpareil, West Steyn
II	S_7S_5	Ballico, Bulbiente, Garbi, Glorieta, Languedoc, Texas (=Mission), Wawona
III	S_5S_7	Baxendale, Durango, Granada, Harvey, Le Grand, Mono, Robson, Sauret no. 2, Thompson, Wassum, Wood Colony
IV	S_7S_7	Alrich, Jenette, Merced, Ne Plus Ultra, Norman, Pearl, Price, Ripon, Rosetta, Sano
V	S_5S_8	Carmel, Jubilee, Livingston, Monarch, Reams, Sauret no. 1, Tioga
VI	S_7S_8	Avalon, Bigelow, Blue Gum, Butte, Dottie Won, Duro Amarelo, Folsom, Grace, Kutsch, Monterey, Northland, Plateau, Rivers Nonpareil, Sultana
VII	S_8S_{13}	Eureka, Kapareil, Solano, Sonora, Vesta
VIII	S_7S_3	Ferragnes, Ferralise, Mourisca
IX	S_7S_{14}	Harpareil, Jordanolo
X	S_6S_8	Drake, Kochi, Smith XL
XI	S_7S_6	Abizanda, Fritz, Peerless, Ruby, Rumbeta-2
XII	S_2S_9	Anxaneta, Tarragones
XIII	S_7S_{10}	Ardechoise, Coop, Desmayo Langueta, Pep de Juneda, Zahaf
XIV	S_7S_{10}	Achaak, Alnem88, Ferrastar
XV	$S_{12}S_{23}$	Pajarera-2, Pestañeta (=Pestanhietta)
XVI	$S_{22}S_{23}$	Malagueña, Muel, Pau, Planeta Fina, Planeta Roja, Verdeta
XVII	$S_{13}S_{27}$	Garrigues, Pajarera-1
XVIII	S_8S_{12}	AS1, Marcona Flota
XIX	S_3S_{23}	Belle d'Aurons, Peraleja
XX	S_3S_5	Bartre, Castañera
XXI	S_7S_9	Masbovera, Moncayo, Tarraco
XXII	S_7S_4	Casanova, Coelhoinha, Ferraduel
XXIII	S_7S_{13}	Chellastone, Milow
XXIV	$S_{11}S_{22}$	Parada, Pestañeta Menuda
XXV	S_8S_{23}	Pierce
XXVI	$S_{fa}S_{36}$	Alzina, Garondes
XXIX	S_7S_2	Cristomorto
XXX	S_3S_4	Ai
XXXI	S_6S_7	Tokyo
XXXII	S_4S_{12}	Carretas Bajas
XXXVIII	$S_{62}S_{63}$	Safari
XL	S_4S_{13}	CEBAS I, Verdeal
XLI	S_3S_9	Tardive de la Verdere
XLII	S_7S_{21}	Bonita
XLIII	$S_{10}S_{22}$	Angones
XLV	$S_{12}S_{22}$	Del Cid, Esperanza Forta
XLVI	$S_{12}S_{28}$	Colorada, Nano

(continued)

Table 3.1 (continued)

I.G.	S-RNase genotype	Cultivars
XLVII	S_6S_{10}	Pauet
XLVIII	S_7S_{12}	Mollar de Tarragona
XLIX	$S_{12}S_{27}$	Carreró
L	S_5S_{12}	Caima, Tardaneta
0 (cultivars with unique S-genotype)		Aldrich (S_7S_{17}), Asperilla ($S_{10}S_{27}$), Atascada (S_5S_{22}), Atocha ($S_{13}S_{22}$), Avellaneda Gruesa ($S_{22}S_{26}$), Belardino (S_2S_{11}), Bertina (S_6S_{11}), Biota (S_5S_{13}), Boa Casta (S_8S_{21}), Bonita de São Brás (S_8S_{22}), Carrion (S_5S_{14}), Castilla (S_6S_{22}), Colossal (S_7S_{11}), Fascionello ($S_{18}S_{52}$), Fina del Alto ($S_{28}S_{29}$), Fournat de Brezenaud ($S_{24}S_{27}$), Fura Saco (S_4S_{23}), Gabaix ($S_{10}S_{24}$), Harriot (S_6S_{14}), Jiménez Salazar ($S_{21}S_{26}$), Jordi (S_5S_6), La Mona ($S_{23}S_{25}$), Liso ($S_{10}S_{23}$), Marcona ($S_{11}S_{12}$), Marcona de San Joy ($S_{22}S_{27}$), Menut ($S_{10}S_{13}$), Mollar (S_8S_{24}), Mollar de la Princesa ($S_{24}S_{53}$), Padre ($S_{11}S_{18}$), Padre Santo (S_3S_{10}), Pané-Barquets ($S_{11}S_{34}$), Parque Samá ($S_{11}S_{33}$), Planeta de les Garrigues ($S_{22}S_{33}$), Ponç ($S_{16}S_{27}$), Pou d'Establiments ($S_{12}S_{33}$), (S_5S_9), Ramillete (S_6S_{23}), Redonda de Palma (S_3S_{25}), Retsou (S_2S_3), Rof (S_5S_{23}), Rumbeta ($S_{11}S_{21}$), Somerton ($S_{11}S_{23}$), Tejeda-2 ($S_{11}S_{22}$), Tendra Amarga ($S_{21}S_{23}$), Tío Martín ($S_{23}S_{27}$), Titan (S_8S_{14}), Totsol (S_8S_{31}), Verruga (S_5S_{10}), Vivot ($S_{16}S_{23}$), Winters ($S_{11}S_{14}$), Yosemite (S_8S_{10})
Self-compatible cultivars		All-in-One, Almenara, Antòñeta, Aylés, Belona, Blanquerna, Cambra, Constanti, Falsa Barese, Felisia, Filippo Ceo, Francoli, Garden Princess, Genco, Guara, Independence, Lauranne, Mandaline, Mardía, Marinada, Marita, Marta, Matan, Occhiorosso, Penta, Soleta, Steliette, Supernova, Sweetheart, Tardona, Teresa, Tuono, Vairo, Vialfas
References		Kester and Gradziel (1996), Bošković et al. (1997, 1998, 2003) Boutard (1999), Certal et al. (2002), Channuntapipat et al. (2003), Martínez-Gómez et al. (2003), Sánchez-Pérez et al. (2004), Ortega et al. (2005, 2006), Barckley et al. (2006), Kodad et al. (2008a, b), Marchese et al. (2008), Kodad and Socias i Company R (2009), Kodad et al. (2010), Gómez et al. (2019)

3.6 External Factors Affecting Flowering and Pollination

Alterations in flower biology may lead to failure in pollination and subsequent lack of fertilization and fruit set. These may occur at flower bud development or at flowering. During the onset of flower differentiation, high temperatures may cause various floral abnormalities, as double pistil formation and appendices with pistil or petal appearance at the end of the stamen filaments instead of the anthers or with a leafy appearance in different floral organs (Herrero et al. 2017).

As other temperate fruit trees, *Prunus* spp. need to accumulate chilling during winter dormancy to break endodormancy. Once the cultivar-specific chilling requirements are fulfilled, flower buds accumulate heat and enter ecodormancy (Lang et al.

Table 3.2 Incompatibility groups and *S*-genotype of 135 sweet cherry cultivars

I.G.	<i>S</i> -RNase genotype	Cultivars
I	S_1S_2	Canada Giant (Sumgita), Ferdouce, Starking Hardy Giant, Summit, Tulare
II	S_1S_3	Areko, Black Star, Coral, Cristalina, Early Robin (Doty), Early Van Compact, Lala Star, Prime Giant, Royal Lee, Rosie, Royal Ansel (Royal Bailey), Redstone, Regina, Samba (Sumste), Satin (Sumele), Sonnet, Sumbola, Van, Vera
III	S_3S_4	Belge, Bing, Karina, Lambert, Napoleon (Monzón, Royal Ann), Somerset, Sweet Lorenz, Sweet Valina, Ulster
IV	S_2S_3	Coralise (Gardel), Nimba, Sue, Vega
VI	S_3S_6	Ambrunés, Duroni 3, Ferdiva, Fertard, Fertille, Kordia, Pico Negro, Satonishiki, Stark's Gold (Dönissens Gelbe, Gold), Techlovan
VII	S_3S_5	Hedelfinger
IX	S_1S_4	Ebony Pearl, King, Rainier, Royal Brynn, Royal Lynn, Sweet Gabriel, Sylvia
X	S_6S_9	Folfer, Penny, Ramón Oliva
XIII	S_2S_4	Royalton, Sam, Vic
XIV	S_1S_5	Blanca de Provenza
XV	S_5S_6	Colney
XVI	S_3S_9	Burlat, Chelan, Moreau, Precoco Bernard, SMS-280, Tieton
XVII	S_4S_6	Larian, Royal Hazel, Royal Tenaya (Royal Marie)
XVIII	S_1S_9	Bigisol (Early Bigi), Brooks, Earlise (Rivedel), Marvin (Niran, 4–70), Rocket, Sweet Early (Panaro 1), Tamara
XIX	S_3S_{13}	Reverchon
XX	S_1S_6	Vanda
XXI	S_4S_9	Cashemire, Merchant
XXII	S_3S_{12}	0900-Ziraat, Ferrovia, Schneiders
XXIV	S_6S_{12}	Aida
XXV	S_2S_6	Fercer (Arcina)
XXVII	S_4S_{12}	Margit, Kavics
XXXIII	S_1S_{14}	Fermina
XXXIV	S_4S_{13}	Black Pearl
XL	S_6S_{22}	Pico Colorado
XLIII	S_2S_9	Primulat (Ferprime)
0 (cultivar with unique <i>S</i> -genotype)	S_5S_{22}	Rita
Self-compatible cultivars		Alex, Blackgold, Blaze Star, Celeste, Columbia (Benton), Compact Stella, Early Star (Panaro 2), Frisco, Grace Star, Index, Lapins, New Star, Pacific Red, Royal Elaine, Royal Tioga, Royal Helen, Royal Edie, Sandor, Sandra Rose, Santina, Selah (Liberty bell), Skeena, Sofia (SPC 106), Sonata, Staccato (Summer Charm), Stardust, Starkimson, Stella, Sumesi, Sunburst, Sweet Aryana, Sweet Georgia, Sweet Saretta, Sweet Stephany, Sweet Valentine (Summac), Sweetheart, Symphony
References		Wünsch and Hormaza (2004b), Schuster (2012, 2017, Herrero et al. (2017), Quero-García et al. (2017)

Table 3.3 Incompatibility groups and *S*-genotype of 153 Japanese plum cultivars

I.G.	<i>S</i> -RNase genotype	Cultivars
I	<i>SaSb</i>	A-606, Angelo, Armstrong, Burmosa, Late Soldam, Mammoth, Red Beaut, Soldam, Sordum
II	<i>SbSc</i>	Blackamber, Black Beaut, Delbartazur, Early Sun, Flavor Granade, Fortune, Golden Plum, Golden Plumza, Green Sun, Gulfrose, Honey Red, Jupiter, Laroda, October Sun, Purple Queen, Sugared, Super Giant, TC Sun
III	<i>SbSf</i>	AU Amber, AU Road Side, Emerald, Frontier, Gran Colle, Verna Delicious
IV	<i>SbSh</i>	Betty Ann, Black Gem, Blue Knight, Eldorado, Freedom, Hiromi Red, Larry Ann, Queen Ann, Songria 10, Sundew, Yonemomo
VI	<i>SfSh</i>	Black Ruby, Kelsey Paulista, Kelsey, Mariposa
VII	<i>ScSh</i>	Angeleno, Gaia, Green Sun, Queen Rosa, Royal Diamond, Ruby Crunch, Ruby Queen, Sweet August
VIII	<i>SeSh</i>	African Delight, African Pride, Autumn Pride, Black Diamond, Black Gold, Black Late, Diamex, Earliqueen, John W., Ruby Star, Showtime, Souvenir
IX	<i>SfSg</i>	Golden Japan, Manchurian, Shiro, White Plum
X	<i>ShSk</i>	Elephant Heart, Explorer, Friar, Howard Sun, Golden Kiss, Redgold, Songold
XI	<i>ScSe</i>	AU Rosa, Autumn Giant, Black Splendor, Champion, Kesselman, Royal Garnet, Royal Zee, Roysum, Sweet Autumn, Sybarite
XII	<i>SbSe</i>	Black Jewell, Durado, Flavor King, Freya, Murietta, Pioneer, Sapphire, Sparkly, Tomar
XIII	<i>SeSf</i>	Black Star, Morris, Primetime
XIV	<i>SaSc</i>	Crimson Glo, Sunkiss, White Queen
XV	<i>SgSh</i>	Bonnie, Ruby Sweet
XVI	<i>SfSk</i>	Kelsey, Weikeshum, Wickson
XVII	<i>SbSo</i>	Ambra, Olinda
XVIII	<i>SaSf</i>	Ozarkpremier, Terada
XIX	<i>SbSd</i>	Formosa, Harypickstone
XX	<i>SbSk</i>	Homeking Delicious
XXI	<i>SeSk</i>	Newyorker, Simon
XXII	<i>SaSe</i>	Dolly, Riou
XXIII	<i>ScSd</i>	Oishiwasumomo
0 (cultivars with unique <i>S</i> -genotype)		Abundance* (<i>SaSk</i>), Botan (<i>SaSm</i>), Byron Gold (<i>ShSr</i>), Combination (<i>SgSl</i>), Gaia (<i>ScSh</i>), Joana Red (<i>SrSs</i>), Lantz (<i>SbSl</i>), Mitard (<i>SqSf</i>), October Red (<i>ShSp</i>), Red Heart (<i>ScSo</i>), Summer Queen (<i>ScSf</i>), Songria 15 (<i>SaSh</i>), Starkgold (<i>SgSk</i>), Superior (<i>SaSn</i>), Tecumseh (<i>SfSj</i>)
Self-compatible cultivars		African Rose, Beauty, Casselman, Honey Rosa, Karari, Laetitia, Late Santa Rosa, Methley, Nubiana, Pioneer, Red Rosa, Rio, Rubirosa, Santa Rosa, Simka, Souvenir, Zanzi Sun
References		Yamane et al. (1999), Beppu et al. (2002, 2003), Sapir et al. (2004), Halász et al. (2007a), Jun et al. (2007), Sapir et al. (2007), Zhang et al. (2007), Guerra et al. (2009, 2011, 2012, 2020) Guerra and Rodrigo (2015)

Table 3.4 Incompatibility groups and *S*-genotype of 160 apricot cultivars

I.G.	<i>S</i> -RNase genotype	Cultivars
I	S_1S_2	Castleton, Farmingdale, Giovanniello, Goldrich, Hargrand, Lambertin-1
II	S_8S_9	Perlecot, Pinkcot
III	S_2S_6	Avirine (Bergarouge), Iri Bitirgen, Moniqui
IV	S_2S_7	Artvin P.A., Ouardi, Priana
V	S_2S_8	Alyanak, Holly Cot, Sweet Cot, Ziraat Okulu
VI	S_2S_{19}	Dörtöl 4, Sebbiyiki
VIII	S_6S_9	Cataloglu, Cheyenne, Feria Cot, Ninja, Orangered, Ozal, Soganci, Stark Early Orange, Sunny Cot, Wonder Cot
XVIII	S_1S_3	Cooper Cot, Perfection
XIX	S_2S_3	Mayacot, Sun Glo
XX	S_2S_9	Goldstrike, Magic Cot
XXI	S_3S_8	Lilly Cot, Spring Blush
XXII	S_3S_9	Durobar (Almadulce), Flodea, Henderson, Kosmos, Tsunami
XXIII	S_7S_9	Goldbar
XXIV	S_1S_6	Primaya
0 (cultivars with unique <i>S</i> -genotype)		Castlebrite (S_2S_2), Cow-1 (S_1S_{31}), Cow-2 ($S_{20}S_{31}$), Estrella (S_1S_7), Harcot (S_1S_4), Harlayne (S_3S_{20}), Harmat ($S_{10}S_{11}$), Mariem (S_7S_{20}), Martinet (S_2S_2), Perla (S_2S_{20}), Portici (S_2S_{20}), Shalah (S_5S_{11}), Stella (S_6S_{20}), Veecot (S_2S_{20}), Velázquez (S_5S_{20})
Self-compatible cultivars		Alba, Aprix 20, Aprix 33, Aprix 9, Apriqueen, Bebecou, Beliana, Beliana, Berdejo, Bergecot, Bergeron, Budapest, Búlida, Callatis, Canino, Cebas Red, Charisma, Corbatoy, Cristalí, Currot, Delice Cot, Dorada, Dulcinea, Effect, Fantasma, Faralia, Farbaly, Farbela, Farclo, Fardao, Farfia, Farhial, Farius, Farlis, Fartoli, Flopria, Galta Vermella Valenciana, Galta Roja, Gandía, Ginesta, Gönci magyarkajsz, Grandir, Kalao, Kioto, Lady Cot, Lido, Lito, Lorna, Luizet, Manrí, Mauricio, Medflo, Mediabel, Mediva, Memphis, Milord, Mirlo Anaranjado, Mirlo Blanco, Mirlo Rojo, Mitger, Modesto, Murciana, Ninfa, Oscar, Palabras, Palau, Palsteyn, Patterson, Paviot, Peñafior 02, Pepito del Rubio, Pisana, Pisana, Playa cot, Pricia, Primidi, Primorosa, Rakovszky, Rambo, Regibus, Rojo Carlet, Rouge Cot, Roxana, Rubista, Sam, Sandy cot, Sayeb, Sherpa, Sirena, Soledane, Sulmona, Swired, Tadeo, Tilton, Tirynthos, Tom Cot, Trevatt, Venus, Victor 1, Xirivello, Zaposdolye
References		Egea and Burgos (1996), Burgos et al. (1998), Alburquerque et al. (2002), Halász et al. (2005, 2007b, 2010) Vilanova et al. (2005), Donoso et al. (2009), Egea et al. (2010), Muñoz-Sanz et al. (2017), Herrera et al. (2018a, b), Ruiz et al. (2018)

1987), and after a variable period of mild temperatures, flower bud development reactivates, buds burst, and subsequent flowering proceeds rapidly (Campoy et al. 2011; Fadón et al. 2015).

Chilling requirements of particular cultivars may be estimated by statistical correlations between flowering dates and temperatures before flowering (Alonso et al.

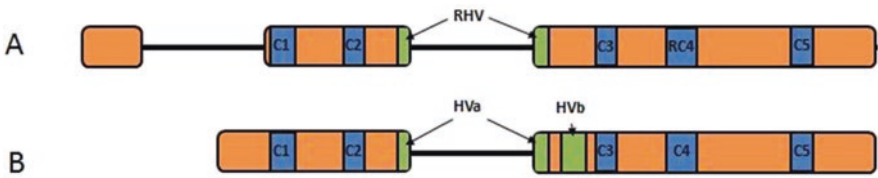


Fig. 3.6 Diagram of the *S-RNase* gene. Genomic sequence of the *S-RNase* gene showing the exons in square, including the five highly conserved regions in Rosaceae (a) and in Plantaginaceae and Solanaceae (b)

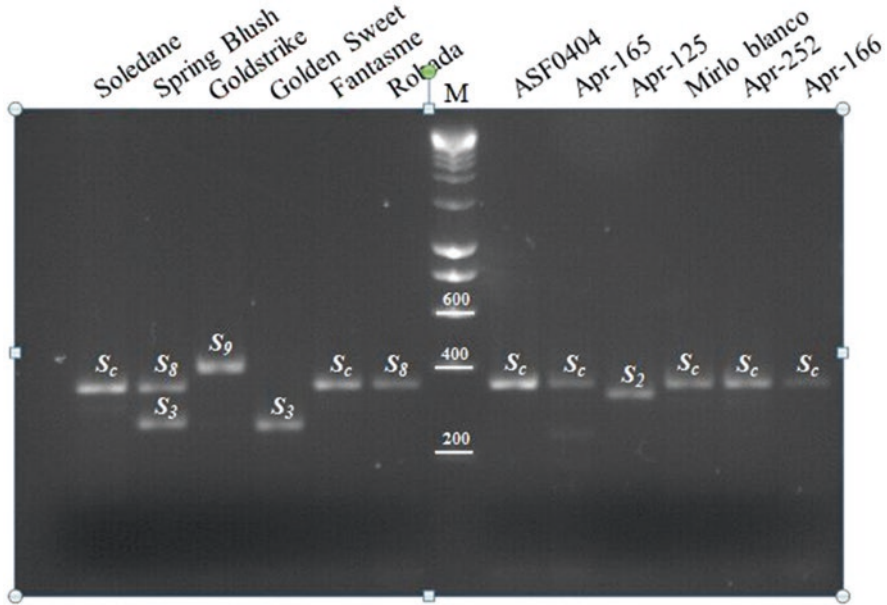


Fig. 3.7 PCR fragment amplification using primer pair primers S_{Rc}-(F/R) for the identification of *S*-alleles in 12 apricot cultivars. Size standard: 1 kb

2005; Luedeling et al. 2009; Luedeling and Gassner 2012) or by cutting shoots at different times during the winter and exposing them to warm temperatures to monitor if the flower buds have recovered their capacity to grow (Fadón and Rodrigo 2018). Once the date of chilling fulfillment is determined, the chilling temperatures previously accumulated are quantified following models such as “chilling hours” (Weinberger 1950), “Utah model” (chill units, Richardson et al. 1974), and “dynamic model” (chill portions, Fishman et al. 1987). These models have been widely used for the estimation of chilling requirements in *Prunus* spp. (Fadón et al. 2020). However, these approaches have some limitations since the methods for determining the time of endodormancy release and for calculating chilling requirements are not standardized and often result in high variations in the estimated values for particular cultivars depending on the location and the experimental conditions (Dennis

Table 3.5 Incompatibility groups and *S*-genotype of 51 European plum cultivars

<i>S</i> -RNase genotype	Cultivars
<i>S</i> ₂ <i>S</i> ₅ <i>S</i> ₆ <i>S</i> ₇ <i>S</i> ₁₂	Mulata
<i>S</i> ₄ <i>S</i> ₆ <i>S</i> ₈ <i>S</i> ₉	Negra
<i>S</i> ₂ <i>S</i> ₅ <i>S</i> ₇ <i>S</i> ₁₀ <i>S</i> ₈ <i>S</i> ₁₂	HuevoChivato
<i>S</i> ₃ <i>S</i> ₅ <i>S</i> ₆ <i>S</i> ₉	Verde
<i>S</i> ₄ <i>S</i> ₆ <i>S</i> ₁₁ <i>S</i> ₉	Negra del país pequeña
<i>S</i> ₁ <i>S</i> ₅ <i>S</i> ₇ <i>S</i> ₉	President
<i>S</i> ₁ <i>S</i> ₃ <i>S</i> ₅ <i>S</i> ₆ <i>S</i> ₁₂	Ruth Gestteter
<i>S</i> ₁ <i>S</i> ₃ <i>S</i> ₂ <i>S</i> ₁₁ <i>S</i> ₉	RC Dorada
<i>S</i> ₂ <i>S</i> ₈ <i>S</i> ₁₀ <i>S</i> ₆ <i>S</i> ₁₂	Fraila
<i>S</i> ₁ <i>S</i> ₃ <i>S</i> ₆ <i>S</i> ₉	Alcor-1
<i>S</i> ₁ <i>S</i> ₅ <i>S</i> ₇ <i>S</i> ₁₁ <i>S</i> ₉	F-4 A-4
Self-compatible cultivars	Anna Späth, Belle de Louvain, Blufre, Cacanska Lepotica, California Blue, Cacanska Rodna, Czar, de Ente, Denniston Superb, Double Robe, Elena, Early Transparent, Early Mirabelle, German Prune, Giant, Gisborne's, Golden Transparent, Goliath, Guthrie's Late, Hanita, Harbella, Haroma, Herman, Jelica, Italian Prune, Jojo, Katinca, Martin, Monarch, Oullin's Golden Gage, Požegaca, Presenta, Reine Claude de Bavay, Reine Claude de Oullins, Ruth Gerstetter, Stanley, Tegera, Valjevka, Victoria, Wangenheims Frühzwetsche
References	Crane (1925), Cambra (1982), Nikolić and Milatović (2010), Neumüller (2011), Abdallah et al. (2019)

Table 3.6 Incompatibility groups and *S*-genotype of 58 sour cherry cultivars

<i>S</i> -RNase genotype	Cultivars
<i>S</i> ₁ <i>S</i> ₄ <i>S</i> ₃₅ <i>S</i> _{36b}	Crisana
<i>S</i> ₁ <i>S</i> ₁₂ <i>S</i> ₁₃ <i>S</i> _{36b}	Erdi Nagygyumolcsu
<i>S</i> ₁ <i>S</i> ₄ <i>S</i> ₃₅ <i>S</i> _{36b}	Pandy 38
<i>S</i> ₁ <i>S</i> ₄ <i>S</i> ₃₅ <i>S</i> _{36b}	Pandy 114
<i>S</i> ₁ <i>S</i> ₄ <i>S</i> _{36a} <i>S</i> _{36b}	Tarina
<i>S</i> ₉ <i>S</i> ₁₃ <i>S</i> ₃₅ <i>S</i> _{36b2}	Tschernokorka
<i>S</i> ₁ <i>S</i> ₁₃ <i>S</i> ₂₆ <i>S</i> ₃₅	Agat
<i>S</i> ₁ <i>S</i> ₁₂ <i>S</i> ₁₃ <i>S</i> _{36b}	Gubenska
<i>S</i> ₆ <i>S</i> ₉ <i>S</i> ₁₃ <i>S</i> _{36b2}	Griot Moskowskij
<i>S</i> ₉ <i>S</i> ₁₃ <i>S</i> ₂₆ <i>S</i> _{36a}	Vladimirska
<i>S</i> ₆ <i>S</i> ₁₃ <i>S</i> ₁₄ <i>S</i> _{36a}	Spanka
Self-compatible cultivars	Ametyst, Cigány 59, Diemitzer Amarelle, Dradem, Early Richmond, Englaise Timpurii, Erdi Botermo, Erdi Jubileum, Espera, Fanal, Galena, Ihruska, Karneol, Korund, Meteor, Montmorency, Morina, MSU III 18 (12), Nana, Obłańska, Pitic de Iasi, Plodorodnaja Mitschurina, Rheinische Schattenmorelle, Safir, Surefire, Tamaris, Topas, Topaz, Újfehértói fürtös, Vowi, Vstryecha, Kelleriis 16, Stevnbaer, Achat, Bucovina, Carmine Jewel, Coraline, Kutahya, Gerema Ilva, Jachim, Jade, Lyubskaya, Nefris, Northstar, Sabina, Šumadinka, Tiki
References	Tsukamoto et al. (2008), Lisek et al. (2017), Schuster et al. (2017), Sebolt et al. (2017)

2003). However, the use of these models has been very useful for the selection of cultivars in particular areas, although in most *Prunus* species an increasing number of new cultivars of unknown requirements are being released (Fadón et al. 2020).

Low temperature stress resulting in death of flower organs is one of the main limiting factors affecting fruit production in stone fruits in some regions and seasons. Freeze injury may occur before bud acclimation in autumn and, especially, during deacclimation, bud burst, and flowering (Rodrigo 2000). High temperatures in the days before flowering can also cause floral malformations such as underdeveloped pistils (Rodrigo and Herrero 2002b).

When pollinizer cultivars are needed, an overlap in flowering time must occur between the main fruit producing cultivar and their pollinizers to allow the transfer of compatible pollen from the anthers to the stigma by pollinators. Insects play a main role in pollination for fruit production in most fruit tree crops, including stone fruits (Gallai et al. 2009). Among them, the major group of animal pollinators in commercial fruit orchards in temperate regions are bees (Kevan 1999), especially the European honeybee (*Apis mellifera* L. [Hymenoptera: Apidae]), but honeybee decline is a fact in different regions and environments (Goulson et al. 2015). Besides the effort needed to analyze and mitigate the causes behind honeybee decline, a complementary approach involves studying additional pollinating insects, such as solitary bees (Koh et al. 2018) or species of *Bombus* (Calzoni and Speranza 1998) that could diversify the availability of pollinators in order to optimize fruit production (Garibaldi et al. 2013; Kleijn et al. 2015). Different species of solitary bees have been shown to play a role in pollination of stone fruits, and a proper management of this diversity is necessary to deal with the diverse problems that affect honeybees, related to climate change, parasites, or diseases (Koh et al. 2018).

The decline of wild pollinating insects is making the introduction of honeybees or bumblebees in commercial orchards essential for ensuring fruit set in an increasing number of situations, not only in self-incompatible but also in self-compatible cultivars. If necessary, managed beehives should be placed in the orchard when the first flowers have already been opened, to prevent bees from establishing their foraging habits in other flowering species (Thompson 1996).

The optimum number of beehives in an orchard depends firstly on the size of the colonies. A good colony should show over 100 bees per minute entering the hive under adequate weather conditions (temperature above 18 °C without wind). A second important factor to determine the number of beehives is the number of trees per hectare and the flower density of the trees. In warm, sunny, and calm conditions, the observation of 25–35 bees working on a mature tree at full bloom can indicate enough pollinators in the orchard (Koumanov and Long 2017). Although ideally the beehives should be uniformly distributed in the orchard for optimal pollination efficiency, they may be placed in groups to facilitate handling, adjusting the distance between groups according to the needs (Thompson 1996).

3.7 Concluding Remarks and Perspectives

Adequate pollination during the short flower life span is essential to ensure fruit production in stone fruit crops. In many areas, climate change is negatively affecting different aspects of the reproductive biology of fruit trees jeopardizing fruit production, which is highlighting the impact of adequate pollination management in commercial orchards (Hedhly et al. 2009; Hedhly 2011). The reduction of chilling during winter is causing a delay for some cultivars to fulfill their chilling requirements or even not fulfill them. This often results in a delay in flowering and causes that inter-compatible cultivars with different chilling requirements do not coincide at blooming in those seasons of insufficient winter chilling (Luedeling et al. 2011; Luedeling 2012; Atkinson et al. 2013). Therefore, some pollinizer cultivars become inadequate and need to be replaced by others. Thus, knowing the pollination requirements of particular cultivars is increasingly necessary. Although self-(in)compatibility and inter-incompatibility between cultivars are known in many of the cultivars currently grown (see Sects. 3.4 and 3.5), especially in species such as sweet cherry, almond, or Japanese plum, the constant varietal renewal, with the introduction of a number of new cultivars each year, makes necessary to continue studying the pollination requirements in the self-incompatible species (Fig. 3.8).

Results obtained in the last decades have allowed establishing the pollination requirements of most commercial cultivars in the main stone fruit crops. For the coming years, it will be necessary to continue determining the self-(in)compatibility and the incompatibility relationships of the new releases. While the available technology allows a fast determination of the *S*-allele genotype of any new cultivar or offspring in the main stone fruit species, the complexity to establish self-(in)

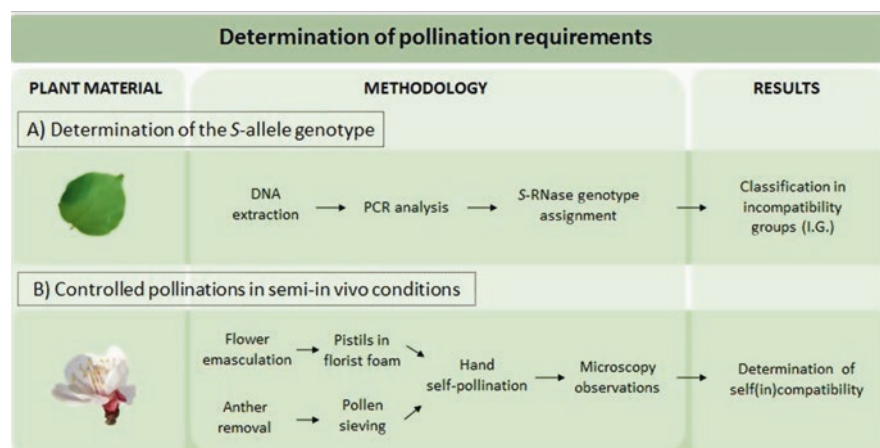


Fig. 3.8 Diagram of the experimental design to determine pollination requirements in stone fruit crops. Workflow of the *S*-allele identification by molecular approaches (a). Workflow of self-(in) compatibility determination by controlled pollinations in semi-in vivo conditions in the laboratory (b)

compatibility differs between species. Particular *S*-alleles associated with self-compatibility have been reported in almond (Kodad et al. 2010; Fernández i Martí et al. 2014), sour cherry (Yamane et al. 2003), and sweet cherry (Bošković and Tobutt 1996; Wünsch and Hormaza 2004a; Cachi and Wünsch 2014). In these species, the identification of *S*-alleles of each cultivar allows not only knowing its incompatibility relationships with other cultivars but also establishing its self-(in) compatibility. This situation has also been previously considered in apricot (Halász et al. 2005; Vilanova et al. 2006), although recent reports have shown self-compatible cultivars whose *S*-genotype does not include the *S*-allele previously associated with self-compatibility (Zuriaga et al. 2013; Muñoz-Sanz et al. 2017; Herrera et al. 2018a). In Japanese plum, self-compatibility was initially associated with the presence of particular *S*-alleles (Beppu et al. 2005, 2010; 2012a, b; Guerra et al. 2009). However, several cultivars carrying these *S*-alleles have been described as self-incompatible. Thus, in apricot, Japanese plum, European plum, and sour cherry, observing the pollen tube behavior under the microscope in self-pollinated pistils is still a recommended tool to assess self-(in)compatibility (Herrera et al. 2018a, 2020; Guerra et al. 2020).

On the other hand, there is a lack of information on the chilling requirements in most cultivars of stone fruit crops. In the next years, this character will be increasingly important to know the adaptability of cultivars to different geographical areas due to the changing conditions caused by climate change, which are causing insufficient yield in some cultivars previously considered as well adapted to particular regions (Campoy et al. 2011; Luedeling et al. 2011; Atkinson et al. 2013). The lack of estimations of chilling requirements is mainly due to the absence of a fast and reliable approach to quantify both chilling and heat requirements (Dennis 2003; Fadón et al. 2020) but also to the lack of a biological marker that allows knowing whether the chilling requirements have been fulfilled under orchard conditions (Fadón and Rodrigo 2018). Finding a biological marker by quantifying a physiological parameter or the expression of particular genes would clarify this situation, which in many cases is accentuated by the expansion of cultivars to new warmer areas looking for early ripening.

3.8 Conclusion

Under the generic name of stone fruit crops, different plant species of the *Prunus* genus in the Rosaceae that produce a drupe type of fruit are included, mainly almonds, apricots, cherries, peaches, and plums. As in most crops in which the main commercial interest is in the fruits, the reproductive phase is probably the main limiting factor for profitable production in stone fruit crops and, more specifically, the days following flower opening, the progamic phase that takes place from pollination to fertilization. The importance of appropriate pollination management approaches is increasing in recent years in these crops due to the release of new cultivars with unknown pollination requirements, the expansion of stone fruit crops to new cultivation areas, and the decrease in winter chilling in many regions caused

by climate change. Consequently, a proper understanding of the pollination requirements of cultivars of the different stone fruits is urgently needed in order to optimize production. Although there is a high variability among stone fruit species in flower characteristics, chilling and heat requirements, pollination behavior, and flowering times, the main biological processes are conserved, and, consequently, a common approach to manage pollination problems can be followed.

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ANEXO II

Herrera, S., Lora, J., Hormaza, J. I., and Rodrigo, J. (2020b). Necesidades de polinización en variedades de albaricoquero (Parte I): autoincompatibilidad. *Revista de Fruticultura* 77, 52–61



Necesidades de polinización en variedades de albaricoquero (Parte I): autoincompatibilidad

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RESUMEN

En albaricoquero se ha producido una importante renovación varietal, con la introducción de muchas variedades procedentes de distintos programas de mejora. La mayoría de las variedades tradicionales son auto-compatibles y sus flores se pueden fecundar con su propio polen para producir fruto. Sin embargo, algunas de las nuevas variedades son autoincompatibles y necesitan ser cultivadas junto a árboles polinizadores de otras variedades compatibles y coincidentes en floración. El objetivo de este trabajo es determinar el carácter de auto(in)compatibilidad en las principales variedades cultivadas en la actualidad y en las nuevas de reciente introducción para conocer cuales se pueden polinizar con su propio polen y cuales necesitan polinización cruzada para fructificar. De las 55 variedades más cultivadas en la actualidad, se han identificado 31 variedades autocompatibles (54%) y 24 variedades autoincompatibles (42%).

Palabras clave: Albaricoquero, Autoincompatibilidad, Polinización, *Prunus armeniaca*, Tubos polínicos.

ABSTRACT

Pollination requirements in apricot cultivars (Part I): self(in)compatibility. An important varietal renewal is taking place in apricot with the introduction of new releases from different breeding programs. Most traditional cultivars are self-compatible and their flowers can be fertilized with their own pollen to set fruit. However, some of the new cultivars are self-incompatible and must be interplanted with other compatible cultivars that overlap in flowering time. The objective of this work is to determine the self(in) compatibility of the main cultivars currently grown in Spain and of the new releases to know those that can be pollinated with their own pollen and those that need cross pollination to set fruit. Of the 55 main cultivars grown in 2019, 31 were identified as self-compatible (54%) and the other 24 as self-incompatible (42%).

Key words: Apricot, Pollen tubes, Pollination, *Prunus armeniaca*, Self(in)compatibility.

El albaricoquero (*Prunus armeniaca* L.) produce en España más de 176.000 toneladas al año, ocupando el tercer lugar entre los frutales de hueso tras el melocotonero y el almendro. Las principales zonas productoras se localizan en la cuenca mediterránea (Región de Murcia y Comunidad Valenciana, con 97.755 y 24.435 toneladas, respectivamente) y Aragón, con 14.173 toneladas (MAPA, 2020). El cultivo ha sufrido grandes fluctuaciones en las últimas décadas. En 1991 llegó a alcanzar una producción de 210.900 toneladas. Sin embargo, a mediados de los años 90 miles de árboles fueron arrancados debido a la incidencia del virus de la sharka, que provocó una reducción del 27% de la superficie de cultivo, con el consiguiente drástico descenso en la producción y ocasionando graves pérdidas económicas (EGEA, CAMPOY y RUIZ, 2010). Ante esta situación, se pusieron en marcha distintos programas de mejora genética públicos y privados, con el objetivo de obtener nuevas variedades resistentes a la sharka y adaptadas a nuestras condiciones edafoclimáticas (LLÁCER, 2005; MARTÍNEZ-CALVO *et al.*, 2009; EGEA *et al.*, 2010). En la actualidad existe un abanico de nuevas variedades que han sustituido con éxito a gran número de las variedades tradicionales susceptibles a la sharka.

Para la obtención de las nuevas variedades, en muchos casos se han utilizado como parentales variedades norteamericanas resistentes a la sharka, que no se podían cultivar en las principales zonas de cultivo nacionales por tener ele-

Cuadro 1. Producción, superficie cultivada y auto(in)compatibilidad de las variedades de albaricoquero más cultivadas en España en 2019.

Variedad	Superficie cultivada (ha)	Superficie cultivada (%)	Producción (t)	Producción (%)	Auto(in)compatibilidad
Colorado	1.016	7,8	14.960	9,1	Autoincompatible
Búlida	1.004	7,7	9.326	5,7	Autocompatible
Mirlo Anaranjado	762	5,9	10.146	6,2	Autocompatible
Flopria	750	5,8	12.136	7,4	Autocompatible
Mitger	571	4,4	4.400	2,7	Autocompatible
Lady Cot	547	4,2	7.203	4,4	Autocompatible
Mogador	511	3,9	7.110	4,3	Autocompatible
Resto de Valencianos	327	2,5	4.614	2,8	Autocompatible
Flodea	322	2,5	5.175	3,2	Autoincompatible
Magic Cot	291	2,2	4.133	2,5	Autoincompatible
Faralia	254	2,0	3.151	1,9	Autocompatible
Lilly Cot	251	1,9	3.679	2,2	Autoincompatible
Moniqui	235	1,8	2.133	1,3	Autoincompatible
Murciana	235	1,8	2.867	1,7	Autocompatible
Kioto	230	1,8	3.682	2,2	Autocompatible
Wonder Cot	220	1,7	2.961	1,8	Autoincompatible
Galta Rocha	189	1,5	1.840	1,1	Autocompatible
Flavor Cot	150	1,2	1.391	0,8	Autocompatible
Madison	148	1,1	1.976	1,2	Autoincompatible
Mirlo Blanco	145	1,1	2.581	1,6	Autocompatible
Pepito	137	1,1	1.687	1,0	Autocompatible
Luca	132	1,0	1.929	1,2	Autoincompatible
Tsunami	123	0,9	1.502	0,9	Autoincompatible
Orangered	122	0,9	1.566	1,0	Autoincompatible
Rambo	121	0,9	1.478	0,9	Autocompatible
Orange ruby	119	0,9	2.019	1,2	Autocompatible
Rojo Pasión	115	0,9	1.099	0,7	Autocompatible
Mambo	111	0,9	2.013	1,2	Autoincompatible
Valorange	107	0,8	1.509	0,9	Autocompatible

vadas necesidades de frío (RODRIGO y HORMAZA, 2005). Pero estas variedades también son auto-incompatibles, por lo que necesitan polinización cruzada para producir fruto, a diferencia de la mayoría de variedades cultivadas en España antes de la llegada de la sharka a nuestro país, que eran autocompatibles. Como resultado de esta situación, muchas de las descendencias obtenidas de estos cruzamientos han heredado este carácter y son autoincompatibles. Aunque algunos programas de mejora registran únicamente variedades autocompatibles, en otros casos se han introducido nuevas variedades autoincom-

patibles que presentan problemas de producción si no se polinizan adecuadamente.

En la actualidad, las variedades más cultivadas en España incluyen variedades tradicionales como 'Búlida', 'Mitger' o 'Moniqui', junto a otras que se han ido introduciendo en los últimos años ('Colorado', 'Mirlo Naranja' o 'Lady Cot') (Cuadro 1). El objetivo de este trabajo es determinar el carácter de autocompatibilidad en las principales variedades cultivadas y en las nuevas de reciente introducción para conocer cuáles se pueden polinizar con su propio polen y cuáles necesitan polinización cruzada para fructificar.

Cuadro 1. (Continuación).

Variedad	Superficie cultivada (ha)	Superficie cultivada (%)	Producción (t)	Producción (%)	Auto(in)compatibilidad
Farely	102	0,8	1.394	0,8	Autoincompatible
Arquer	94	0,7	1.416	0,9	Autocompatible
Fartoli	92	0,7	1.255	0,8	Autocompatible
Big Red	91	0,7	1.537	0,9	Autocompatible
Megatea	88	0,7	1.499	0,9	Autoincompatible
Mirlo Rojo	84	0,6	1.239	0,8	Autocompatible
Samourai	83	0,6	845	0,5	Autoincompatible
Almabar	81	0,6	1.222	0,7	Autocompatible
Paviot	78	0,6	394	0,2	Autocompatible
Sublime	78	0,6	1.166	0,7	Autoincompatible
Robada	67	0,5	1.011	0,6	Autoincompatible
Medaga	67	0,5	884	0,5	Autoincompatible
Goldbar	60	0,5	793	0,5	Autoincompatible
Sweet Cot	54	0,4	440	0,3	Autoincompatible
Tom Cot	52	0,4	602	0,4	Autocompatible
Tornado	51	0,4	446	0,3	Autoincompatible
Sunny Cot	42	0,3	457	0,3	Autoincompatible
Alba	42	0,3	768	0,5	Autocompatible
Perle Cot	41	0,3	482	0,3	Autoincompatible
Dorada	36	0,3	284	0,2	Autocompatible
Soledane	27	0,2	329	0,2	Autocompatible
Flash Cot	27	0,2	366	0,2	Autoincompatible
Colorados (Antones)	25	0,2	220	0,1	Autocompatible
Floneca	19	0,1	252	0,2	Autoincompatible
Kou	16	0,1	208	0,1	No determinada
Rojo de Carlet	13	0,1	125	0,1	Autocompatible
Delice Cot	8	0,1	125	0,1	Autocompatible
Otras	2.239	17,2	24.221	14,7	
Total	13.002	100	164.245	100	

Fuente: Agrosseguro (Datos de superficie asegurada).

El proceso de polinización y fecundación

La floración del albaricoquero, como en otros frutales de hueso, está condicionada tanto por factores externos (condiciones ambientales, presencia de polinizadores) como por factores internos (estado nutricional de las yemas, factores genéticos). Las condiciones meteorológicas como la temperatura y la humedad influyen en el establecimiento del cuajado. Las heladas primaverales pueden causar grandes caídas de flores mientras que las altas temperaturas durante la diferenciación floral provocan malformacio-

nes en las estructuras femeninas y masculinas de la flor y pueden adelantar la floración. También se pueden producir problemas en floración debido a la lluvia ya que afecta a la adhesión del polen al estigma.

Para que se produzca el cuajado de fruto, es también necesaria una polinización adecuada de la flor. Para ello, es imprescindible la presencia de insectos polinizadores como abejas (Foto 1) o abejorros para que los granos de polen se transporten adecuadamente hasta el estigma de la flor, donde germinan emitiendo los tubos polínicos que deben crecer a través del esti-



Foto 1. Abeja polinizando flores de albaricoquero.

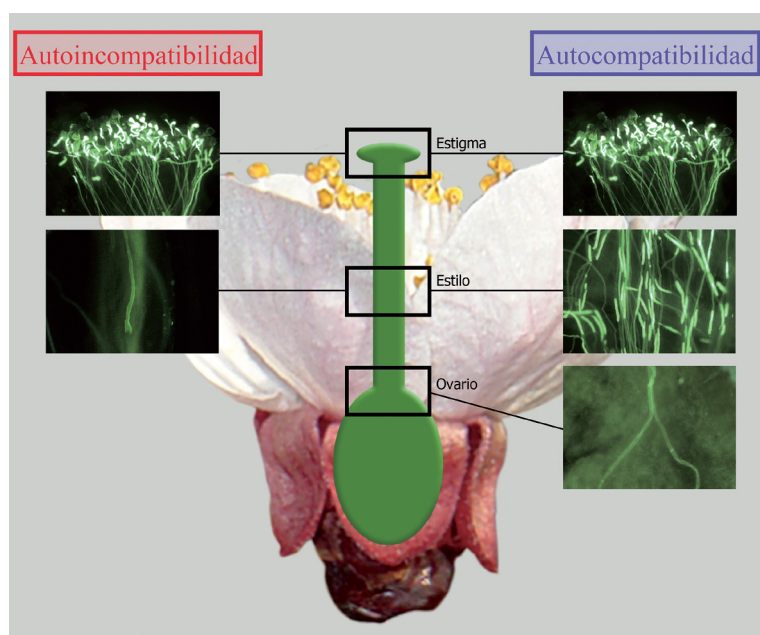


Figura 1. Proceso de polinización a fecundación en una flor de albaricoquero. Comportamiento de los tubos polínicos en el pistilo en una variedad autoincompatible (izda.) y en una variedad autocompatible (dcha.).

lo, alcanzar el ovario y fecundar uno de los dos óvulos (Figura 1).

En algunos frutales existe incompatibilidad floral, que es una barrera genética que actúa para evitar la fecundación de la flor con su propio polen, promoviendo la variabilidad genética en la descendencia. La autoincompatibilidad se produce cuando el polen es incapaz de fecundar la flor de la propia variedad. Este mecanismo se ma-

nifiesta mediante la detención de los tubos polínicos en el estilo antes de llegar al ovario, impidiendo así la fecundación. Para producir fruto, las variedades autoincompatibles deben ser cultivadas junto con variedades con las que sean intercompatibles, para que sus flores se puedan polinizar con polen compatible. Por otro lado, en las variedades autocompatibles, los granos de polen son compatibles con el pistilo de la propia flor,

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Figura 2. Determinación de la auto(in)compatibilidad en albaricoquero.

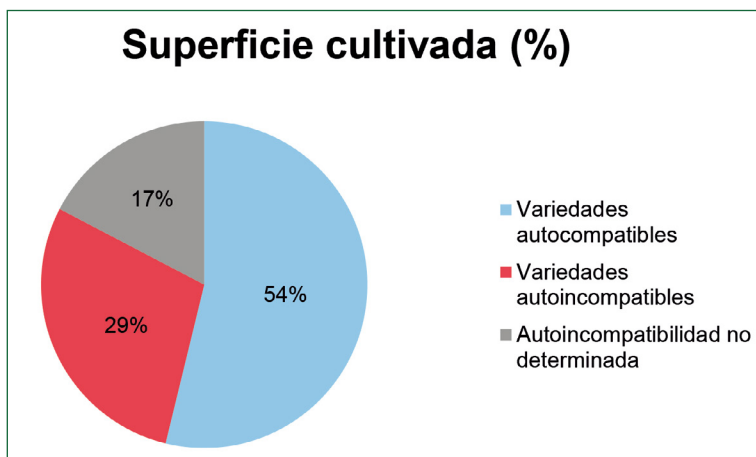


Figura 3. Porcentaje de la superficie cultivada de albaricoquero en España en 2019 ocupada por variedades autocompatibles y autoincompatibles.

por lo que los tubos polínicos procedentes de polen de la misma flor o de flores de la misma variedad crecen a lo largo del estilo hasta el ovario, donde se produce la fecundación del óvulo.

En albaricoquero existen tanto variedades autocompatibles como autoincompatibles, por lo que es necesario conocer las necesidades de polinización de cada variedad para poder diseñar adecuadamente las plantaciones asegurando la presencia de polen compatible.

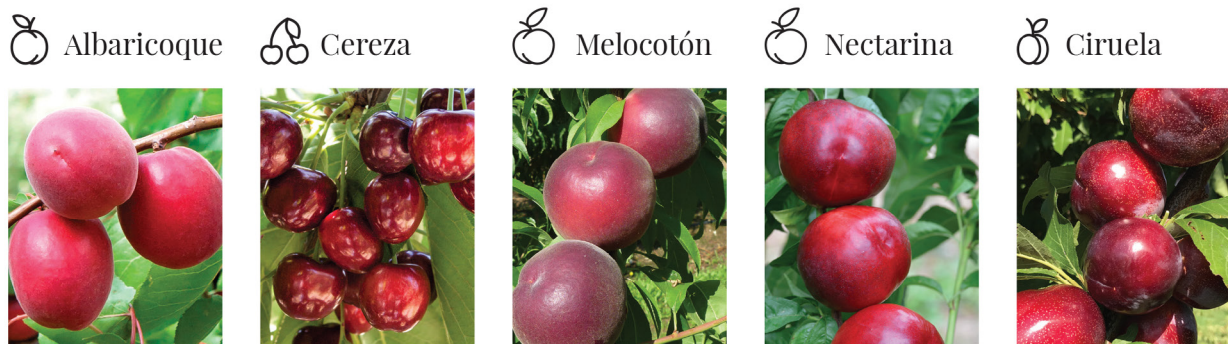
Determinación de la auto(in) compatibilidad

La autoincompatibilidad se ha estudiado tradicionalmente mediante la polinización controlada de flores en campo y la posterior determinación del cuajado de frutos. Sin embargo, el proceso de fructificación puede verse afectado por factores externos, como las condiciones meteorológicas adversas, que pueden interferir en los resultados de estos ensayos. Para evitarlo, se ha desarrollado una metodología en

laboratorio que permite determinar la auto(in) compatibilidad de cada variedad sin que se vea afectada por las condiciones externas. Para ello, se recogen flores en estado de botón globoso, para asegurar que las flores no han sido visitadas por insectos polinizadores y, por tanto, solo tienen su propio polen en el interior. Las flores se preparan en bandejas en el laboratorio en condiciones controladas y se polinizan con su propio polen. A los tres días, que es el tiempo medio que tarda un tubo polínico compatible en alcanzar el óvulo en albaricoquero, las flores se fijan y se preparan para la observación del crecimiento de los tubos polínicos al microscopio (Figura 2).

Observaciones al microscopio

Para determinar la autoincompatibilidad de cada variedad, se realizaron dos cruzamientos controlados, uno con el polen de la misma variedad y otro con polen de una variedad conocida compatible que se utilizó como control.



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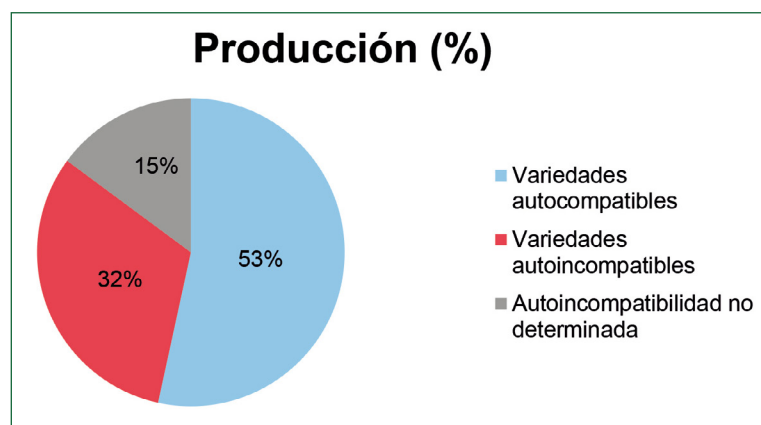


Figura 4. Porcentaje de la producción de albaricoquero en España en 2019 ocupada por variedades autocompatibles y autoincompatibles.

Cuadro 2. Variedades de albaricoquero autocompatibles y autoincompatibles.

Variedades Autoincompatibles			Variedades Autocompatibles				
Aurora	Holly Cot	Priabel	Alba	Currot	Golden Sweet	Mirlo Rojo	Rojo de Carlet
Bergarouge	Lambertín-1	Robada	Almabar	Delice Cot	Gönci Magyar	Mitger	Rojo Pasión
Colorado	Lilly Cot	Samourai	Aprix 20	Dorada	Harval	Modesto	Rouge Cot
Cooper Cot	Luca	Spring Blush	Aprix 33	Faralia	Hatíf Colomer	Mogador	Rouge de Roussillon
Almadulce	Madison	Stark E. Orange	Aprix 9	Farbaly	Katy	Murciana	Sandy Cot
Early Blush	Magic Cot	Stella	Arquer	Farbela	Kioto	Ninfa	Soledane
Farely	Mambo	Sublime	Bergecot	Farclo	Lady Cot	Orange ruby	Swired
Feria Cot	Maya Cot	Sundrop	Bergeron	Fardao	Lito	Palabras	Tadeo
Flash Cot	Medaga	Sun Glo	Big Red	Farfia	Lorna	Palau	Tilton
Flodea	Megatea	Sunny Cot	Búlida	Farhial	Luizet	Palstein	Tirynthos
Floneca	Moniquí	Sweet Cot	Canino	Farius	Mauricio	Paviot	Tom Cot
Goldbar	Monster Cot	Tornado	Castlebrite	Farlis	Medflo	Pepito del Rubio	Trevatt
Goldrich	Muñoz	Tsunami	Charisma	Fartoli	Mediabel	Playa Cot	Valorange
Goldstrike	Orangered	Vanilla Cot	Colorados (Antones)*	Flavor Cot	Mediva	Pricia	Victor-1
Harcot	Pandora	Veecot		Flopria	Mirlo Blanco	Primidi	
Hargrand	Perle Cot	Velázquez	Corbato	Galta Rocha	Mirlo Anaranjado	Rambo	
Henderson	Pinkcot	Wonder Cot	Cristali	Ginesta		Real fino	

Las variedades en las que se observaron los tubos polínicos detenidos en mitad del estilo en las flores autopolinizadas se consideraron variedades autoincompatibles. Por otro lado, aquellas variedades en las que se observaron tubos polínicos creciendo hasta alcanzar el final del estilo e introduciéndose en el ovario, donde tiene lugar la fecundación, se consideraron variedades autocompatibles (Figura 1).

Para evitar posibles errores en el diagnóstico de la autoincompatibilidad debidos a polen no

germinado, se debe comprobar la viabilidad del polen de cada variedad. Para ello, después de polinizar las flores se sembró el polen utilizado en placas con medio de cultivo, analizando su germinación al microscopio tras 24 horas en el medio de germinación.

En todas las variedades se observó una correcta germinación del polen en condiciones in vitro, y también polen germinado en el estigma de las flores analizadas, por lo que se descartó que la falta de tubos polínicos en el ova-

rio fuera debida a problemas de germinación del polen. De las 55 variedades más cultivadas en la actualidad (*Cuadro 1*), 31 variedades (54%) presentaron tubos polínicos a lo largo del estilo que alcanzaron el ovario en las flores polinizadas con su propio polen y se consideraron variedades autocompatibles. Por otro lado, en 24 variedades (42%) se observó que los tubos polínicos detuvieron su crecimiento a lo largo del estilo y se consideraron variedades autoincompatibles (*Figura 1*). Las variedades autoincompatibles de albaricoquero ocuparon en 2019 una superficie de más de 3.700 ha (*Cuadro 1*), el 29 % de la superficie total asegurada (*Figura 3*), y una producción de más de 50.000 toneladas (*Cuadro 1*), el 32% de la producción total (*Figura 4*). Esta situación es muy diferente a la existente antes de la llegada de la sharka, cuando la mayoría de las variedades cultivadas eran autocompatibles.

En la actualidad se conoce la auto(in)compatibilidad de más de 150 variedades, incluyendo variedades tradicionales de distintos países y variedades comerciales de reciente introducción. En el *Cuadro 2* se ha recopilado información de la mayoría de las variedades cultivadas en España, incluyendo los resultados obtenidos en este trabajo y en trabajos anteriores (BURGOS *et al.*, 1997; EGEA *et al.*, 2010b; MUÑOZ-SANZ *et al.*, 2017; HERRERA *et al.*, 2018), mostrando 80 variedades autocompatibles y 52 variedades autoincompatibles.

Conclusiones

Aunque ya se conocía la presencia de algunas variedades autoincompatibles, los resultados obtenidos han mostrado un porcentaje de variedades autoincompatibles muy alto. Ya que la tendencia es que las nuevas variedades, en muchos casos muy productivas y con características de fruto muy atractivas, continúen sustituyendo a las tradicionales, es previsible que la presencia de nuevas variedades autoincompatibles se mantenga o aumente en los próximos años. Esto hace que, además de la autoincompatibilidad, sea necesario conocer las relaciones de intercompatibilidad entre variedades para poder realizar un diseño adecuado de las

nuevas plantaciones, y también para identificar problemas de producción asociados a falta de polen compatible en las plantaciones ya existentes. En el siguiente número de Revista de Fruticultura (noviembre/diciembre de 2020) se publicará un artículo ("Necesidades de polinización en variedades de albaricoquero (Parte II): Selección de variedades polinizadoras") incluyendo las relaciones de incompatibilidad de la mayoría de las variedades de albaricoquero cultivadas. •

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ANEXO III

Herrera, S., Lora, J., Hormaza, J. I., and Rodrigo, J. (2020c). Necesidades de polinización en variedades de albaricoquero (Parte II): selección de variedades polinizantes. *Revista de Fruticultura* 78, 6-11.

Necesidades de polinización en variedades de albaricoquero (Parte II): selección de variedades polinizantes

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RESUMEN

En los últimos años se han registrado muchas variedades de albaricoquero de las que apenas se tiene información sobre sus necesidades de polinización. Muchas de las nuevas variedades son autoincompatibles y necesitan ser cultivadas junto a otras variedades con las que muestren incompatibilidad. El desarrollo de nuevas herramientas basadas en marcadores moleculares permite determinar las relaciones de incompatibilidad de las variedades mediante la identificación de los alelos *S* y clasificar las variedades autoincompatibles en grupos de incompatibilidad. En este trabajo se describen las necesidades de polinización de 129 variedades, 33 autoincompatibles y 91 autocompatibles. Las variedades autoincompatibles se han agrupado en 13 grupos de incompatibilidad. Esta información permite escoger la combinación de variedades más idónea para una correcta polinización. Las variedades autoincompatibles deben plantarse junto a variedades de un grupo de incompatibilidad diferente, que además coincidan en floración.

Palabras clave: *Prunus armeniaca*, Autoincompatibilidad, Marcadores moleculares, alelos *S*, Grupos de incompatibilidad.

ABSTRACT

Pollination requirements in apricot cultivars (Part II): selection of appropriate pollinizers.

The number of new apricot cultivars has been increasing in the last years. However, the pollination requirements of most of those new apricot cultivars are still unknown. Many of them are self-incompatible and need to be interplanted with cross-compatible cultivars. The development of new tools based on molecular markers have allowed to determine the incompatibility relationships among the apricot cultivars by the characterization of *S*-alleles. In this work, we analyze the pollination requirements of 129 apricot cultivars, including 33 self-incompatible and 91 self-compatible cultivars. Self-incompatible cultivars have been allocated according to the *S*-genotype in 13 incompatibility groups. Incompatibility identification of the new apricot cultivars is a valuable information for the appropriate selection of cultivars in commercial orchards and apricot breeding programs. Thus, self-incompatible cultivars must be interplanted with a cultivar of a different Incompatibility Group and with overlapping flowering times.

Key words: *Prunus armeniaca*, Self(in) compatibility, Molecular markers, *S*-alleles, Incompatibility groups.

Como en la mayoría de los frutales, en albaricoquero es imprescindible que se realice una polinización adecuada para que se produzca el cuajado. Para ello, es necesaria la presencia de insectos polinizadores (Foto 1) que permitan con éxito la llegada de los granos de polen al estigma de la flor, donde cada grano de polen emite un tubo polínico. La parte femenina de la flor, además del estigma, presenta un estilo y un ovario con dos óvulos. Los tubos polínicos crecen a través del estilo y llegan al ovario, donde pueden fecundar uno de los dos óvulos. Para el diseño de las plantaciones, es necesario conocer las necesidades de polinización de cada variedad para evitar problemas de cuajado. Las variedades de albaricoquero, al igual que otros frutales, pueden ser autocompatibles en las flores y pueden fecundarse con el polen de la propia variedad, o autoincompatibles, donde es necesaria la polinización cruzada con polen de otra variedad compatible para obtener fruto. La incompatibilidad se manifiesta mediante la inhibición del crecimiento del tubo polínico en el estilo, lo que impide la fecundación del óvulo.

En el anterior número de Revista de Fruticultura (septiembre/octubre de 2020) se publicó el artículo "Necesidades de polinización en variedades de albaricoquero (Parte I): autoincompatibilidad" (HERRERA *et al.*, 2020), en el que se determinaron las necesidades de polinización de las variedades más cultivadas en la actualidad mediante cruzamientos controlados en laboratorio y el estudio del crecimiento de los tubos polínicos a través de microscopía. En este número, se



Foto 1. Polinización de flores de albaricoquero mediante insectos.

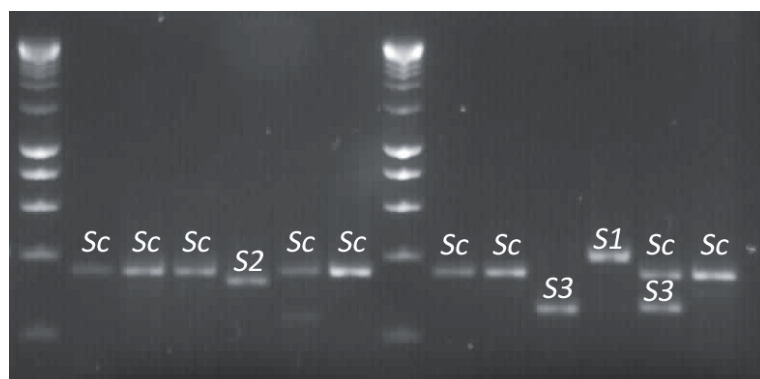


Foto 2. Análisis de alelos S con la técnica PCR.

completa el estudio anterior con las relaciones de incompatibilidad de 129 variedades.

Relaciones de incompatibilidad entre variedades

En albaricoquero, como en otros frutales, el carácter de auto(in)compatibilidad está determinado genéticamente por una región del genoma denominada locus S. Esta región codifica los genes que determinan el genotipo del pistilo y del grano de polen, expresados como alelos S. En las variedades autoincompatibles, cuando el ale-

lo S del grano de polen es igual a alguno de los dos alelos expresados en el pistilo, se produce la detención del crecimiento del tubo polínico, impidiendo así la fecundación. Las variedades son compatibles entre sí cuando tienen al menos un alelo diferente. Conocer los alelos S de cada variedad permite predecir las relaciones de compatibilidad o incompatibilidad entre variedades y facilita la elección de variedades polinizantes adecuadas.

En los últimos años, los avances en biología molecular han permitido desarrollar herra-



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Cuadro 1. Alelos S de las principales variedades de albaricoquero y clasificación en grupos de incompatibilidad (G.I.).

Grupos de incompatibilidad (G.I.)	Alelos S	Variedades
I	S ₁ S ₂	'Castleton', 'Goldrich', 'Hargrand', 'Lambertín-1'
II	S ₈ S ₉	'Perle Cot', 'Pinkcot'
III	S ₂ S ₆	'Avirine' (Bergarouge), 'Moniqui'
IV	S ₂ S ₇	'Ouardi', 'Priana'
V	S ₂ S ₈	'Holly Cot', 'Sweet Cot'
VIII	S ₆ S ₉	'Cheyenne', 'Feria Cot', 'Orangered', 'Stark Early Orange', 'Sunny Cot', 'Wonder Cot', 'Flash Cot'
XVIII	S ₁ S ₃	'Cooper Cot', 'Perfection'
XIX	S ₂ S ₃	'Maya Cot', 'Sun Glo'
XX	S ₂ S ₉	'Goldstrike', 'Magic Cot'
XXI	S ₃ S ₈	'Lilly Cot', 'Spring Blush'
XXII	S ₃ S ₉	'Durobar' (Almadulce), 'Flodea', 'Henderson', 'Kosmos'
XXIII ¹	S ₇ S ₉	'Goldbar'
XXIV ¹	S ₁ S ₆	'Primaya'
Grupo 0		'Estrella' (S ₁ S ₇), 'Harcot' (S ₁ S ₄), 'Stella' (S ₆ S ₂₀), 'Veecot' (S ₂ S ₂₀), 'Velázquez' (S ₅ S ₂₀)
Variedades autocompatibles		'Alba', 'Aprix 20', 'Aprix 33', 'Aprix 9', 'Apriqueen', 'Arquer', 'Bebecou', 'Beliana', 'Berdejo', 'Bergecot', 'Bergeron', 'Búlida', 'Canino', 'Cebas Red', 'Charisma', 'Corbato', 'Cristali', 'Currot', 'Delice Cot', 'Dorada', 'Dulcinea', 'Fantasme', 'Faralia', 'Farbaly', 'Farbela', 'Farclo', 'Fardao', 'Farfia', 'Farhial', 'Farius', 'Farlis', 'Fartoli', 'Floppia', 'Galta Vermella Valenciana', 'GaltaRoja', 'Gandia', 'Ginesta', 'Gönci magyarkajsz', 'Hatif de Colomer', 'Katy', 'Kioto', 'Lady Cot', 'Lido', 'Lito', 'Lorna', 'Luizet', 'Mauricio', 'Medflo', 'Mediabel', 'Mediva', 'Memphis', 'Milord', 'Mirlo Anaranjado', 'Mirlo Blanco', 'Mirlo Rojo', 'Mitger', 'Modesto', 'Mogador', 'Murciana', 'Ninfa', 'Oscar', 'Palabras', 'Palau', 'Palsteyn', 'Patterson', 'Paviot', 'Pepito del Rubio', 'Pisana', 'Playa Cot', 'Pricia', 'Primidi', 'Primorosa', 'Rambo', 'Real Fino', 'Regibus', 'Rojo Carlet', 'Rojo Pasión', 'Rouge Cot', 'Rouge de Roussillon', 'Roxana', 'Rubista', 'Sandy Cot', 'Sherpa', 'Soledane', 'Swired', 'Tadeo', 'Tilton', 'Tirynthos', 'Tom Cot', 'Trevatt', 'Victor 1'

ALBURQUERQUE *et al.*, 2002; VILANOVA *et al.*, 2005; HALÁSZ *et al.*, 2007; MUÑOZ-SANZ *et al.*, 2017; HERRERA *et al.*, 2018a; 2018b; HERRERA *et al.*, 2020.

(1) En estos grupos solo han sido incluidas las variedades más significativas.

mientas para la identificación del genotipo de incompatibilidad mediante el uso de marcadores moleculares. Para ello, se extrae el ADN de la planta y se analiza el locus S mediante la técnica de PCR (Foto 2). En albaricoquero se han identificado 33 alelos, relacionándose algunos de ellos, como el alelo Sc, con el carácter de autocompatibilidad. Estos avances metodológicos permiten determinar de forma precoz las relaciones de incompatibilidad entre variedades sin necesidad de realizar cruzamientos ni analizar flores, ya que se puede extraer ADN de cualquier muestra de material vegetal.

Grupos de incompatibilidad

Las variedades autoincompatibles se han clasificado en grupos de incompatibilidad (G.I.) según sus alelos S. Las variedades autoincom-

patibles con los mismos alelos S son incompatibles entre sí y se agrupan en el mismo grupo de incompatibilidad, pero son compatibles con las variedades de cualquiera de los otros grupos. En la actualidad existen 26 grupos de incompatibilidad (I-XXVI) (ALBURQUERQUE *et al.*, 2002; VILANOVA *et al.*, 2005; HALÁSZ *et al.*, 2007; MUÑOZ-SANZ *et al.*, 2017; HERRERA *et al.*, 2018a y b; 2020). En este trabajo, las 33 variedades autoincompatibles se han clasificado en 13 grupos de incompatibilidad (Cuadro 1). En el grupo 0 se agrupan variedades que presentan una combinación de alelos que no se encuentran en ninguna otra variedad, por lo que serían compatibles con cualquier otra. Las variedades autocompatibles que no necesitan otras variedades polinizantes se agrupan en la última fila por separado y tienen la ventaja de ser polini-



Foto 3. Falta de sincronía en el periodo de floración entre dos variedades de albaricoquero.

zantes universales, pudiendo polinizar con éxito las flores de la propia variedad y de cualquier otra. La información recogida en el *Cuadro 1* permite elegir los polinizantes más adecuados para el diseño de nuevas plantaciones, asegurándose la compatibilidad entre ellos. También permite solucionar problemas de producción causados por falta de polinización en plantaciones ya establecidas y diseñar cruzamientos en programas de mejora genética.

Además de conocer las relaciones de compatibilidad entre las variedades, es necesario que las variedades que se van a interplantar coincidan en la época de floración. La época de floración puede variar entre zonas y años, por lo que es importante conocer el comportamiento de cada variedad en cada zona de cultivo. Cuando esta información no está disponible, es conveniente utilizar más de una variedad polinizante para evitar falta de solapamiento, ya que variedades que no coincidan en floración (Foto 3) pese a ser compatibles entre sí presentarán problemas de cuajado. ●

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ANEXO IV

Fadón, E., Herrera, S., Guerrero, B. I., Guerra, M. E., Rodrigo, J. (2020b). Chilling and Heat Requirements of Temperate Stone Fruits Trees (*Prunus* sp.). *Agronomy* 10, 409. <https://doi.org/10.3390/agronomy10030409>.

Review

Chilling and Heat Requirements of Temperate Stone Fruit Trees (*Prunus* sp.)

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Abstract: Stone fruit trees of genus *Prunus*, like other temperate woody species, need to accumulate a cultivar-specific amount of chilling during endodormancy, and of heat during ecodormancy to flower properly in spring. Knowing the requirements of a cultivar can be critical in determining if it can be adapted to a particular area. Growers can use this information to anticipate the future performance of their orchards and the adaptation of new cultivars to their region. In this work, the available information on chilling- and heat-requirements of almond, apricot, plum, peach, and sweet cherry cultivars is reviewed. We pay special attention to the method used for the determination of breaking dormancy, the method used to quantify chilling and heat temperatures, and the place where experiments were conducted. The results reveal different gaps in the information available, both in the lack of information of cultivars with unknown requirements and in the methodologies used. The main emerging challenges are the standardization of the conditions of each methodology and the search for biological markers for dormancy. These will help to deal with the growing number of new cultivars and the reduction of winter cold in many areas due to global warming.

Keywords: almond; apricot; chilling hours; chilling units; chilling portions; European plum; growing degree hours; Japanese apricot; Japanese plum; peach; sour cherry; sweet cherry

1. Introduction

Temperate stone fruits belong to the genus *Prunus* in the Rosaceae and produce a fruit called drupe, whose seed is covered by the woody endocarp which in turn is covered by the endocarp. In most cultivated *Prunus* species, the edible part of the fruit is the endocarp, which includes the fleshy pulp (mesocarp) and skin (exocarp) such as apricot (*P. armeniaca* L.), European plum (*P. domestica* L.), Japanese apricot (*P. mume* Siebold and Zucc.), Japanese plum (*P. salicina* Lindl.), peach (*P. persica* L. Batsch), sour cherry (*P. cerasus* L.) and sweet cherry (*P. avium* L.) [1]. On the other hand, in almond (*P. dulcis* (Mill.) D.A. Webb), the edible part of the fruit is the seed. The annual global stone fruit production reached in 2017 more than 47 million t in 7.3 million ha [2]. The most cultivated species are peach (*P. persica* L. Batsch) (24.6 million t in 1.5 million ha), plum (including European and Japanese plum) (11.7 million t in 2.6 million ha), apricot (4.2 million t in 0.5 million ha), sweet cherry

(2.4 million t in 0.4 million ha), almond (2.2 million t in 1.9 million ha) and sour cherry (1.2 million t in 0.2 million ha) [2].

Stone fruit trees, like other temperate woody species, need to accumulate a cultivar-specific amount of chilling during winter to overcome dormancy and then experience warm temperatures to finally flower in spring [3–5]. These conditions the adaptation of species and cultivars to each region [6] and it is the main drawback for their extension to warmer latitudes [7]. Knowing the temperature requirements of a cultivar can be useful for growers to anticipate the future performance of their orchards and to design new orchards taking into account the predicted global warming [7–9]. In this work, the available information on chilling- and heat-requirements of cultivars of the most cultivated stone fruit crops (almond, apricot, peach, plum and cherry) is reviewed, paying special attention to the approach used for the determination of breaking dormancy, the method used to quantify chilling and heat temperatures, and the place where the experiments were conducted. There is extensive information available about chilling and heat requirements that has purposefully been omitted from this review. We have only included those studies that a) obtained results by using an experimental methodology (i.e., transferring shoots into a growth chamber sequentially during winter) or computational/statistical approaches that relate flowering dates to temperature data over a sufficiently long time series, and b) quantified chilling and heat temperatures using the common models (Chilling Hours model, Utah model or Dynamic model for chilling requirements, Growing Degree Hours for heat requirements).

2. Dormancy: Definition and Description

Stone fruit trees adapt to temperate regions by establishing a dormancy state during winter that allows surviving at low temperatures [10]. Dormancy characterizes by the absence of growth since flower primordia remain protected inside the buds. Growth is not only suppressed by the low temperatures since dormant trees do not respond to suitable conditions to grow and need exposure to a certain period of low temperatures to overcome dormancy [11]. It seems clear that dormancy is triggered by internal factors inherent to the plant [12]; however, up to now, most of these physiological factors remain unclear. The exposition to chilling temperatures allows a progressive restoration of growth capability. However, growth is not immediately restored [3], since low temperatures could continue and prevent buds from growing, and the exposure to warm temperatures is needed to grow after dormancy release [13]. Chilling and heat requirements are genetically determined and therefore are cultivar specific [14,15].

The phases of chilling and warm temperature accumulation are differently named, although they occur at the same phenological stage [16]. Lang et al. proposed one of the most used terms, naming the stages “endo-dormancy” while chilling accumulates, and “eco-dormancy” while heat accumulates and chilling prevents the plant from growing [11]. On the other hand, a recent proposal by Considine and Considine considered that dormancy only refers to when it is internally caused, and the lack of growth by external factors correspond to a quiescence state [17].

Dormancy has acquiring rising importance for a sustainable fruit production under a global warming context [8,9,18–20]. However, the physiological processes behind remain unknown and a reliable biological factor linked to the dormancy breaking is still missing [12].

3. Dormancy prediction

The characterization of the temperature requirements of a cultivar is crucial for the design and management of the fruit orchards since they determine the flowering date and a flowering overlap is needed for cross-pollination in case of self-incompatible cultivars [21]. It is also a key trait in breeding programs since it determines both the adaptation to different climates and the blooming and ripening dates [22]. However, the lack of a whole understanding of the process makes obtaining reliable data complex [6]. In most works, the determination of the temperature requirements of a particular cultivar consists of two phases (Figure 1): first, the establishment of the dormancy and forcing periods

(Figure 1a), either experimentally (Figure 1a.1) [23,24] or statistically (Figure 1a.2) [25–27]; and the subsequent temperature quantification in both phases (Figure 1b) by using temperature-based models for chilling (Figure 1b.1) [28–30] and heat quantification (Figure 1b.2) [29]. One of the main challenges of determining the temperature requirements is establishing the transition from dormancy to eco-dormancy (Figure 1a) and thus the periods in which chilling (Figure 1b.1) and warm temperatures (Figure 1b.2) are quantified.

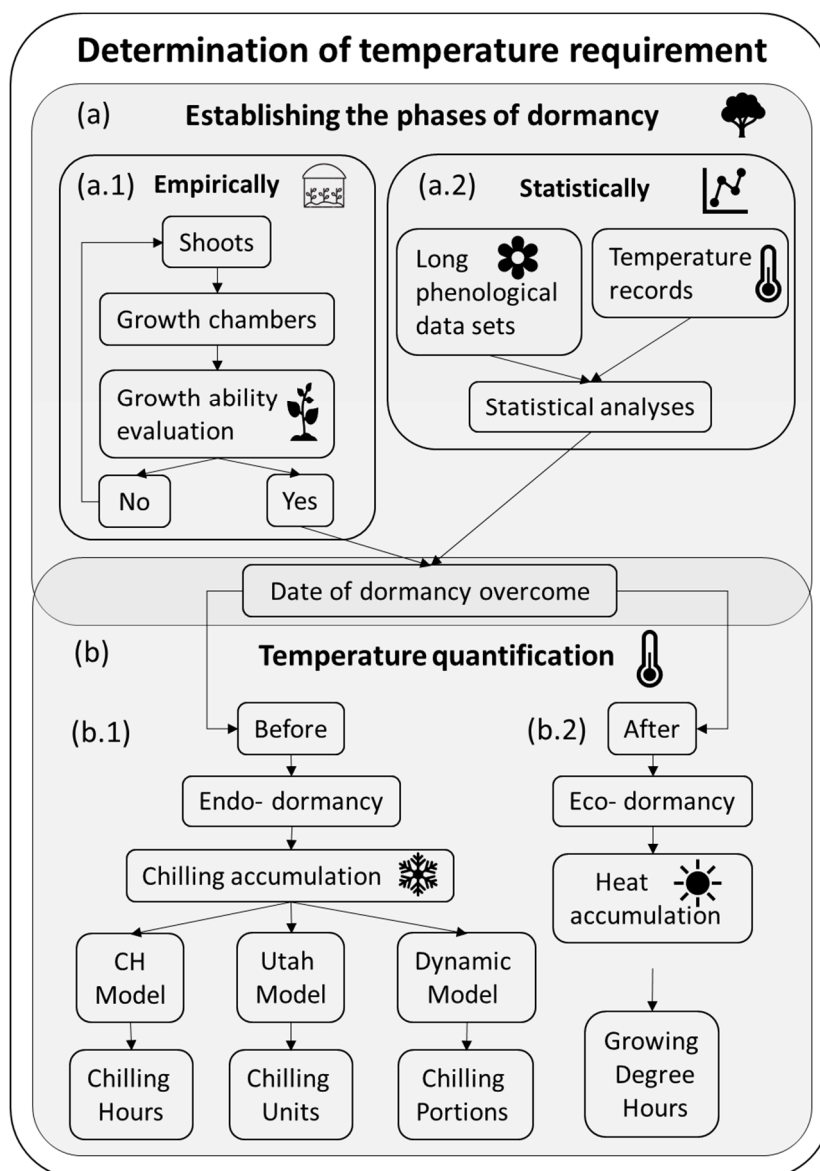


Figure 1. Determination of the temperature (chill and heat) requirements in temperate fruit trees: a workflow. (a) Determination of the phases of dormancy: (a.1) empirically or (a.2) statistically. (b) Temperature quantification: (b.1) chilling quantification during endo-dormancy and (b.2) heat quantification over eco-dormancy.

The experimental determination of dormancy consists of evaluating when the buds recover the capacity to grow (Figure 1a.1). This is usually performed by transferring shoots into a growth chamber sequentially during winter, thus after different chilling exposures. Shoots remain a certain period in the warm conditions, and then bud growth is evaluated. This approach has been widely used from

early [24] to recent studies [31] that determine the chilling requirements of the cultivars. Furthermore, these experiments serve as a base for physiological studies on dormancy [6].

The statistical approach estimates the date of chilling fulfillment based on a long series of phenological observations (flowering dates) and relating them with the previous temperature records (Figure 1a.2). Tabuenca et al. established a statistical methodology by calculating the correlation coefficients between the maximum, minimum and mean temperatures of certain time periods and flowering dates in apple, apricot, cherry, peach, pear, plum [32], and almonds [33]. Then, Alonso et al. determined the temperature requirements correlations between the flowering dates of almond cultivars and daily minimum, mean and maximum temperatures calculated as the mean of the surrounding 5, 10, 15 ... until 30 days, with a set of data from 7 years. The endo-dormancy to eco-dormancy transition was considered to be when the significant correlation coefficients change from being mainly positive to be mainly negative [26]. Ashcroft et al. firstly estimated chilling and heat accumulation of peaches [29] based on when the chilling and heat accumulation presented the least squared residuals methods [25]. A new approach has been recently developed based on the statistical analysis of long-term phenological records and temperature series. The application of partial least squares (PLS) regression leads to the estimation of the agroclimatic requirements. PLS regression is especially applicable when the number of independent variables (daily temperatures, 365 data per year) substantially exceeds the number of dependent variables (one flowering date per cultivar and year). The results of these analyses include the model coefficients and variable-importance-in-the-projection. Significant positive model coefficients correspond with the chilling accumulation, endo- dormancy, while negative coefficients correspond with the heat accumulation, eco- dormancy [34]. It was initially applied in sweet cherry [34] and later in other fruit trees as almond [35,36], pistachio [19], apricot [27] or apple [36].

4. Temperature Based Models for Phenology Prediction

Three main models are currently used in agriculture to quantify chilling over the dormancy period [37]. They were developed in peach: the Chilling Hours model [28], the Utah model [29], and the Dynamic model [30] (Figure 1b.1). The Chilling Hours model was developed in the early fifties of the 20th century, and it has been widely used up to now due to its simplicity and easy comprehension and calculation. This model establishes that a Chilling Hour (CH) corresponds to an hour at temperature between 0 and 7.2 °C (45 °F), since this range of temperatures is considered to affect dormancy completion. While temperatures below 0 °C are assumed not contributing due to at such low temperatures biological processes were considered slowed or not occurring, temperatures over 7.2 °C (45 F) were considered not low enough to affect dormancy completion [28].

The Utah model bases on the quantification of Chilling Units (CU) and establishes different ranges of temperatures with a different contribution to dormancy completion. A chilling unit corresponds to one hour under temperatures between 2.5–9.1 °C, a range that is considered the most effective temperatures on dormancy completion. Other ranges of temperatures are considered to have half (1.5–2.4 °C and 9.2–12.4°C), null (<1.4 °C and 12.5–15.9 °C) or negative (>16 °C) contribution to dormancy [29].

The Dynamic model, dated back from the 1980s [30], is based on a series of experiments that evaluated the effect of different series of temperatures on dormancy release [38–40]. This model proposed the accumulation of an intermediate product promoted by cold temperatures that can be reversed by warm temperatures (first step). Once this intermediate product has reached a certain level, the chill portions are permanently fixed and are considered not affected by warm temperatures [30]. The model is based on a possible biological process in which a thermally unstable precursor would lead to the accumulation of a factor in the buds. This process would follow the Arrhenius law that fits the mathematical relationship between temperature and the rate of a chemical reaction.

Once dormancy is predicted allowing the quantification of chilling, it is also needed to quantified warm temperatures after dormancy for flowering to occur (Figure 1b.2). The modelization of warm temperatures was early developed in agriculture to predict the different phenological stages of crops [41].

The combination of a chilling model with a heat model to predict flowering was firstly described with the combination of the Utah model with the Growing Degree Hours (GDH) quantification [25], and then this combination was also applied with the other chilling models. A GDH is defined as one hour at 1 °C above the base temperature (4.5 °C), this linearly progresses until the upper limit (25° C) [13]. One of the main drawbacks of using these models is the necessity of hourly temperature data records, whose availability is limited. Thus, equivalent models have been developed based on maximum and minimum temperatures [42].

5. Chilling and Heat Requirements

5.1. Almond (*P. dulcis*)

Although North America is the main area for almond production (over 1 million t/year) [2], temperature requirements have been calculated in the Mediterranean area, in Spain [26,33,36,43], the second world producer (0.6 million t/year) [2] and Tunisia [35]. Temperature requirements (chilling and heat) are available for a total of 106 almond cultivars [26,33,35,36,43] (Table 1). The chilling requirements varies between ‘Achaak’ (8 CH/ -297CU/ 3.4CP) [35] and ‘R1000’ (996 CU) [43]. The heat requirements range from 2894 GDH for ‘Pizzuta’ to 10201 GDH for ‘Primorskiy’ [36].

In this species, the most data (96 out of 106 cultivars) were calculated with statistical approaches, which contrast with the other *Prunus* sp. reported in this work. Almond data were obtained according to three different statistical methodologies [26,33,34]. The initial phenological data set also differed between works: the PLS analysis was performed over the date of flowering initiation (BBCH phenological stage 61, 10% flowers open) during 30 years [34,35], while the other approaches based on the dates of full bloom (BBCH phenological stage 65, 50% flowers open) over 7 [26] and 4–10 years [33].

A comparison between experimental (E) [43] and statistical (S) [26] approaches reveals similar results of chilling requirements and heat requirements for ‘Ferragnès’ (558 and 444 CU, 7309 and 8051 GDH), ‘Marcona’ (435 and 428 CU, 6681 and 6603 GDH) and ‘Ramillete’ (326 and 444 CU, 6538 and 5947 GDH) in Spain. Unfortunately, it is not possible to make more comparisons due to the different models used to quantify chilling and the different cultivars used in each study.

Table 1. Chilling and heat requirements of almond cultivars.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
A-258	-	-	17 ± 4.5	8725 ± 1712	S	Spain	[36]
Abiodh de Sfax	12	−284	4.6	6206	S	Tunisia	[35]
Abiodh Ras Djebel	59	−53	15.5	7324	S	Tunisia	[35]
Achaak	-	266	-	6444	E	Spain	[43]
	8	−297	3.4	8703	S	Tunisia	[35]
Aï	-	444	-	8051	S	Spain	[26]
	169	-	-	Very high	S	Spain	[33]
Alicante	-	-	21.7 ± 4.7	6940 ± 1400	S	Spain	[36]
Alzina	-	463	-	6757	S	Spain	[26]
	408	-	-	Very low	S	Spain	[33]
Amargo	169	-	-	Low	S	Spain	[33]
Andreu	151	-	-	High	S	Spain	[33]
Antoñeta	-	514	-	7512	E	Spain	[43]
Ardechoise	-	-	21.8 ± 4.5	6994 ± 1546	S	Spain	[36]

Table 1. Cont.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Avola	50	46	13.6	6673	S	Tunisia	[35]
Aylés	-	481	-	7909	S	Spain	[26]
Bertina	-	463	-	8536	S	Spain	[26]
Blanquerna	-	463	-	6906	S	Spain	[26]
Bonifacio	61	101	15.8	7559	S	Tunisia	[35]
Bruantine	34	-219	10.4	8548	S	Tunisia	[35]
Cambra	-	463	-	7697	S	Spain	[26]
Cavallera	34	-219	10.4	7042	S	Tunisia	[35]
	-	-	11.6 ± 4.0	7452±1601	S	Spain	[36]
Chellastone	-	463	-	6168	S	Spain	[26]
Chine	151	-	-	Low	S	Spain	[33]
Constantini	-	444	-	5345	S	Spain	[26]
Cristomorto	83	-29	22.6	5872	S	Tunisia	[35]
	-	428	-	8027	S	Spain	[26]
	-	-	20.7 ± 4.7	8236 ± 1482	S	Spain	[36]
Desmayo	-	309	-	5942	E	Spain	[43]
	169	-	-	Medium	S	Spain	[33]
Desmayo Langueta	-	428	-	5458	S	Spain	[26]
	-	-	8.4 ± 3.7	8552±1741	S	Spain	[36]
Desmayo Rojo	-	463	-	6418	S	Spain	[26]
	169	-	-	High	S	Spain	[33]
Dorée	46	-174	12.7	8867	S	Tunisia	[35]
Drake	169	-	-	Very high	S	Spain	[33]
Durán	151	-	-	Medium	S	Spain	[33]
Faggoussi	54	-148	14.5	3962	S	Tunisia	[35]
Fakhfekh	33	-219	10.4	5979	S	Tunisia	[35]
Fasciuneddu	34	-219	10.4	7027	S	Tunisia	[35]
Felisia	-	428	-	9352	S	Spain	[26]
Ferraduel	54	59	14.4	9272	S	Tunisia	[35]
	-	-	52.9 ± 6.0	7285 ± 1362	S	Spain	[36]
Ferragnès	-	558	-	7309	E	Spain	[43]
	54	59	14.4	9215	S	Tunisia	[35]
	-	444	-	8051	S	Spain	[26]
	-	-	20.7 ± 4.7	8696 ± 1543	S	Spain	[36]
Filippo Ceo	-	463	-	7558	S	Spain	[26]
	483	-	-	Low	S	Spain	[33]
Fourcouronne	169	-	-	High	S	Spain	[33]
Fournat de Brézénau	169	-	-	Very high	S	Spain	[33]
	-	416	-	7367	S	Spain	[26]
	79	-50	21.1	5368	S	Tunisia	[35]
Gabaix	-	-	13.4 ± 4.4	6824 ± 1421	S	Spain	[36]
Garbí	-	-	52.9 ± 6	7040 ± 1312	S	Spain	[36]
Garnghzel	12	-284	4.6	8703	S	Tunisia	[35]

Table 1. Cont.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Garrigues	-	-	22.0 ± 4.7	8054 ± 1811	S	Spain	[36]
Genco Taronto	80	194	21.4	6148	S	Tunisia	[35]
	-	-	28.7 ± 4.9	5971 ± 1189	S	Spain	[36]
Glorieta	-	-	51.6 ± 5.9	5654 ± 1177	S	Spain	[36]
Guara	-	463	-	7978	S	Spain	[26]
Jordi	-	428	-	6488	S	Spain	[26]
	151	-	-	Medium	S	Spain	[33]
Khoukhi	31	-227	9.9	8873	S	Tunisia	[35]
Ksontini	21	-258	7.3	7071	S	Tunisia	[35]
Languedoc	23	-174	7.7	9097	S	Tunisia	[35]
Lauranne	-	428	-	8569	S	Spain	[26]
LeGrand	-	428	-	8027	S	Spain	[26]
Lluch		-		Very high	S	Spain	[33]
Malagueña	23	-82	7.6	9224	S	Tunisia	[35]
Marcona	169	-	-	High	S	Spain	[33]
	-	435	-	6681	E	Spain	[43]
	-	428	-	6603	S	Spain	[26]
	-	-	22.0 ± 4.7	6378 ± 1341	S	Spain	[36]
Marta	-	478	-	7577	E	Spain	[43]
Masbovera	-	463	-	7841	S	Spain	[26]
	-	-	28.6 ± 4.9	6232 ± 1221	S	Spain	[36]
Mazzetto	54	-68	14.5	9507	S	Tunisia	[35]
Miagkoskorlupij	-	463	-	7439	S	Spain	[26]
	631	-	-	Very low	S	Spain	[33]
Mollar de Tarragona	-	-	20.0 ± 4.7	6718 ± 1378	S	Spain	[36]
Moncayo	-	463	-	8696	S	Spain	[26]
Montrone	31	-227	9.9	9694	S	Tunisia	[35]
Morskoi	233	-	-	Very high	S	Spain	[33]
Ne Plus Ultra	169	-	-	Medium	S	Spain	[33]
	50	11	13.6	6847	S	Tunisia	[35]
	-	463	-	6635	S	Spain	[26]
Nonpareil	83	-29	22.6	6045	S	Tunisia	[35]
	-	403	-	7758	S	Spain	[26]
	169	-	-	High	S	Spain	[33]
	-	-	21.7 ± 4.7	7062 ± 1399	S	Spain	[36]
Picantilli	-	428	-	7386	S	Spain	[26]
	561	-	-	Very low	S	Spain	[33]
Pizzuta	83	-29	22.6	2894	S	Tunisia	[35]
Ponç	-	428	-	6210	S	Spain	[26]
	101	-	-	High	S	Spain	[33]
Poleta	151	-	-	High	S	Spain	[33]
Pou de Felanitz	-	392	-	5419	S	Spain	[26]
	101	-	-	Medium	S	Spain	[33]

Table 1. Cont.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Princesa	169	-	-	High	S	Spain	[33]
Primorskij	-	428	-	8434	S	Spain	[26]
	-	-	52.85 ± 5.95	10201 ± 1834	S	Spain	[36]
R1000	-	996	-	7438	E	Spain	[43]
Rachele	233	-	-	Very high	S	Spain	[33]
	-	376	-	8302	S	Spain	[26]
	47	-167	13.3	6374	S	Tunisia	[35]
Ramillete	-	444	-	5947	S	Spain	[26]
	-	326	-	6538	E	Spain	[43]
	-	-	20.7 ± 4.7	6998 ± 1540	S	Spain	[36]
Ramlet R249	33	18	10.3	6812	S	Tunisia	[35]
Ramlet R250	19	-266	6.7	6812	S	Tunisia	[35]
Rana	-	-	20.0 ± 4.7	6518 ± 1292	S	Spain	[36]
Rof	-	463	-	6418	S	Spain	[26]
	169	-	-	High	S	Spain	[33]
	-	-	20.8 ± 4.7	6965 ± 1355	S	Spain	[36]
Rotjet	151	-	-	Low	S	Spain	[33]
S2332	-	417	-	6481	E	Spain	[43]
S5133	-	973	-	7003	E	Spain	[43]
Sicilia	151	-	-	High	S	Spain	[33]
Soukaret	77	-57	20.6	5960	S	Tunisia	[35]
Tardive de la Verdère	-	358	-	8814	S	Spain	[26]
Tardy Nonpareil	-	-	55.4 ± 5.9	9444 ± 1658	S	Spain	[36]
Tarragona	83	-29	22.6	5830	S	Tunisia	[35]
Tarragones	-	-	51.6 ± 5.9	6370 ± 1238	S	Spain	[36]
Tamarite 2	169	-	-	High	S	Spain	[33]
Texas	-	463	-	7697	S	Spain	[26]
	233	-	-	High	S	Spain	[33]
	-	-	51.6 ± 5.9	6280 ± 1225	S	Spain	[36]
Thompson	-	463	-	7697	S	Spain	[26]
Titan	-	444	-	8457	S	Spain	[26]
Tokyo	-	463	-	7558	S	Spain	[26]
Totsol	-	428	-	6943	S	Spain	[26]
	101	-	-	High	S	Spain	[33]
Tozeur 1	12	-284	4.6	8124	S	Tunisia	[35]
Tozeur 2	33	-219	10.4	6309	S	Tunisia	[35]
Tozeur 4	12	-284	4.6	6698	S	Tunisia	[35]
Trell	77	-57	20.6	6003	S	Tunisia	[35]
	233	-	-	Medium	S	Spain	[33]
Tuono Taronto	-	463	-	7978	S	Spain	[26]
	50	46	13.6	6148	S	Tunisia	[35]
	-	-	52.9 ± 6.0	6870 ± 1319	S	Spain	[36]

Table 1. Cont.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Verdereta	-	416	-	6606	S	Spain	[26]
	101	-	-	High	S	Spain	[33]
Verdiere	169	-	-	Very high	S	Spain	[33]
Vinagrilla	408	-	-	Very low	S	Spain	[33]
Vivot	-	428	-	6603	S	Spain	[26]
	151	-	-	High	S	Spain	[33]
Xina	-	403	-	5815	S	Spain	[26]
Yaltano	713	-	-	Very low	S	Spain	[33]
Yaltinskij	-	463	-	8536	S	Spain	[26]
Zahaf	31	-227	9.9	6279	S	Tunisia	[35]
	-	392	-	5611	S	Spain	[26]

5.2. European and Japanese Apricot (*P. armeniaca* and *P. mume*)

European apricot is one of the most economically important fruit crops in temperate regions worldwide [44]. It is mainly produced in the Mediterranean area and the Middle East, being the higher producers Turkey, Uzbekistan, Italy, Algeria, and Iran [2]. A total of 15 works have experimentally evaluated the chilling requirements of 68 apricot cultivars all around the world (Iran, Italy, Serbia, South Africa, Spain, and the USA) (Table 2). The range of chilling requirements is between 274 CU in ‘Palsteyn’ [45] to 1450–1600 CU in ‘Orangered’ [46]. This crop is cultivated mainly in Mediterranean regions and it has traditionally been considered that most cultivars had low chilling requirements. However, some traditional cultivars showed high chilling requirements as ‘Búlida’ (1048 CU), ‘Canino’ (806 CU), ‘Currot’ (642 CU) or ‘Moniqui’ (1139 CU) [42,43] (Table 2).

In the last decades, an important renewal is taking place due to sharka, a disease caused by the Plum Pox Virus (PPV). High chilling PPV-resistant cultivars from North America, such as ‘Goldrich’ (950–108 CU / 65–59 CP), ‘Harcot’ (920–1665 CP), ‘Orangered’ (568–1481 CH / 902–1600CU / 55–69 CP), and ‘Stark Early Orange’ (1411 CU / 79 CP) (Table 2), have been used as parentals in different breeding programs with the aim of introducing a source of the resistance to the disease. The release of a high number of new cultivars is resulting in a lack of information about the chilling and heat requirements of the majority of the new commercial cultivars [44,47].

Some cultivars such as ‘Aurora’, ‘Bergeron’, ‘Currot’, ‘Dorada’, ‘Goldrich’, ‘Laycot’, ‘Luizet’, ‘Moniqui’, ‘Murciana’, ‘Paviot’, ‘Rojo Pasión’, ‘Royal’, ‘San Castrese’, and ‘Selene’ show homogeneous results in the different studies (Table 2). However, highly variable results have been reported in other cultivars as ‘Cafona’, ‘Canino’, ‘Harcot’, ‘Orangered’, ‘Palsteyn’, ‘Polonais’, ‘Precoz de Colomber’, and ‘Tonda di Costiglione’, showing heterogeneity among the different approaches (Table 2). Likewise, high differences have been reported when the experiments were carried out in different locations [45,48,49] or years [48,50,51]. Even when the same cultivars (‘Canino’, ‘Orangered’ and ‘Palsteyn’) were evaluated using the same approach in two environments with different climatic conditions, the results obtained showed high differences, with higher values in Spain (806/1172/631 CU) than in South Africa (304/957/274 CU) [45] (Table 2). Heat requirements ranged from 485 GDH in ‘Goldrich’ [49] to values above 6000 GDH in ‘Canino’ [48], ‘Dorada’, ‘Palsteyn’, and ‘Rojo Pasión’ [45]. Some cultivars showed high differences between seasons, as ‘Cafona’ (2499–5800 GDH), ‘Canino’ (2547–6729 GDH), and ‘Precoz de Colomber’ (2320–5304 GDH) [48].

Japanese apricot originated in China and has been widely cultivated for about 3000 years in Asian countries as China, Japan, and Korea. However, this crop is hardly known in other countries probably due to its poor adaptation to other areas of different climatic conditions, since it requires warmer and more humid conditions than European apricot [44].

Chilling requirements range from 26 ± 7 CP for ‘Shuangshuidaroumei’ [52] to 78.5 CP for ‘Sichuangqingmei’ and ‘Tengwulang’ [53] (Table 3). Heat requirements vary from 822 GDH for ‘Dayu’ to 2378 GDH for ‘Jietianmei’, ‘Sichuangqingmei’, and ‘Tengwulang’ (Table 3) [53]. Japanese apricot shows high chilling requirements and extremely low heat requirements when compared with the other *Prunus* sp. reported in this study.

Some cultivars with a wide range of chilling requirements, such as ‘Nanko’, a high-chilling cultivar from Japan, and ‘Ellching’, a low-chilling cultivar from the subtropical region in Taiwan, have been used in studies on dormancy physiology [54] and genetic regulation [55–59].

Table 2. Chilling and heat requirements of European apricot cultivars.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Abricot Pêche 1708 A.D.	1015–1105	-	-	-	E	Spain	[60]
Alessandrino	-	1000–1140	-	3825	E	Italy	[46]
Amabile Vecchioni	-	1140	-	2950	E	Italy	[46]
Amoscatelado	711–806	-	-	-	E	Spain	[60]
Asgarabad	710	652	-	3465	E	Iran	[61]
Aurora	-	1140	-	2750	E	Italy	[46]
	1237	1296	-	2490–2812	E	Italy	[62]
Baracca	-	1000–1140	-	4680	E	Italy	[46]
Bebeco	-	1030–1125	-	3775	E	Italy	[46]
Bergeron	-	1225	-	4300	E	Italy	[46]
	699	1176	64.8	4526	E	Spain	[50]
	762	1134	61.7	5150	E	Spain	[45]
	-	1122–1224	-	-	E	Serbia	[51]
Blanc Rose	1105–1185	-	-	-	E	Spain	[60]
Búlida	1050	-	-	-	E	Spain	[63]
	950–983	-	-	-	E	Spain	[60]
	976–1015	-	-	-	E	Spain	[60]
	830–926	-	-	-	E	Spain	[60]
	1133 $\pm$ 170	1296 $\pm$ 145	-	-	E	Spain	[64]
	560	968	53.8	5146	E	Spain	[50]
	708	1048	56.4	5294	E	Spain	[45]
Cafona	-	1200 $\pm$ 35	-	3433	E	Italy	[48]
	-	824–1515	-	2499–5800	E	Spain	[48]
Canino	≤ 750	-	-	-	E	Spain	[63]
	787–878	-	-	-	E	Spain	[60]
	< 779	-	-	-	E	Spain	[60]
	771–779	-	-	-	E	Spain	[60]
	-	964–1370	-	2477 - 3087	E	Italy	[48]
	-	725–1350	-	2547 - 6729	E	Spain	[48]
	-	1030	-	3275	E	Italy	[46]
	532	806	45	5724	E	Spain	[45]
	488	304	29.8	-	E	South Africa	[45]

Table 2. Cont.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Cegledy arany	-	1122–1310	-	-	E	Serbia	[51]
Charisma	188	290	31.7	-	E	South Africa	[45]
Comice de Toulon	806–878	-	-	-	E	Spain	[60]
Corbato	≤750	-	-	-	E	Spain	[63]
Currot	≤750	-	-	-	E	Spain	[63]
	354–507	-	-	-	E	Spain	[60]
	267	596	34.3	5879	E	Spain	[50]
	-	621	40.4	1611–2083	E	Italy	[49]
	-	634	38.8	2114–3168	E	Spain	[49]
	-	726–669	-	-	E	Italy	[65]
	409	642	37.8	5774	E	Spain	[45]
D’Alessandria	-	1000–1140	-	4150	E	Italy	[46]
Doctor Mascle	592–711	-	-	-	E	Spain	[60]
Dorada	594	1007	56.2	5079	E	Spain	[50]
	720	1069	57.7	6189	E	Spain	[45]
Early Blush	1407	-	-	2969	E	Italy	[66]
Galta Rocha	372–592	-	-	-	E	Spain	[60]
Giletano	771–806	-	-	-	E	Spain	[60]
Goldrich	-	950–1030	-	3950	E	Italy	[46]
	-	1084	65.2	485–913	E	Italy	[49]
		992	58.6	2067–3431	E	Spain	[49]
	-	834–846	-	-	E	Serbia	[51]
Harcot	-	1275–1530	-	2267–2988	E	Italy	[48]
	-	920–1665	-	3731–5355	E	Spain	[48]
Hatif de Sig	902–976	-	-	-	E	Spain	[60]
Hoja de Parra	668–787	-	-	-	E	Spain	[60]
Koiska	1050	-	-	-	E	Spain	[63]
Laycot	1214	-	-	3533	E	Italy	[66]
	1045	1157	-	3252–3481	E	Italy	[62]
Luicet	1150	-	-	-	E	Spain	[63]
	1074–1140	-	-	-	E	Spain	[60]
	1058–1116	-	-	-	E	Spain	[60]
Magyar kajski	-	1122–1310	-	-	E	Serbia	[51]
Moniqui	850	-	-	-	E	Spain	[63]
	779–926	-	-	-	E	Spain	[60]
	954 ± 103	1139 ± 96	-	-	E	Spain	[64]
	-	930–1140	-	3250	E	Italy	[46]
Moongold	-	910	-	2712	E	USA	[67]
Moonpark	1074–1105	-	-	-	E	Spain	[60]

Table 2. Cont.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Murciana	585	1009	55.9	4440	E	Spain	[50]
	690	1030	55.6	5392	E	Spain	[45]
Ninfa	-	834–846	-	-	E	Serbia	[51]
Orangered	1587	-	-	3448	E	Italy	[66]
	-	1450–1600	-	2700	E	Italy	[46]
	1481	1467	-	2654–3136	E	Italy	[62]
	738	1266	69.1	4362	E	Spain	[50]
	-	902	55.5	2421–3398	E	Italy	[49]
	-	1146	67.2	1443–1505	E	Spain	[49]
	777	1172	64.3	4916	E	Spain	[45]
	568	957	55.4	-	E	South Africa	[45]
Palsteyn	171	274	31.6	-	E	South Africa	[45]
	413	631	37.1	6247	E	Spain	[45]
Patriarca de Hueso Dulce	664–729	-	-	-	E	Spain	[60]
Paviot	1050	-	-	-	E	Spain	[63]
	995–1075	-	-	-	E	Spain	[60]
	1148 ± 148	1318 ± 145	-	-	E	Spain	[64]
Perfection	-	844	-	2593	E	USA	[67]
Perla	1074–1105	-	-	-	E	Spain	[60]
Phelps	-	857	-	2206	E	USA	[67]
Pike	-	895	-	2753	E	USA	[67]
Pisana	-	1113–1122	-	-	E	Serbia	[51]
Polonais	1058–1116	-	-	-	E	Spain	[60]
	-	1175–1450	-	2611 - 2823	E	Italy	[48]
	-	920–1665	-	4047 - 5753	E	Spain	[48]
	-	1300	-	2850	E	Italy	[46]
Precoz de Colomer	950	-	-	-	E	Spain	[63]
	< 779	-	-	-	E	Spain	[60]
	-	1175–1250	-	2503–2563	E	Italy	[48]
	-	690–1190	-	2320–5304	E	Spain	[48]
Priana	-	926 ± 26	-	2189	E	Spain	[48]
Rapareddu	-	1250	-	2850	E	Italy	[46]
Re Umberto	-	1126–1442	-	-	E	Serbia	[51]
Rojo Pasión	531	917	51.2	4670	E	Spain	[50]
	566	874	48.2	6078	E	Spain	[45]
Rouge de Rousillon	950 - 1005	-	-	-	E	Spain	[60]
Royal	875	-	-	-	E	USA	[23]
	850	-	-	-	E	Spain	[63]
	779 - 950	-	-	-	E	Spain	[60]

Table 2. Cont.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
San Castrese	-	964–1100	-	1410–3289	E	Italy	[48]
	-	725 ± 23	-	4134	E	Spain	[48]
	1044	-	-	3558	E	Italy	[66]
	-	870–930	-	3425	E	Italy	[46]
	788	894	-	2705–3874	E	Italy	[62]
	-	880	54	1615–3326	E	Italy	[49]
	-	981	56.5	2116–2967	E	Spain	[49]
S. Nicola Grosso	-	1140	-	3350	E	Italy	[46]
Sarritzu I°	-	950–1140	-	3950	E	Italy	[46]
Selene	590	1018	57.4	4078	E	Spain	[50]
	705	1057	56.9	4605	E	Spain	[45]
Shakarpore	862	746	-	3171	E	Iran	[61]
Shamlo	1130	826	-	2987	E	Iran	[61]
Stark Early Orange	-	1411	78.9		E	Italy	[49]
Sundrop	-	964–967	-	-	E	Serbia	[51]
Sylred	-	967–1019	-	-	E	Serbia	[51]
Tabarze ghermez	1130	826	-	2987	E	Iran	[61]
Tilton	1000	-	-	-	E	USA	[23]
Tirynthos		935–1000	-	1898–3289	E	Italy	[48]
Tom Cot	-	834–846	-	-	E	Serbia	[51]
Tonda di Costiglione	1812	-	-	3643	E	Italy	[66]
	1586	1561	-	3402–3696	E	Italy	[62]

Table 3. Chilling and heat requirements of Japanese apricot cultivars.

Cultivar	Chilling requirements			Heat req. GDH	Method	Loc.	Ref.
	CH	CU	CP				
67	-	-	69	994	E	China	[53]
Baijiahe	-	-	59	1316	E	China	[53]
Changnong17	-	-	69	994	E	China	[53]
Dabaimei	-	-	56	1669	E	China	[53]
Dali	-	-	40	1192	E	China	[53]
Danfenghou	-	-	73	2054	E	China	[53]
Daqiandi	-	-	66.5	1096	E	China	[53]
Daroumei	-	-	38.5	1100	E	China	[53]
Dayezhugan	-	-	73	1651	E	China	[53]
Dayu	-	-	69	822	E	China	[53]
Dongqing	875 ± 147	1054 ± 256	58±9	1018±174	E	China	[52]
Dongshanlimei	-	-	50	1250	E	China	[53]
Ellching	300	-	-	-	E	Japan	[54]
Fenghou	1148 ± 162	1323 ± 247	73±8	1697±1697	E	China	[52]
Fenghualime	-	-	56	1533	E	China	[53]

Table 3. Cont.

Cultivar	Chilling requirements			Heat req.	Method	Loc.	Ref.
	CH	CU	CP	GDH			
Gaotianfenghou	-	-	73	2054	E	China	[53]
Gaotiangmei	-	-	64.5	1099	E	China	[53]
Guangdonghuangpi	-	-	40	1192	E	China	[53]
Gucheng	-	-	59	1383	E	China	[53]
Hangzhoubaimei	-	-	60	1231	E	China	[53]
Henghe	-	-	34	1287	E	China	[53]
Hongding	-	-	69	860	E	China	[53]
Hongmei	-	-	73	1835	E	China	[53]
Hongnong	-	-	75	1675	E	China	[53]
Huangxiaoda	-	-	56	1533	E	China	[53]
Huaxiangshi	-	-	33.5	1072	E	China	[53]
Jiazhouxiaomei	-	-	69	1733	E	China	[53]
Jiazhouzuixiao	-	-	62	1116	E	China	[53]
Jietianmei	-	-	77	2378	E	China	[53]
Jiuzhongmei	-	-	66.5	1096	E	China	[53]
Lizimei	-	-	69	977	E	China	[53]
Longyan	-	-	73	1835	E	China	[53]
Lve	-	-	29	1268	E	China	[53]
Nanhong	-	-	50	1583	E	China	[53]
Nanko	500	-	-	-	E	Japan	[54]
Pinzhimei	-	-	73	1835	E	China	[53]
Qijiangxingmei	-	-	56	1533	E	China	[53]
Qingjia2	-	-	75	1675	E	China	[53]
Qixingmei	-	-	60	1232	E	China	[53]
Ruantiaohongmei	-	-	73	1835	E	China	[53]
Shuangshuidaroumei	239 ± 84	479 ± 180	26±7	1235±77	E	China	[52]
Shuangtaomei	-	-	36.5	1079	E	China	[53]
Sichuangbaimei	-	-	60	1231	E	China	[53]
Sichuanghuangmei	-	-	60	1231	E	China	[53]
Sichuangqingmei	-	-	78.5	2378	E	China	[53]
Siyuemei	-	-	60	1231	E	China	[53]
Taihu1	-	-	69	977	E	China	[53]
Taihu3	-	-	50	1575	E	China	[53]
Taoxingmei	332 ± 110	567 ± 198	32 ± 8	110 ± 199	E	China	[52]
Tengwulang	-	-	78.5	2378	E	China	[53]
Tonglv	-	-	73	1835	E	China	[53]
Touguhong	-	-	62	986	E	China	[53]
Wanhong	-	-	73	1835	E	China	[53]
Weishanzhong	-	-	50	1574	E	China	[53]
Xianmimei	-	-	40	1205	E	China	[53]
Xiaomei	-	-	50	1369	E	China	[53]
Xiaoougongfen	-	-	42.5	1037	E	China	[53]

Table 3. Cont.

Cultivar	Chilling requirements			Heat req. GDH	Method	Loc.	Ref.
	CH	CU	CP				
Xiaoqing	-	-	73	1432	E	China	[53]
Xiaoyezhugan	-	-	56	1533	E	China	[53]
Xingnongxiaomei	-	-	62	1116	E	China	[53]
Xiyeqing	828 ± 139	1040 ± 231	64 ± 9	1179 ± 230	E	China	[52]
Xuemei	-	-	69	1405	E	China	[53]
Yanglao1	-	-	59	1530	E	China	[53]
Yanglao2	-	-	34	1297	E	China	[53]
Yanglao3	-	-	56	1669	E	China	[53]
Yanhua	1141 ± 253	1321 ± 328	76 ± 6	1250 ± 213	E	China	[52]
Yanzhimei	-	-	69	977	E	China	[53]
Yeliqing	-	-	69	994	E	China	[53]
Yingsu	-	-	59	1463	E	China	[53]
Yinnafenghou	-	-	73	2054	E	China	[53]
Yueshijie	-	-	62	1116	E	China	[53]
Yunnanxingmei	-	-	73	1835	E	China	[53]
Yuying	-	-	62	1116	E	China	[53]
Zaohong	-	-	50	1583	E	China	[53]
Zaohua	-	-	56	1403	E	China	[53]
Zhizhimei	-	-	69	1069	E	China	[53]
Zhonghong	-	-	53	1542	E	China	[53]

5.3. Peach (*P. persica*)

Peach is the stone fruit crop with higher economic importance. It has been confined traditionally to latitudes between 30° and 50° North and South [68], but in the last years, there is an increasing interest to expand it to warmer areas, including tropical and subtropical regions [68–70]. In recent decades, intense breeding has led to the release of an enormous number of cultivars of different types of fruit, including pubescent (peaches) or glabrous skin (nectarines), round or flat shape, white or yellow flesh, and freestone or clingstone [70] (Table 4). Several peach cultivars have been used to develop models in dormancy studies, both in experimental approaches to determine the date of breaking of endodormancy [23,38,71] and in models to quantify chilling and forcing temperatures [13,28,29,72]. The *DORMANCY-ASSOCIATED MAD-BOX (DAM)* genes that regulate dormancy were first reported in an ‘evergreen’ peach mutant [73,74].

This work compiles the chilling requirements of 216 cultivars, including seven flat peach cultivars, 25 nectarine cultivars and 172 peach cultivars, showing high differences in the range 239–536 CH, 354–861 CU and 22.3–48.5 CP for flat peaches, 90–426 CH/ 45–1050CU/9–47CP for nectarines and 71–1390 CH/ 5–1220 CU/ 1–1221.8 CP for peaches. The heat requirements of 44 cultivars have been compiled, ranging from 5853 to 9338 GDH for nectarine and between 3476 and 16493 GDH for peach.

Table 4. Chilling and heat requirements of peach cultivars.

	Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
		CH	CU	CP				
Flat peach	Carioca	368	582	35.5	-	E	Spain	[75]
	Siroco 10	305	480	28.9	-	E	Spain	[75]
	Siroco 5	239	355	22.3	-	E	Spain	[75]
	Sweet Cap	536	86	47.6	-	E	Spain	[75]
	UFO 2	432	681	40.2	-	E	Spain	[75]
	UFO 3	451	741	43.8	-	E	Spain	[75]
	UFO 4	484	803	48.5	-	E	Spain	[75]
Nectarine	Caldessi 2000	316	210	33	9002	S	Argentina	[76]
	Carolina	326	-	-	-	S	Argentina	[77]
	Cheonhong	-	800	-	-	S	Korea	[78]
	Collins	-	950	-	-	S	Korea	[78]
	Cortez	-	750	-	-	S	Korea	[78]
	Derby	-	750	-	-	S	Korea	[78]
	Earliscarlet	-	800	-	-	S	Korea	[78]
	Early Giant	342	249	46	8677	S	Argentina	[76]
	Fantasia	-	750	-	-	S	Korea	[78]
	Firebrite	308	198	34	7498	S	Argentina	[76]
	Flavortop	-	750	-	-	S	Korea	[78]
	Garden State	-	1050	-	-	S	Korea	[78]
	Hahong	-	700	-	-	S	Korea	[78]
	Hardired	-	950	-	-	S	Korea	[78]
	Lara	350	-	-	-	S	Argentina	[77]
		93	47	9	9338	S	Argentina	[76]
	María Anna	426	392	46	5853	S	Argentina	[76]
	María Lucía	413	244	47	6777	S	Argentina	[76]
	May Glo	98	84	12	8242	S	Argentina	[76]
	May Grand	-	800	-	-	S	Korea	[78]
	Redgold	-	850	-	-	S	Korea	[78]
	Roseprincess	313	207	33	9000	S	Argentina	[76]
	Suhong	-	700	-	-	S	Korea	[78]
	Sunfre	-	500	-	-	S	Korea	[78]
	Sungem	-	425	-	-	S	Korea	[78]
	Sunraycer	90	45	10	9086	S	Argentina	[76]
Peach	Afterglow	750	-	-	-	S	USA	[28]
	Akatsuki	1176	1074	-	5675	E	Japan	[79]
	Anjiry Asali	862	746	-	4232	E	Iran	[61]
	Anjiry Zafarany	973	805	-	4099	E	Iran	[61]
	Armking	-	600	-	-	S	Korea	[78]
	Autumnglo	-	950	-	-	S	Korea	[78]
	Babygold 5	498	364	53	8505	S	Argentina	[76]

Table 4. Cont.

	Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
		CH	CU	CP				
Peach	Belle	850	-	-	-	S	USA	[28]
	Best May	850	-	-	-	S	USA	[28]
	Big top	363	716	45.2	-	E	Spain	[80]
	Bonão	142.3	46.6	532	-	S	Brazil	[81]
	BR-1	<300	-	-	-	E	Brazil	[82]
	BR-3	305	265	982.6	-	S	Brazil	[81]
	Cambará do sul	371	295	1221.8	-	S	Brazil	[81]
	Camdem	-	750	-	-	S	Korea	[78]
	Canadian	-	750	-	-	S	Korea	[78]
	Candoka	850	-	-	-	S	USA	[28]
	Catherina	793	1220	62.4	-	E	Spain	[80]
	Changbangjosaeng	232	-	-	-	S	China	[83]
	Changhowon Hwangdo	-	850	-	-	S	Korea	[78]
	Cheonghong	146 - 261	-	-	-	S	China	[83]
	Cheonjoongdo	137	-	-	-	S	China	[83]
	Chinese cling	850	-	-	-	S	China	[84]
	Chiyohime	820	-	-	-	E	Japan	[85]
	Colora	1050	-	-	-	S	USA	[28]
	Coral	354	32	1137.8	-	S	Brazil	[81]
	Cresthaven	-	950	-	-	E	USA	[86]
	Cumberland	850	-	-	-	S	USA	[28]
	Delicioso	200	-	-	-	E	Brazil	[82]
	Della Nona	400	-	-	-	E	Brazil	[82]
	Diamante	294	228	875.6	-	S	Brazil	[81]
	Dixigem	850	-	-	-	S	USA	[28]
	Dixigold	850	-	-	-	S	USA	[28]
	Dixired	950	-	-	-	S	USA	[28]
	Duke of York	1150	-	-	-	S	USA	[28]
	Early Elberta	-	850	-	-	S	Korea	[78]
		850	-	-	-	S	USA	[28]
	Early Halegaven	850	-	-	-	S	USA	[28]
	Early Hiley	750	-	-	-	S	USA	[28]
	Early Jubilee	850	-	-	-	S	USA	[28]
	Early May Crest	300	600	40	-	E	Tunisia	[87]
	Early Rose	1150	-	-	-	S	USA	[28]
	Early Vedette	950	-	-	-	S	USA	[28]
	Early Wheeler	950	-	-	-	S	USA	[28]
	Eclipse	950	-	-	-	S	USA	[28]
	Elberta	850	-	-	-	S	USA	[28]
		790	-	-	5110	E	USA	[27]

Table 4. Cont.

	Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
		CH	CU	CP				
Peach	Eldorado	300	-	-	-	E	Brazil	[82]
	Elegant Lady	806	-	-	4692	E	Italy	[66]
	Erly-Red-Fre	850	-	-	-	S	USA	[28]
	Fairhaven	-	850	-	-	S	Korea	[78]
	Fairprince	-	850	-	-	S	Korea	[78]
	Fairs Beauty	1050	-	-	-	S	USA	[28]
	Fay Elberta	750	-	-	-	S	USA	[28]
	Feicheng Bai Li 10	1100	-	-	-	S	China	[84]
	Fergold	921	861	52.8	-	E	Spain	[80]
	Fireglow	750	-	-	-	S	USA	[28]
	Fireprince	341	226	36	8488	S	Argentina	[76]
	Fisher	-	950	-	-	S	Korea	[78]
		950	-	-	-	S	USA	[28]
	Fla. 91-8c	100	-	-	-	S	Argentina	[77]
	Flaming Gold	750	-	-	-	S	USA	[28]
	Flavorcrest	-	750	-	-	S	Korea	[78]
	Flordaglo	79	11	1	8394	S	Argentina	[76]
	Flordastar	-	225	-	3476 ± 57	E	Spain	[88]
	Franca	818	-	-	4887	E	Italy	[66]
	Fuzzless Berta	1150	-	-	-	S	USA	[28]
	Gage	750	-	-	-	S	USA	[28]
	GaLa	306	206	30	7415	S	Argentina	[76]
	Gemmers Elberta	750	-	-	-	S	USA	[28]
	Golden Jubilee	-	850	-	-	S	Korea	[78]
		850	-	-	-	S	USA	[28]
	Goldeneast	1050	-	-	-	S	USA	[28]
	Guglielmina	488	381	51	8543	S	Argentina	[76]
	Haj kamzemi	1390	868	-	4543	E	Iran	[61]
	Halberta Giant	850	-	-	-	S	USA	[28]
	Halegold	850	-	-	-	S	USA	[28]
	Halehaven	850	-	-	-	S	USA	[28]
	Halford	-	900	-	-	S	Korea	[78]
	Harbrite	-	850	-	-	S	Korea	[78]
	Harken	-	750	-	-	S	Korea	[78]
	Harland	-	850	-	-	S	Korea	[78]
	Harrow Beauty	-	775	-	-	S	Korea	[78]
	Herbhale	850	-	-	-	S	USA	[28]
	Hikawahakuhou	1173	1079	-	5505	E	Japan	[79]
	Hiley	750	-	-	-	S	USA	[28]
	Ideal	850	-	-	-	S	USA	[28]
	J.H. Hale	850	-	-	-	S	USA	[28]

Table 4. Cont.

	Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
		CH	CU	CP				
Peach	Janghowon	134	-	-	-	S	China	[83]
	Jerseyglo	-	750	-	-	S	Korea	[78]
	Jerseyland	-	850	-	-	S	Korea	[78]
	Jinmi	-	850	-	-	S	Korea	[78]
	July Elberta	750	-	-	-	S	USA	[28]
	Juneprince	-	650	-	-	E	USA	[86]
		-	625	-	-	S	Korea	[78]
	Kalhaven	950	-	-	-	S	USA	[28]
	Kosary	1390	868	-	4543	E	Iran	[61]
	Late Dwarf	71	5	6	16493	S	Argentina	[76]
	Levante 30	321	523	31.7	4592	S	Spain	[89]
	Levante 40	341	536	33.0	6824	S	Spain	[89]
	Lizzie	950	-	-	-	S	USA	[28]
	Loring	-	800	-	-	S	Korea	[78]
	Lovell	-	850	-	-	S	Korea	[78]
	Majestic	-	750	-	-	S	Korea	[78]
	Maravilha	203	124	692.4	-	S	Brazil	[81]
	Marfim	313	287	1018.2	-	S	Brazil	[81]
	María Delizia	338	223	47	11504	S	Argentina	[76]
	María Marta	327	212	34	9252	S	Argentina	[76]
	Maruja	572	809	51.8	-	E	Spain	[80]
	Maxine	1050	-	-	-	S	USA	[28]
	Mayflower	1150	-	-	-	S	USA	[28]
	Mibaekdo	-	850	-	-	S	Korea	[78]
	Michelini	884	-	-	5333	E	Italy	[66]
	Midway	850	-	-	-	S	USA	[28]
	Mihong	-	850	-	-	S	Korea	[78]
	Misshong	-	850	-	-	S	Korea	[78]
	Mistral 30	402	659	40.5	7103	S	Spain	[89]
	Momo tsukuba 127	555	652	-	5259	E	Japan	[79]
	Nectaross	925	-	-	5218	E	Italy	[66]
	New Yorker	-	850	-	-	S	Korea	[78]
	Newday	750	-	-	-	S	USA	[28]
	Okinawa 1	319	443	-	4691	E	Japan	[79]
	Pacemaker	750	-	-	-	S	USA	[28]
	Pepita	204	118	687.3	-	S	Brazil	[81]
	Planalto	400 - 500	-	-	-	E	Brazil	[82]
		216	139	720.4	-	S	Brazil	[81]
	Precocinho	150	-	-	-	E	Brazil	[72]

Table 4. Cont.

	Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
		CH	CU	CP				
Peach	Qingzhou Bai Pi Mi Tao	1100	-	-	-	S	China	[84]
	Raritan Rose	-	1050	-	-	S	Korea	[78]
		950	-	-	-	S	USA	[28]
	Red Globed	-	850	-	-	E	USA	[86]
		-	850	-	-	S	Korea	[78]
	Redelberta	750	-	-	-	S	USA	[28]
	Redhaven	850	-	-	-	S	USA	[28]
		870	-	-	4922	E	USA	[27]
	Redheaven	-	950	-	-	S	Korea	[78]
	Redrose	850	-	-	-	S	USA	[28]
	Reliance	-	1050	-	-	S	Korea	[78]
	Rich Lady	73	6	11	14086	S	Argentina	[76]
	Richhaven	-	950	-	-	S	Korea	[78]
	Rio Oso Gem	850	-	-	-	S	USA	[28]
	Riograndense	300	-	-	-	E	Brazil	[82]
	Rosa del West	434	378	51	9879	S	Argentina	[76]
	Ruiguang 03	-	777	-	-	S	Korea	[78]
	Salberta	850	-	-	-	S	USA	[28]
	Salwy	1050	-	-	-	S	USA	[28]
	Sentry	-	875	-	-	S	Korea	[78]
	Shipper Late Red	850	-	-	-	S	USA	[28]
	Siroco 5	246	427	25.8	7025	S	Spain	[89]
	Siroco 20	344	561	35.8	7384	S	Spain	[89]
	Siroco 30	308	539	31.2	6815	S	Spain	[89]
	Siroco 40	310	509	30.3	7463	S	Spain	[89]
	Siroco 43	370	593	36.4	6552	S	Spain	[89]
	Southland	750	-	-	-	S	USA	[28]
	Spring Belle	650	-	-	-	S	Spain	[90]
	Spring Lady	331	625	40.8	-	E	Spain	[80]
	Springtime	-	650	-	-	S	Korea	[78]
	Stark Red Gold	898	-	-	5188	E	Italy	[66]
	Starking Delicious	-	750	-	-	S	Korea	[78]
	Sullivan	850	-	-	-	S	USA	[28]
	Summercrest	950	-	-	-	S	USA	[28]
	Sunglo	-	850	-	-	S	Korea	[78]
	Sunhigh	750	-	-	-	S	USA	[28]
	Sunland	-	750	-	-	E	USA	[86]
		-	750	-	-	S	Korea	[78]
	Triogem	850	-	-	-	S	USA	[28]

Table 4. Cont.

	Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
		CH	CU	CP				
Peach	Tropic Beauty	-	150	-	-	S	Korea	[78]
	Tropic Snow	-	200	-	-	S	Korea	[78]
	Turmalina	250	184	801.8	-	S	Brazil	[81]
	Up-to-date	850	-	-	-	S	USA	[28]
	Valiant	850	-	-	-	S	USA	[28]
	Vedette	1050	-	-	-	S	USA	[28]
	Veeglo	-	950	-	-	S	Korea	[78]
	Veteran	1050	-	-	-	S	USA	[28]
	Vivid	-	950	-	-	S	Korea	[78]
	Worlds Earliest	750	-	-	-	S	USA	[28]
	Yanguang	-	780	-	-	S	Korea	[78]
	Youmyeong	150 - 277	-	-	-	S	China	[83]
	Yu Hua Lu	800	-	-	-	S	China	[84]
	Yumi	-	800	-	-	S	Korea	[78]
	Yumyeong	-	850	-	-	S	Korea	[78]
	Zoud Ras	1130	826	-	4384	E	Iran	[61]

5.4. European and Japanese plum (*P. domestica* and *P. salicina*).

World production of plums increased by almost 20% in the last 10 years (from 9.5 million tons in 2007 to 12 million tons in 2017) [2]. These data include European plums, Japanese plums and hybrids between different *Prunus* sp. In spite of the economic importance of this crop, temperature requirements are little studied, with data available for only nine cultivars of European plum (Table 5) [91] and 16 cultivars of Japanese plum (Table 6) from two studies performed in Spain [31,91]. The experimental procedure used was slightly different between studies, with variation in the temperature of the growing chamber and in the growth evaluation procedure.

European plums are cultivated in colder climates [92], showing higher chilling requirements (579–1323 CH) than Japanese plums (118–685 CH) (Tables 5 and 6). The chilling requirements of European plum ranged from 579–678 CH for ‘Reine Claude d’Oullins’ to 1116–1323 CH for ‘Reine Claude Noir’ [93]. There is no data available on heat requirements in this species.

The Japanese plum cultivars with lower chilling requirements are ‘Methley’ (118–239 CH [91] and ‘Pioneer’ (181–231 CH / 297–358 CU / 18.4–27.6 CP), which also showed the lower values of heat requirements (5261–6720 GDH) [31]. ‘Songold’ showed higher values both for chilling (561–630 CH / 974–1001 CU / 60.4–61.1 CP) and heat requirements (8588–10034 GDH). There are only data from different reports for ‘Golden Japan’ and ‘Santa Rosa’, showing high differences between them (Table 6).

Table 5. Chilling and heat requirements of European plum cultivars.

Cultivar	Chilling requirements			Heat req. GDH	Method	Loc.	Ref.
	CH	CU	CP				
Beauty Plum of Catalogne	678–819	-	-	-	E	Spain	[91]
Coe’s Golden Drop	984–1157	-	-	-	E	Spain	[91]
Real de Calahorra	976–1029	-	-	-	E	Spain	[91]
Reine Claude de Bavay	984–1157	-	-	-	E	Spain	[91]
Reine Claudie Noir	1116–1323	-	-	-	E	Spain	[91]
Reine Claude d’Oullins	579–678	-	-	-	E	Spain	[91]
Reine Claude Verte	976–1275	-	-	-	E	Spain	[91]
Reine Claude Violetta d’Agen	819–984	-	-	-	E	Spain	[91]
Reine Claude Washington	976–1275	-	-	-	E	Spain	[91]

Table 6. Chilling and heat requirements of Japanese plum cultivars.

Cultivar	Chilling requirements			Heat req. GDH	Method	Loc.	Ref.
	CH	CU	CP				
Angeleno	434–447	750–779	39.7–50.1	7300–8180	E	Spain	[31]
Apex	486–678	-	-	-	E	Spain	[91]
Black Diamond	389–432	630–750	38.7–47.1	6994–8068	E	Spain	[31]
Black Splendor	213–324	437–605	29.1–31.2	5744–6705	E	Spain	[31]
Burbank	486–678	-	-	-	E	Spain	[91]
Formosa	486–678	-	-	-	E	Spain	[91]
Fortune	436–447	750–769	39.7–46.7	6681–8506	E	Spain	[31]
Golden Globe	473–685	872–1053	44.8–63.5	7300–9151	E	Spain	[31]
Golden Japan	118–287	-	-	-	E	Spain	[91]
	384–454	701–829	35.4–52.1	6939–7855	E	Spain	[31]
Laetitia	436–454	750–829	39.7–52.1	7894–8020	E	Spain	[31]
Methley	118–239	-	-	-	E	Spain	[91]
Pioneer	181–231	297–358	18.4–27.6	5261–6720	E	Spain	[31]
Red Beauty	265–369	500–688	25.6–46	6727–7183	E	Spain	[31]
Santa Rosa	372–627	-	-	-	E	Spain	[91]
	436–459	750–829	41.8–52.1	6591–9099	E	Spain	[31]
Songold	561–630	974–1001	60.4–61.1	8588–10034	E	Spain	[31]
Wickson	345–627	-	-	-	E	Spain	[91]

5.5. Sweet and Sour Cherry (*P. avium* and *P. cerasus*)

Sweet cherries are mainly produced in Mediterranean countries as Turkey (0.63 million t/year), Italy (0.12 million t/year), Spain (0.11 million t/year) and Greece (0.90 million t/year), in Middle Eastern countries such as Iran (0.14 million t/year) and Uzbekistan (0.14 million t/year), in the United States (0.40 million t/year), and in Chile (0.13 million t/year) [2]. Chilling requirements are available for 53 cultivars [34,94–97], while the heat requirements were only calculated for 16 of them (Table 7) [34,96]. Chilling requirements are mainly quantified with CH in 48 cultivars [94,96] that range from 176 CH of ‘Cristobalina’ [96] to more than 1100 CH for ‘Garrafal de Lérida’, ‘Hedelfinger’, ‘Lambert’, ‘Napoleón’, and ‘Vignola’ [94]. Data available in CU in 21 cultivars range between 94 CU for ‘Lapins’ and ‘Larian’ [97] and 1559 ± 53 CU for ‘Skeena’ [95], and 8 cultivars in CP from to 30.4 CP for ‘Cristobalina’ to 57.6 CP for ‘Marvin’ [96]. Chilling requirements for ‘Burlat’ and ‘Cristobalina’ were experimentally analyzed in two studies, resulting in higher values in the north of Spain (‘Burlat’ 900–100 CH and ‘Cristobalina’ <800 CH) [94] than in the Mediterranean area (‘Burlat’ 618 CH and ‘Cristobalina’

176 CH) [96]. Estimations of heat requirements showed high variation between cultivars and locations: 3473 $\pm$ 1236 GDH for ‘Schneider’ in Germany [34], and 15500–16000 GDH are for ‘Lapins’, ‘Larian’, and ‘0900 Ziraat’ in Turkey [97].

The experimental methodology [94–97] showed slight differences among studies: the frequency of field sampling (every 2 [97] or 7 [94] days, or every 50–100 CU [96]), the temperature of the chamber (24 ± 1 °C [96,97] or 20 ± 1 °C [94]), the period in the chamber (7 [94,95], 10 [96] or 21 [97] days), and also in the bud growth evaluation (dry weight [94], fresh weight [95], or phenology [96,97]). A statistical methodology was developed based on ‘Schneiders’ in Germany [34].

Sour cherry requirements have been poorly studied. There is only data of cultivar Montmorency, which showed 954 CU and 6130 GDH [42] (Table 7).

Table 7. Chilling and heat requirements of sour and sweet cherry cultivars.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Ambrunés	1000–1100	-	-	-	E	Spain	[94]
Bing	1000–1100	-	-	-	E	Spain	[94]
	-	1082 $\pm$ 27	-	-	E	Spain	[95]
Brooks	411.5	556	36.7	7863.2	E	Spain	[96]
Burlat	900–1000	-	-	-	E	Spain	[94]
	618	806	48	8750.2	E	Spain	[96]
	-	981 $\pm$ 83	-	-	E	Spain	[95]
	-	-	86	-	E	Spain	[98]
Cherovina	900–1000	-	-	-	E	Spain	[94]
Cristobalina	<800	-	-	-	E	Spain	[94]
	176	397	30.4	9195	E	Spain	[96]
	-	687 $\pm$ 83	-	-	E	Spain	[95]
	-	-	29	-	E	Spain	[98]
Daiber	1000–1100	-	-	-	E	Spain	[94]
Earlise	-	981 $\pm$ 83	-	-	E	Spain	[95]
Early Rivers	800–900	-	-	-	E	Spain	[94]
Fertard	-	-	101	-	E	Spain	[98]
Garrafal de Lérida	> 1100	-	-	-	E	Spain	[94]
Guillaume	1000–1100	-	-	-	E	Spain	[94]
Hedelfingen	> 1100	-	-	-	E	Spain	[94]
Jaboulay	1000–1100	-	-	-	E	Spain	[94]
Jarandilla	> 1100	-	-	-	E	Spain	[94]
Kordia	700–750	150	-	14000	E	Turkey	[97]
Lambert	> 1100	-	-	-	E	Spain	[94]
Lampé (Ramillete)	900–1000	-	-	-	E	Spain	[94]
Lapins	400–450	94	-	15500–16000	E	Turkey	[97]
Larian	450	94	-	15500–16000	E	Turkey	[97]
Marmotte	1000–1100	-	-	-	E	Spain	[94]
Marvin	788	1002	57.6	9450	E	Spain	[96]
Merton Glory	900–1000	-	-	-	E	Spain	[94]
Mollar de cáceres	900–1000	-	-	-	E	Spain	[94]
Moreau	1000–1100	-	-	-	E	Spain	[94]
Nafrina	500–550	120	-	15000–15500	E	Turkey	[97]
Napoleón	> 1100	-	-	-	E	Spain	[94]
New Star	709	909	53.5	8257	E	Spain	[96]

Table 7. Cont.

Cultivar	Chilling requirements			Heat requirements GDH	Method	Loc.	Ref.
	CH	CU	CP				
Noir de Guben	600–650	110	-	14000–14500	E	Turkey	[97]
Pico Colorado	1000–1100	-	-	-	E	Spain	[94]
Producta	1000–1100	-	-	-	E	Spain	[94]
Ramón Oliva	900–1000	-	-	-	E	Spain	[94]
Regina	-	-	86	-	E	Spain	[98]
Reverchon	1000–1100	-	-	-	E	Spain	[94]
Ripolla	800–900	-	-	-	E	Spain	[94]
Ruby	618	806	48	7326	E	Spain	[96]
Somerset	618	806	48	8625.2	E	Spain	[96]
Schneiders	698 ± 151	794 ± 17	45.7 ± 5.4	3473 ± 1236	S	Germany	[34]
Skeena	-	1559 ± 63	-	-	E	Spain	[95]
Stark Hardy Giant	1000–1100	-	-	-	E	Spain	[94]
Summit	650	125	-	15000	E	Turkey	[97]
Sunburst	650–700	141	-	14000–14500	E	Turkey	[97]
Taleguera Brillante	1000–1100	-	-	-	E	Spain	[94]
Temprana de Sot	1000–1100	-	-	-	E	Spain	[94]
Tigré	900–1000	-	-	-	E	Spain	[94]
Van	1000–1100	-	-	-	E	Spain	[94]
Vernon	1000–1100	-	-	-	E	Spain	[94]
Vignola	> 1100	-	-	-	E	Spain	[94]
Villareta	900–1000	-	-	-	E	Spain	[94]
0900 Ziraat	600–650	134	-	15500–16000	E	Turkey	[97]
Montmorency(sour cherry)	-	954	-	6130	S	USA	[42]

6. Concluding Remarks and Perspectives

This study compiles the temperature requirements of a total of 530 cultivars of eight *Prunus* ssp. Most of the data correspond to peach (204 cultivars), almond (106 cultivars), Japanese apricot (77 cultivars), European apricot (68 cultivars), and sweet cherry (49 cultivars) since there is little information available for European plum (nine cultivars), Japanese plum (16 cultivars), and sour cherry (1 cultivar) (Table 8). These data represent a very small percentage of the existing commercial cultivars and, in addition, 84 out of 530 cultivars came from studies published more than 25 years ago (Table 8). Therefore, temperature requirements are only available for very current growing cultivars. To serve as a reference, more than 1500 stone fruit cultivars were registered in the European Union in the last 25 years, including 946 for peach, 320 for apricot, 132 for Japanese plum, and 130 for sweet cherry [99]. Two main methodologies, statistical and experimental, have been used to obtain the temperature requirements reported here. Chilling requirements were experimentally determined for all the cultivars of European and Japanese apricot and European and Japanese plum. In contrast, most of the data available for cultivars of almond (153 statistical data vs. 56 experimental data) and peach (173 statistical data vs. 42 experimental data) were statistically determined (Table 8).

Table 8. For each stone fruit crop, number of studies included in this work, number of cultivars with available data of chilling and/or heat requirements, and number of cultivars according to the methodology used.

Specie	Studies	Cultivars		Methodology	
		Total n°	> 25 years old	Statistical	Experimental
Almond	5	106	17	98	10
European apricot	15	68	23	0	69
Japanese apricot	3	77	0	0	77
Peach	Flat peach	1	7	1	0
	Nectarine	3	25	3	0
	Peach	18	172	7	11
European plum	1	9	9	0	9
Japanese plum	2	16	5	0	16
Sweet and Sour cherry	7	50	29	1	49

Chilling quantification was performed according to different temperature models: 382 data were calculated with CH, 342 with CP and 241 with CP. Heat requirements are available for 328 out of 530 cultivars (Table 9). Among the different stone fruit crops, peach cultivars presented a wider range of chilling requirements (71–1390 CH, 5–1220 CU, and 1–1222 CP), while flat peach cultivars showed the least narrow range (239–536 CH, 354–861 CU, and 22.3–48.8 CP). Some almond cultivars showed the lowest chilling requirements, while some cultivars of European apricot, European plum, peach, and sweet cherry showed the highest chilling requirements. Japanese apricot cultivars showed lower heat requirements, while some peach cultivars showed the highest values (16493 GDH) (Table 9).

Table 9. For each stone fruit crop, the number of cultivars and range of both chilling and heat requirements according to the model used.

Specie	Chilling requirements						Heat requirements		
	CH		CU		CP		GDH		
	Data	Range	Data	Range	Data	Range	Data	Range	
Almond	73	8–713	91	284–996	62	3.4–55.4	107	2862–10201	
Eur. apricot	78	171–1812	88	274–1665	31	29.8–78.9	74	485–6729	
Jap. apricot	8	239–1148	6	479–1323	75	29–78.5	75	822–2378	
Peach	Flat peach	7	239–536	7	354–861	7	22.3–48.5	0	-
	Nectarine	11	90–426	24	45–1050	9	9–47	9	5853–9338
	Peach	132	71–1390	93	5–1220	34	1–122	35	3476–16493
Eur. plum	9	579–1323	0	-	0	-	0	-	
Jap. plum	16	118–685	11	297–1053	11	18–64	11	5261–10034	
Sweet cherry	48	176–1100	22	94–1559	12	29–107	17	3473–16000	

The empirical methodology to determine dormancy by monitoring shoots in forcing conditions is the unique currently available, although it was designed more than 60 years ago [23]. This method has been applied to determine chilling requirements in early [32] and recent studies [31], but it has also been used to infer the dormant stage in research aimed at studying the physiology of dormancy [6,54,59,100–102]. The variability on the experimental designs among studies had often resulted in inconsistencies in the data obtained. This variability is noted in the frequency of shoot sampling along winter (e.g., weekly, every 10 days or every certain amount of chilling accumulated), but also in the environmental conditions of the growth chamber: temperature (20 °C, 25 °C), temperature regime (constant or with day/night variation), and photoperiod. Subsequently, the evaluation of

bud growth is performed after different periods in the growth chamber (a week, 10 days, 20 days), and it is based on analyzing vegetative [103] or flower buds [95]. Furthermore, several criteria are used to determine dormancy overcome. These can consist of significant increases in fresh [23] or dry weight [60], and/or phenological changes in bud phenology [24], which can result in an underestimation of the chilling requirements [98]. All these variations make this methodology easily adaptable to the characteristics of each fruit species and regional variations, but the results obtained under specific conditions should be taken with caution when applied to other regions or climates.

The statistical determination of dormancy is mainly based on two approaches. On one side, the correlation of winter temperatures with the flowering dates, which has been used to establish the chilling requirements of almond [26,33], apricot, peach, plum and sweet cherry [33] cultivars in Spain. On the other side, PLS analysis has been recently developed in a sweet cherry cultivar in Germany [34], and was subsequently applied to other temperate fruit crops as almond in Tunisia [35] and Spain [36], and apricot in China [27,104]. This methodology has reported interesting results on predicting phenology under future scenarios of global warming [19,104]. However, statistical analyses present low applicability on new cultivars released from breeding programs, since they are based on a long series of flowering dates records (more than 20 years).

Once the endo- and eco- dormancy periods have been established, specific temperature models are used to calculate the duration of each phase. Although these models were specially designed to quantify chilling or warm temperatures, they present numerous drawbacks. The Chilling Hours model is easy to understand and calculate and is commonly used by growers who often know the accumulated CH in their location, despite the lack of information about the requirements of their cultivars. This model does not fit perfectly with the behavior of trees, especially in mild and warm areas [105,106]. The Utah model attempts to be more accurate by weighting the temperature ranges. However, the fact that it establishes negative values for warm temperatures hampers its applicability in mild winter conditions. The different criteria for establishing the starting point to quantify temperatures, either an established date for dormancy starting (e.g., November 1st) [35] or the date with the maximum negative value [29], had resulted in high differences even for the same cultivar as occurs in almond [26,35,43] or European apricot [45,48,50], making the comparison between studies difficult. The Dynamic model has been proposed as the best model available but also presents limitations on fitting the plant responses to chill [37]. It was designed as a process-based model, however, the physiological process behind is still unknown [30,40,72]. Finally, the Growing Degree Hours model allows quantifying forcing temperatures in a wide range of biological processes, such as phenological stages of annual crops or even insect growth [107]. The results of GDH quantification are highly variable between species, especially at different locations [34]). This could be due to both the model and the interaction between chill and heat accumulation [108–110].

In spite of both the methodologies to determine dormancy periods and the temperature models have numerous pitfalls, the available data of temperature requirements are useful to predict the adaptability of a particular cultivar to a certain area. However, very few of the cultivars currently grown have known temperature requirements. This means that, in most cases, the flowering period is the unique information available to assess the adaptation of a cultivar. Flowering periods are usually related to a reference cultivar (e.g., 'Burlat' in sweet cherry) [5] and successfully used for spring frost risk assessment and for pollination purposes, to predict flowering overlap between pollinating and pollinated cultivars. However, assessing the possible adaptation to an area based on relative flowering dates has many limitations, and temperature requirements offer a more reliable approach [111]. However, this review shows that information is not available for the most important cultivars nowadays, and, when available, they are usually imprecise estimations based only on flowering dates. Furthermore, temperature requirements are scarcely evaluated in most breeding programs, which could lead to an increasing lack of information in the coming years.

In conclusion, numerous improvements may be needed to obtain an accurate determination of the temperature requirements of stone fruit cultivars. The standardization of the experimental

conditions would allow obtaining more robust and comparable data. However, the increasing number of new cultivars in most *Prunus* species and the expected reduction of winter chill due to global warming emphasize the necessity of a proper biological marker for dormancy. This would allow the analysis of samples collected directly from the field, without depending on external factors such as forcing conditions in the experimental approach or the availability of a large set of phenological data. Recent reports have revealed several processes as promising candidates for dormancy markers such as the expression of the *DORMANCY-ASSOCIATED MAD-BOX (DAM)* genes in peach [112], starch accumulation within the ovary primordia cell in sweet cherry [113], anther meiosis in apricot [114,115], and hormone regulation in sweet cherry [116]. Establishing the relationship between temperature records and a biological dormancy marker would lead to a process-based model that would allow direct determination of dormancy and a more accurate estimation of the temperature requirements of particular cultivars.

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ANEXO V

Herrera, S., Lora, J., Hormaza, J. I., and Rodrigo, J. (2021c). Albaricoquero: renovación varietal y necesidades de polinización. *Agricultura* 1047, 52-56.

Albaricoquero: renovación varietal y necesidades de polinización

La reconversión varietal que se está produciendo en el albaricoquero es la respuesta ante diversos problemas que ha presentado el cultivo en los últimos años. El rápido lanzamiento de nuevas variedades desarrolladas en programas de mejora de todo el mundo ha provocado que las necesidades de polinización sean un factor importante a tener cada vez más en cuenta en el cultivo.

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El cultivo del albaricoquero en España ha sufrido grandes fluctuaciones en las últimas décadas. En 1991, el año de mayor producción, se llegaron a alcanzar las 210.900 t en 25.800 ha. La expansión del virus de la sharka (*Plum pox virus*) provocó una considerable reducción del cultivo en los años posteriores, ya que todas las variedades cultivadas hasta entonces en nuestro país eran sensibles a la sharka, por lo que hubo que arrancar millones de árboles (Martínez-Calvo et al., 2011). La superficie cultivada fue reduciéndose hasta alcanzar un mínimo de 18.150 ha y una producción de 156.872 t en 2006. Desde entonces, la puesta en marcha de programas de mejora, como los del CEBAS (CSIC) en Murcia, el IVIA en Valencia y otros de iniciativa privada, han permitido introducir nuevas variedades resistentes a la sharka, lo que ha provocado una recuperación del cultivo en las zonas tradicionales y la expansión a nuevas zonas de cultivo. En la actualidad se cultivan 20.567 ha con una producción anual de unas 176.200 t en 2018, principalmente en la Región de Murcia (97.755 t y 8.852 ha), la Comunidad Valenciana (24.435 t y 3.715 ha), Aragón (14.173 t y 2.636 ha) y Castilla la Mancha (13.985 t y 1.582 ha) (MAPA, 2021).

Renovación varietal

A la necesidad de sustituir las variedades tradicionales por variedades resistentes a la sharka en muchas zonas, se han unido otros factores como la reducción de la demanda de frutos para la industria o el cambio en las preferencias de los consumidores, especialmente en el norte de Europa, hacia frutos de pulpa naranja y piel naranja o rojiza, lo que ha provocado una importante reconversión varietal en el cultivo (Egea et al., 2006; Egea y Ruiz, 2014). Cada año se introducen nuevas variedades procedentes de programas de mejora tanto de financiación pública como privada de distintos países, lo que se refleja en la solicitud de más de 300 variedades para su registro en la Oficina Comunitaria de Variedades Vegetales de la Unión Europea (CPVO) en los últimos 20 años (CPVO, 2021).



Foto 1
Abeja polinizando
flores de albaricoquero

Tabla 1.
Superficie cultivada y origen las variedades
autocompatibles de albaricoquero más cultivadas en
España en 2019 según los datos de superficie asegurada
(Agroseguro)

VARIEDAD	ORIGEN (OBTENTOR O EDITOR)	SUPERFICIE CULTIVADA (HA)
Búlida	Tradicional	1004
Mirlo Anaranjado	CEBAS	762
Flopria	PBS	750
Mitger	Tradicional	571
Lady Cot	COT	547
Mogador	PBS	511
Resto de valencianos	Tradicional	327
Faralia	IPS	254
Murciana	CEBAS	235
Kioto	Escande	230
Galta Rocha	Tradicional	189
Flavor Cot	COT	150
Mirlo Blanco	CEBAS	145
Pepito	Tradicional	137
Rambo	PBS	121
Orange Ruby	Europépinières	119
Rojo Pasión	CEBAS	115
Valorange	CEBAS	107
Arquer	Tradicional	94
Fartoli	IPS	92
Big Red	Escande	91
Mirlo Rojo	CEBAS	84
Almabar	Frutaria	81
Paviot	Tradicional	78
Tom Cot	COT	52
Albas	Tradicional	42
Dorada	CEBAS	36
Soledane	IPS	27
Colorados (Antones)	Tradicional	25
Rojo Carlet	Tradicional	13
Delicot	COT	8

Tabla 2.
Superficie cultivada y origen las variedades
autoincompatibles de albaricoquero más cultivadas en
España en 2019 según los datos de superficie asegurada
(Agroseguro)

VARIEDAD	ORIGEN (OBTENTOR O EDITOR)	SUPERFICIE CULTIVADA (HA)
Colorado	PBS	1016
Flodea	PBS	322
Magic Cot	COT	291
Lilly Cot	COT	251
Moniqui	Tradicional	235
Wonder Cot	COT	220
Madison	PBS	148
Luca	PBS	132
Tsunami	Escande	123
Orangered	Rutgers	122
Mambo	PBS	111
Farely	IPS	102
Megatea	PBS	88
Samourai	Escande	83
Sublime	CEBAS	78
Robada	USDA	67
Medaga	IPS	67
Goldbar	COT	60
Sweet Cot	COT	54
Tornado	Escande	51
Sunny Cot	COT	42
Perle Cot	COT	41
Flash Cot	COT	27
Floneca	PBS	19

Uno de los principales objetivos de los programas de mejora de albaricoquero es la obtención de variedades resistentes al virus de la sharka. Para ello, en la mayoría de programas se utilizaron como parentales variedades resistentes seleccionadas en Norteamérica como 'Stark Early Orange', 'Sunglo' o 'Goldrich'. Estas variedades son autoincompatibles y, por tanto, necesitan polinización cruzada para producir fruto, al contrario que la mayoría de variedades tradicionales que se venían cultivando en España, que son autocompatibles y sus flores pueden polinizarse con éxito con su propio polen. Como consecuencia de esta situación, muchas de las descendencias obtenidas en los cruzamientos de los principales programas de mejora resultaron ser también autoincompatibles. Mientras algunos programas de mejora se han centrado en la selección de variedades autocompatibles, otros programas han

En los últimos años se han introducido un gran número de nuevas variedades de albaricoquero procedentes de programas de mejora nacionales y extranjeros

introducido nuevas variedades de necesidades de polinización desconocidas. En las **Tablas 1 y 2** se muestran las variedades más cultivadas en España según los datos de la superficie asegurada en 2019 (Agroseguro, 2021). En nuestro país hay varios programas de mejora genética de albaricoquero tanto públicos como privados. El programa del CEBAS-CSIC de Murcia tiene como principal objetivo

el desarrollo de variedades resistentes a la sharka, autocompatibles y de frutos de gran calidad que, además, permitan ampliar el calendario de producción (Egea et al., 2010). Como resultado de este programa, se han registrado variedades que han permitido dar una alternativa a las variedades tradicionales que se cultivaban en la Región de Murcia, cubriendo todo el calendario de recolección. Destaca la

serie de los “mirlos” (‘Mirlo Anaranjado’, ‘Mirlo Blanco’, ‘Mirlo Rojo’), ‘Murciana’, ‘Rojo Pasión’, ‘Sublime’, ‘Dorada’ y, recientemente, nuevas obtenciones como ‘Cebased’ o ‘Primorosa’ (CEBASfruit, 2021), que suponen actualmente más del 12% de la superficie cultivada de albaricoquero (**Tabla 1**). El programa del IVIA en Valencia presenta objetivos similares y ha introducido variedades como ‘Moixent’, ‘Dama Rosa’ y ‘Dama Taronja’ (Bádenes y Martínez-Calvo, 2014; IVIA, 2021).

Además, existen programas de mejora privados de empresas radicadas en España y otros países como Estados Unidos, Francia o Italia. Las variedades más cultivadas de PBS Producción Vegetal (Murcia) son ‘Colorado’, ‘Flopria’, ‘Mogador’, ‘Flodea’, ‘Madison’, ‘Luca’, ‘Rambo’, ‘Mambo’, ‘Floneca’ y ‘Megatea’ (25% de la superficie cultivada). Entre los programas de otros países y editores internacionales destacan COT Internacional, con una amplia variedad de variedades como ‘Lady Cot’, ‘Magic Cot’, ‘Lilly Cot’, ‘Wonder Cot’, ‘Flavor Cot’, ‘Goldbar’, ‘Sweet Cot’, ‘Tom Cot’, ‘Sunny Cot’, ‘Perle Cot’, ‘Flash Cot’ y ‘Delicot’ (más del 13% de la superficie cultivada), Escande con ‘Kioto’, ‘Tsunami’, ‘Big Red’, ‘Samourai’ y ‘Tornado’ (más del 4% de la superficie cultivada), e International Plant Selection (IPS) con ‘Faralia’, ‘Farely’, ‘Fartoly’ o ‘Medaga’ (4% de la superficie cultivada). Además de las nuevas obtenciones, todavía tienen importancia algunas variedades tradicionales, como ‘Búlida’, ‘Mitger’, ‘Moniqui’, ‘Galta Rocha’, ‘Pepito del Rubio’ y ‘Arquer’, que ocupan más del 38% de la superficie cultivada, aunque su presencia va disminuyendo año tras año (**Tablas 1 y 2**).

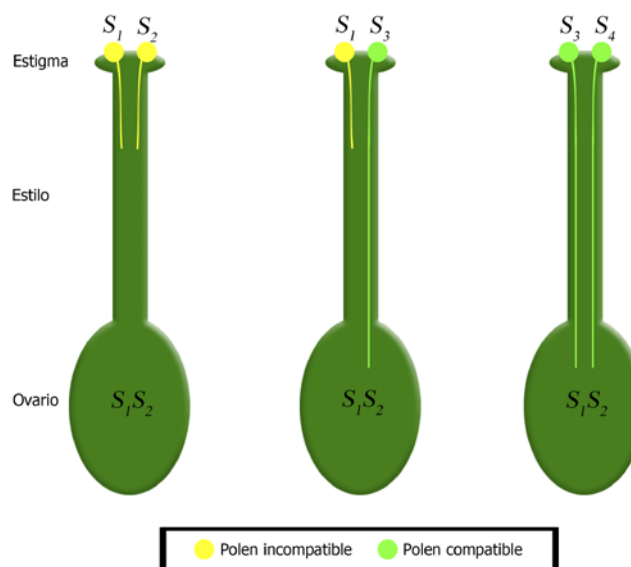
Polinización

La polinización en los frutales de hueso se lleva a cabo mediante insectos, generalmente abejas (*Apis* sp.) y abejorros (*Bombus* sp.). Los insectos polinizadores transportan el polen de la parte masculina de la flor (los estambres) a la parte femenina (pistilo) y lo depositan en el estigma (**Foto 1**).

La introducción de algunas nuevas variedades autoincompatibles ha puesto de manifiesto la necesidad de conocer sus necesidades de polinización, aspecto al que tradicionalmente no se prestaba mucha atención en el cultivo. En albari-

Figura 1.

Sistema de incompatibilidad entre el pistilo y el polen en albaricoquero. En las variedades autoincompatibles, cuando el alelo S del grano de polen coincide con uno de los alelos del pistilo, los tubos polínicos se detienen sin alcanzar el ovario, impidiendo así la fecundación del óvulo. Cuando el alelo S del polen es diferente a los alelos del pistilo, el tubo polínico puede crecer hasta el ovario y fecundar el óvulo.



coquero, como en otros frutales de hueso como el cerezo, el ciruelo o el almendro, existen variedades autocompatibles y autoincompatibles. Si el polen es compatible con el pistilo, el grano de polen germina en el estigma y emite un tubo polínico que crece a través del estilo hasta alcanzar el ovario donde puede fecundar uno de los dos óvulos, comenzando el proceso de formación del fruto. Cuando el polen es incompatible con el pistilo, los granos de polen también germinan en el estigma y emiten un tubo polínico que se dirige al estilo. Sin embargo, el crecimiento del tubo incompatible se detiene en la parte media del estilo, impidiendo su llegada al ovario y la fecundación del óvulo (**Figura 1**). Las flores no fecundadas se caen en los días o semanas siguientes sin llegar a cuajar.

El carácter de auto(in)compatibilidad se puede determinar mediante cruza-mientos controlados y la observación en el microscopio del comportamiento de los tubos polínicos en el pistilo de flores autopollinizadas (Herrera et al., 2020a) o mediante análisis genéticos (Herrera et al., 2020b). El mecanismo de incompatibilidad está regulado genéticamente por una región del genoma llamada locus S. En las variedades autoincompatibles, el pistilo de la flor es capaz de reconocer

su propio polen y rechazarlo para evitar la autofecundación y promover la fecundación cruzada. Cada variedad diploide tiene dos alelos S. En el pistilo (diploide) se expresan los dos alelos, mientras que en el polen (haploide) se expresa un solo alelo S. En las variedades autoincompatibles, cuando el mismo alelo S es expresado en el polen y en el pistilo se produce una reacción de incompatibilidad que origina la inhibición del crecimiento del tubo polínico impidiendo así la fecundación del óvulo y, por tanto, el cuajado del fruto. Por otro lado, si el polen y el pistilo presentan alelos S distintos, no se produce la reacción de incompatibilidad y el tubo polínico no detiene su crecimiento y puede fecundar el óvulo (Herrera et al., 2021) (**Figura 1**).

En los últimos años se ha determinado el genotipo S de las principales variedades de albaricoquero mediante técnicas basadas en el desarrollo de marcadores moleculares (Herrera et al., 2018a y b; 2020). Determinar los alelos S de cada variedad permite clasificar las variedades autoincompatibles en grupos de incompatibilidad, con lo que se establecen las relaciones de incompatibilidad entre variedades. En cada grupo de incompatibilidad se agrupan las variedades autoincompatibles con los mismos alelos



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Tabla 3.
Variedades autoincompatibles de albaricoquero agrupadas en grupos de incompatibilidad (G.I.) según sus alelos S

G.I.	ALELOS S	VARIEDADES AUTOINCOMPATIBLES
I	S ₁ S ₂	Castleton, Goldrich, Hargrand, Lambertin-1
II	S ₈ S ₉	Perle Cot, Pinkcot
III	S ₂ S ₆	Avirine (Bergarouge), Moniqui
IV	S ₂ S ₇	Ouardi, Priana
V	S ₂ S ₈	Holly Cot, Sweet Cot
VIII	S ₆ S ₉	Cheyenne, Feria Cot, Orangered, Stark Early Orange, Sunny Cot, Wonder Cot, Flash Cot
XVIII	S ₁ S ₃	Cooper Cot, Perfection
XIX	S ₂ S ₃	Maya Cot, Sun Glo
XX	S ₂ S ₉	Goldstrike, Magic Cot
XXI	S ₃ S ₈	Lilly Cot, Spring Blush
XXII	S ₃ S ₉	Durobar (Almadulce), Flodea, Henderson, Kosmos
XXIII	S ₇ S ₉	Goldbar
XXIV	S ₁ S ₆	Primaya

Tabla 4.
Variedades autocompatibles de albaricoquero

VARIEDADES AUTOCOMPATIBLES			
Alba	Faralia	Lito	Pepito del Rubio
Almabar	Farbaly	Lorna	Playa Cot
Aprix 9	Farbela	Luizet	Pricia
Aprix 20	Farclo	Mauricio	Primidi
Aprix 33	Fardao	Medflo	Primorosa
Apriqueen	Farfia	Mediabel	Rambo
Arquer	Farhial	Mediva	Real fino
Beliana	Farius	Memphis	Regibus
Bergecot	Farlis	Milord	Rojo de Carlet
Bergeron	Fartoli	Mirlo Anaranjado	Rojo Pasión
Big Red	Flavor Cot	Mirlo Blanco	Rouge Cot
Búlida	Flopria	Mirlo Rojo	Rouge de Roussillon
Canino	Galta Vermella	Mitger	Rubista
Castlebrite	Galta Rocha	Modesto	Sandy Cot
Cebas Red	Gandía	Mogador	Sherpa
Charisma	Ginesta	Murciana	Soledane
Colorados (Antones)	Golden Sweet	Ninfa	Swired
Corbato	Gönci Magyar	Orange ruby	Tadeo
Cristali	Harval	Oscar	Tilton
Currot	Hatíf Colomer	Palabras	Tirynthos
Delice Cot	Katy	Palau	Tom Cot
Dorada	Kioto	Palsteyn	Trevatt
Dulcinea	Lady Cot	Patterson	Valorange
Fantasme	Lido	Paviot	Victor-1

S que son incompatibles entre sí, por lo que no pueden actuar de polinizadores entre ellas, pero que son compatibles con cualquier variedad perteneciente a otro grupo (**Tabla 3**). En la actualidad, se han descrito 33 alelos de autoincompatibilidad en albaricoquero, y un alelo (Sc) relacionado con la autocompatibilidad (Herrera *et al.*, 2020b). Para ser buenas variedades polinizadoras, además de ser compatibles entre sí deben coincidir en el periodo de floración.

Las flores de las variedades autocompatibles (**Tabla 4**) pueden fecundarse con su propio polen, por lo que no necesitan la presencia de árboles de otras variedades polinizadoras. Además, el polen de estas variedades es compatible con cualquier otra variedad, por lo que se pueden utilizar como polinizadoras de cualquier variedad autoincompatible siempre que coincidan en floración.

Para determinar la incidencia de las variedades autoincompatibles en el cultivo de albaricoquero en la actualidad, en las **Tablas 1 y 2** se han agrupado por separado las variedades autocompatibles y las variedades autoincompatibles. Los datos de superficie asegurada muestran que más de 29% de la superficie cultivada en España está ocupada por variedades

Es necesario conocer las necesidades de polinización de cada variedad para evitar problemas de producción asociados a la falta de polinización

autoincompatibles, en su mayoría de nuevas variedades registradas en los últimos años. Es previsible que esta situación continúe en los próximos años debido a la utilización de variedades autoincompatibles en las nuevas plantaciones comerciales que todavía no han entrado en producción y al descenso de la importancia de las variedades tradicionales, que son en su mayoría autocompatibles. Estos datos resaltan la importancia de determinar el carácter de auto(in)compatibilidad de las nuevas obtenciones y actualizar las relaciones de intercompatibilidad para evitar problemas de producción asociados a la falta de polen compatible en las plantaciones.

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Bibliografía

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