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Evaluación agronómica y estudio de la calidad del fruto en melocotonero [*Prunus persica* (L.) Batsch]. Variabilidad y genética de asociación

Memoria presentada por Carolina Font Forcada, Ingeniero Agrónomo, para optar al grado de Doctor por la Universidad de Lleida.

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CERTIFICAN

Que la Tesis Doctoral titulada "**Evaluación agronómica y estudio de la calidad del fruto en melocotonero [*Prunus persica* (L.) Batsch]. Variabilidad y genética de asociación**", ha sido realizada por la Ingeniero Agrónomo **Carolina Font Forcada**, en el Departamento de Pomología de la Estación Experimental de Aula Dei del Consejo Superior de Investigaciones Científicas bajo su dirección y reúne, a su juicio, las condiciones requeridas para optar al Grado de Doctor.

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RESUMEN

El melocotonero [*Prunus persica* (L.) Batsch] es la especie frutal de hueso más importante en España y la que aporta la mayor producción dentro del conjunto de todas las especies de hueso y de pepita. Actualmente, España es el tercer productor a nivel mundial y el segundo a nivel europeo. En los últimos 15 años la producción española se ha duplicado, como consecuencia de la introducción de nuevas variedades y patrones, y de la mejora tecnológica. Sin embargo, los problemas de calidad del fruto obligan a seleccionar nuevas variedades con mayor calidad organoléptica y mejor adaptadas a las condiciones de cultivo mediterráneas.

El presente estudio tiene como objetivo general la caracterización morfológica, agronómica, bioquímica y molecular de variedades de melocotonero y nectarina de la colección de germoplasma existente en la Estación Experimental de Aula Dei (EEAD-CSIC), con el fin de profundizar en el conocimiento de los factores que se asocian con el control genético de la calidad organoléptica del fruto. Además, se pretende determinar la influencia de diversos patrones *Prunus* sobre los parámetros de calidad del fruto de variedades de melocotonero y nectarina.

Se ha evaluado el comportamiento agronómico y la calidad organoléptica y nutricional del fruto de 94 variedades de melocotón y nectarina, que incluyen 43 autóctonas españolas y 51 extranjeras en su mayoría procedentes de EE.UU. Este estudio mostró una gran variabilidad fenotípica para los caracteres agronómicos y pomológicos, así como sobre los parámetros básicos de calidad y compuestos nutricionales del fruto. Esta variabilidad podrá ser utilizada en los programas de mejora destinados a la creación de nuevas variedades.

Además, se han realizado análisis de genética de asociación utilizando marcadores moleculares del tipo SSRs y SNPs y los caracteres pomológicos y de calidad del fruto de las 94 variedades de melocotón y nectarina. Con la utilización de los 40 SSRs seleccionados se realizó el análisis del desequilibrio de ligamiento mostrando su máximo nivel a los 20cM. También se realizó el estudio de la estructura poblacional mostrando dos grupos principales: el de las variedades modernas y el de las locales. Los análisis de genética de asociación mostraron un total de 55 asociaciones entre los 40 SSRs y los caracteres pomológicos evaluados, de los cuales se seleccionaron, por su posición en el grupo de ligamiento (GL) 4, los marcadores BPPCT015, CPPCT028 y endoPG1 asociados con la fecha de cosecha, firmeza, fenoles, flavonoides, capacidad

antioxidante relativa (RAC), sorbitol y azúcares totales. Por otra parte, con 3.185 SNPs se obtuvieron un total de 347 asociaciones con los diferentes parámetros pomológicos evaluados. Entre ellas, hay que destacar las obtenidas con la fecha de floración y de cosecha, índice de madurez, antocianinas, flavonoides, RAC, sorbitol y azúcares totales. Estos resultados serán de interés para asociar estos marcadores a regiones del genoma más conocidas en el mapa de referencia de *Prunus* y que controlan caracteres organolépticos de interés en melocotonero.

La influencia de diferentes patrones *Prunus* con distinta base genética (*P. amygdalus* x *P. persica* y *P. davidiana* x *P. persica*, y los ciruelos *P. insititia* y *P. domestica*) fue evaluada. Se observó que los híbridos Adarcias y Cadaman, así como los ciruelos Pollizo de Murcia (*P. insititia*) Adesoto y PM 105 AD, presentaban en general un buen comportamiento desde el punto de vista agronómico (vigor, producción y supervivencia de los árboles) y una mayor calidad del fruto (firmeza del fruto, contenido en sólidos solubles, azúcares y compuestos antioxidantes). El menor vigor de estos patrones y/o su mejor adaptación a las condiciones de cultivo podría explicar su influencia positiva sobre la calidad del fruto. Estos resultados apoyan su interés como patrones para melocotonero a nivel comercial, dado el creciente interés hacia productos de mayor calidad en la nutrición humana.

Además de las características agronómicas, morfológicas y de calidad básicas, la composición química del fruto relacionada con factores nutricionales puede aportar un valor añadido en las nuevas variedades. Igualmente, las nuevas técnicas moleculares de asociación genética permitirán identificar y localizar genes asociados a los parámetros agronómicos y nutricionales de calidad del fruto.

ABSTRACT

Peach [*Prunus persica* (L.) Batsch] is the most important temperate fruit tree grown in Spain making a major contribution to total stone and pome fruit production. Currently, Spain is the third largest producer in the world and the second within the European Union. During the last 15 years, Spanish production has doubled as a result of the introduction of new varieties and rootstocks, in combination with new technologies. However, new varieties with improved fruit quality and better adaptation to Mediterranean growing conditions are still required.

This study characterizes morphological, agronomical, biochemical and molecular aspects of the peach and nectarine germplasm collection established at the Estación Experimental de Aula Dei (EEAD-CSIC) in order to gain a better understanding of the genetic control of critical traits contributing to fruit quality. A related objective is the evaluation of the effect of different rootstocks on fruit quality for these diverse peach and nectarine cultivars.

The agronomic, organoleptic and nutritional performance of 94 peach and nectarine cultivars from the EEAD collection were evaluated. This germplasm included 43 native and local Spanish cultivars and 51 modern cultivars primarily from the EE.UU. Results identified considerable variation for agronomic and pomological characteristics, and also for fruit quality and phytochemical composition. This extensive variability could be the basis for breeding programs developing new cultivars.

Marker-trait association analyses were also performed using SSRs and SNPs, and the pomological and fruit quality data from the 94 peach and nectarine cultivars. An analysis of linkage disequilibrium (LD) using a set of 40 SSRs revealed a high level (up to 20 cM) of LD. In addition, a population structure analysis identified two subpopulations: the local and the modern cultivars. A total of 55 significant associations were found between 40 SSRs and fruit and tree traits. Markers BPPCT015, CPPCT028 and endoPG1, positioned in linkage group (LG) 4 were associated with harvest date, firmness, phenols, flavonoids, relative antioxidant capacity (RAC), sorbitol and total sugars. A total of 347 significant associations were obtained with the different traits and 3.185 SNPs. These include associations with blooming and harvest date, ripening index, anthocyanins, flavonoids, RAC, sorbitol and total sugars. Results will be useful for identifying markers, using the well established *Prunus* reference map, for improving selection efficiency for important quality traits.

Prunus rootstocks with diverse genetic background (*P. amygdalus* x *P. persica* and *P. davidiana* x *P. persica*, and the plums *P. insititia* and *P. domestica*) were also evaluated for effect on peach characteristics. Results indicate that the peach-hybrids Adarcias and Cadaman, as well as the plums Pollizo de Murcia (*P. insititia*) Adesoto and PM 105 AD had better overall tree performance (vigour, yield and tree survival) and higher fruit quality (fruit firmness, soluble solids content, sugars and antioxidant compounds). The lower vigour of these rootstocks and/or their better adaptation to the growing conditions appeared to promote higher fruit quality. These results can be immediately applied to the improvement of fruit quality and nutritional value in commercial production through appropriate rootstock use.

The resulting detailed chemical composition data for this large and comprehensive collection of current commercial cultivars will similarly allow the immediate improvement of consumer health through appropriate cultivar selection. Association mapping techniques developed in this project will be very useful for detecting further marker-trait association using this comprehensive data.

RESUM

El presseguer [*Prunus persica* (L.) Batsch] és l'espècie fruitera d'os més important a Espanya, i la qual aporta la major producció dins del conjunt de totes les espècies d'os i de llavor. Actualment, Espanya és el tercer productor mundial i el segon europeu. En els darrers 15 anys la producció espanyola s'ha duplicat, com a conseqüència de la introducció de noves varietats i patrons, així com de la millora tecnològica. No obstant això, els problemes de qualitat del fruit obliguen a seleccionar noves varietats amb major qualitat organolèptica i millor adaptades a les condicions de conreu mediterrànies.

Aquest estudi té com a objectiu general la caracterització morfològica, agronòmica, bioquímica i molecular de varietats de presseguer, incloses nectarines, de la col·lecció de germoplasma existent a l'Estación Experimental de Aula Dei (EEAD-CSIC), amb la finalitat d'aprofundir en el coneixement dels factors que s'associen amb el control genètic de la qualitat organolèptica del fruit. A més a més, es pretén de determinar la influència de diversos patrons *Prunus* sobre els paràmetres de qualitat del fruit de varietats de presseguer, inclosa la nectarina.

S'ha avaluat el comportament agronòmic i la qualitat organolèptica i nutricional del fruit de 94 varietats, repartides entre 43 varietats autòctones espanyoles i 51 estrangeres majoritàriament procedents de EE.UU. Aquest estudi va mostrar una gran variabilitat fenotípica per als caràcters agronòmics i pomològics, així com per als paràmetres bàsics de qualitat i compostos nutricionals del fruit. Aquesta variabilitat es pot utilitzar en els programes de millora destinats a la creació de noves varietats.

A més, s'han realitzat anàlisis de genètica d'associació utilitzant marcadors moleculars del tipus SSRs i SNPs i els caràcters pomològics i de qualitat del fruit d'aquestes 94 varietats. Amb la utilització dels 40 SSRs seleccionats es va realitzar l'anàlisi del desequilibri de lligament mostrant el seu màxim nivell als 20 cM. També, es va realitzar l'estudi de l'estructura poblacional mostrant dos grups principals: el de les varietats modernes i el de les locals. Les anàlisis de genètica d'associació van mostrar un total de 55 associacions entre els 40 SSRs i els caràcters pomològics avaluats, dels quals es van seleccionar, per la seva posició en el grup de lligament (GL) 4, els marcadors BPPCT015, CPPCT028 i endoPG1, associats amb la data de collita, fermesa, fenols, flavonoides, capacitat antioxidant relativa (RAC), sorbitol i sucres totals. D'altra banda, amb 3.185 SNPs es van obtenir un total de 347 associacions entre els diferents

paràmetres pomològics avaluats. Entre aquestes associacions, cal destacar les obtingudes amb les dates de floració i de collita, l'índex de maduresa, el contingut en antocianines, flavonoides, RAC, sorbitol i sucres totals. Aquests resultats seran d'interès per a associar aquests marcadors a les regions del genoma més conegudes al mapa de referència de *Prunus* i que controlen caràcters organolèptics d'interès en presseguer.

A més, es va estudiar la influència de diferents patrons *Prunus* amb distinta base genètica (*P. amygdalus* x *P. persica* i *P. davidiana* x *P. Persica*, i les prunes *P. Insititia* i *P. domestica*) com a patrons per al presseguer. Es va deduir que els patrons híbrids Adarcias i Cadaman, i les pruneres Pollizo de Múrcia (*P. insititia*) 'Adesoto' i PM 105 AD, van presentar en general, un bon comportament des del punt de vista agronòmic (vigor, producció i supervivència dels arbres) i major qualitat del fruit (fermesa del fruit, contingut en sòlids solubles, sucres solubles i compostos antioxidants). El menor vigor d'alguns d'aquests patrons o la seva millor adaptació a les condicions de conreu podria explicar la seva influència positiva sobre la qualitat del fruit. Aquests resultats recolzen el seu interès com a patrons per a presseguer a nivell comercial, donat el creixent interès cap a productes de més qualitat en la nutrició humana.

A més de les característiques agronòmiques, morfològiques i de qualitat bàsiques, la composició química del fruit relacionada amb factors nutricionals pot aportar un valor afegit a les noves varietats. A més, les noves tècniques moleculars d'associació genètica permetran d'identificar i localitzar gens associats als paràmetres agronòmics i nutricionals de qualitat del fruit.

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Abreviaturas

ANOVA	análisis de varianza
DL	desequilibrio de ligamiento
endopG	endopoligalacturonasa
GL	grupo de ligamiento
ha	hectárea
N	newton
NS	no significativo
PF	peso fresco
SAM	selección asistida por marcadores
SS	sólidos solubles

Abbreviations

AFLP	amplified fragment length polimorphism
AsA	ascorbic acid
bp	base pair
C3GE	cyanidin-3-glucoside equivalents
cM	centiMorgan
DNA	desoxirribonucleic acid
dNTP	desoxiribonucleotide
DPPH	2, 2-dipyridyl-1, 1-diphenyl-2-picrylhydrazyl
endopG	endopolygalacturonase
FF	flesh firmness
FW	fresh weight
GAE	gallic acid equivalents
HPLC	high-performance liquid chromatography
IPGI	international peach genome initiative
LD	linkage disequilibrium
LG	linkage group
MAS	marker assisted selection
Mbp	million of base pair
MS	mean square
MSE	mean square error
N	newton
NS	not significant
PCA	principal component analysis
PCR	polymerase chain reaction
Popgene	population genetic analysis
QTLs	quantitative trait loci
RAC	relative antioxidant capacity
RI	ripening index
SE	standard error
SSC	soluble solids content
SSR	simple sequence repeats
SNP	single nucleotide polymorphism
TA	titratable acidity
TCSA	trunk cross-sectional area
UPGMA	un-weighted pair group method average

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Capítulo 1

Introducción general

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El melocotonero es la especie frutal de hueso más cultivada en España (73.100 ha), por detrás del almendro (542.000 ha) y por delante del cerezo (24.000 ha) (FAOSTAT, 2012). Además, con una producción de 1,13 millones de t en 2010, presenta mayor producción que el conjunto de los restantes frutales de hueso y que los frutales de pepita, manzano y peral. Aunque es una especie de origen asiático, el melocotonero se ha adaptado bien al clima seco y templado del área mediterránea, siendo este área una de las máximas productoras a nivel mundial. En España, la producción de melocotonero ha aumentado considerablemente, como consecuencia de la utilización de nuevos patrones y variedades, así como de su adaptación al clima seco y caluroso que caracteriza la mayor parte de las zonas frutícolas de España, en particular las del Valle del Ebro. Además de la innovación varietal, la mejora tecnológica ha propiciado un incremento progresivo en la producción permitiendo un notable aumento y diversificación de la oferta. La disponibilidad de nuevas variedades supone además una innovación constante en tipologías de fruto y fechas de recolección (Byrne et al., 2012). Ello, unido a los menores costes de producción con respecto a otros frutales, ha proporcionado a esta especie una mayor competitividad, que se ha traducido en un incremento progresivo de las exportaciones. Así, España pasó a ser el primer país exportador a nivel mundial alcanzando las 574.000 t en 2010 (FAOSTAT, 2012).

La mayor innovación se ha dado en la mejora de la presentación de los frutos, en especial en lo referido a la coloración, calibre, forma y aptitud a las manipulaciones. En cuanto a la tipología del fruto, la nectarina es el grupo más importante con el 38% de la producción, seguida por el melocotón amarillo (34%) y el melocotón rojo, que junto al de fruto achatado o paraguayo representan el 28%. En el caso de la nectarina, el 76% corresponde a variedades de carne amarilla y el 24% a variedades de carne blanca, mientras que para el melocotón estos porcentajes son del 86% y del 14% para la carne amarilla y blanca, respectivamente (Iglesias y Casals, 2011).

Los programas de mejora genética en el melocotonero tienen como objetivo la obtención de variedades más productivas, mejor adaptadas a las condiciones de cultivo y con elevada calidad organoléptica y sensorial del fruto (Byrne et al., 2012). Por otra parte, se ha demostrado que los componentes nutricionales de las frutas confieren propiedades antioxidantes en la dieta humana y previenen la aparición de enfermedades cardiovasculares y cancerigenas (Prior y Cao, 2000). Por ello, resulta de interés la obtención de nuevas variedades con un mayor contenido en dichos compuestos

nutricionales. Para lograr dichos objetivos es imprescindible disponer de variabilidad genética que contenga los caracteres deseados. Actualmente, se están incorporando nuevas técnicas moleculares en los programas de mejora genética, lo que probablemente va a facilitar el proceso de selección de los genotipos que posean los genes de interés.

1.1. TAXONOMÍA, ORIGEN Y EVOLUCIÓN DEL MELOCOTONERO

El melocotonero [*Prunus persica* (L.) Batsch] pertenece al género *Prunus*, de la familia de las Rosáceas y subfamilia *Prunoideae*. Otras especies de gran importancia económica en el género *Prunus* son el albaricoquero (*P. armeniaca* L.), el almendro (*P. dulcis* Mill), el cerezo (*P. avium* L.), y el ciruelo europeo (*P. domestica* L.) y japonés (*P. salicina* L.). Además de la especie *P. persica, e*xisten otras cuatro especies distintas de melocotonero, *P. davidiana* (Carr.) Franch., *P. mira* Koehne, *P. kansuensis* Rehd. y *P. ferganensis*, pero solo la especie *P. persica* se cultiva por su fruta, con la excepción de usos locales de *P. ferganensis* y *P. mira* (Hancock et al., 2008). Algunas de estas especies se utilizan también como patrones para melocotonero (Byrne et al., 2012). Una clasificación botánica de las especies mencionadas puede verse resumida en la tabla 1.1 (Rehder, 1940).

El melocotonero es una especie diploide con un número de cromosomas 2n=2x=16, que cuenta con un genoma relativamente pequeño de 230 Mbp (Arús et al., 2012), apenas el doble del tamaño del genoma de *Arabidopsis*. Por ello, y por su proximidad taxonómica con otras especies frutales de gran importancia económica y su corto periodo de juvenilidad, el melocotonero se ha convertido en una planta modelo para la investigación en la familia de las Rosáceas (Sosinski et al., 2000).

Se tienen referencias del cultivo del melocotonero a principios del siglo X a.C. en las regiones montañosas de Asia Central y Occidental. En su origen, los árboles producían frutos pequeños y amargos, aunque los agricultores fueron seleccionando las variedades de fruta más grande, jugosa y dulce. El melocotonero se extendió hacia el Mediterráneo a través de las rutas comerciales de Persia, de donde toma su nombre científico *P. persica* (Hedrick, 1917). Posteriormente, en el siglo I a.C., el emperador romano Pompeyo lo introdujo en Roma, desde donde se dispersó rápidamente a través de Europa occidental. Finalmente, durante el siglo XVI los colonizadores españoles y portugueses introdujeron el cultivo en América (Byrne et al., 2012; Hedrick, 1917).

Tabla 1.1. Clasificación botánica según Rehder (1940).

Género	Subgénero	Sección	Especies
Prunus	*Amygdalus* (L.) Benth Hook.	*Euamygdalus*	*P. davidiana, P. dulcis, P. ferganensis, P. kansuensis, P. persica...*
	Prunus = Prunophora (Neck.) Focke	*Euprunus*	*P. cerasifera, P. domestica, P. insititia, P. salicina…*
		Prunocerasus	*P. americana, P. hortelana, P. munsoniana…*
		Armeniaca	*P. armeniaca, P. brigantiaca, P. mandshurica…*
	Cerasus (Adans.) Focke	*Eucerasus*	*P. avium, P. cerasus…*
		Microcerasus	*P. besseyi…*
		Mahaleb	*P. mahaleb…*
	Padus (Moench) Koehne.		*P. padus…*
	Laurocerasus (Ser.) Rehd.		*P. lusitanica…*

Aunque el melocotonero fue introducido en España por los romanos, hasta principios del siglo XIX fue considerado como un cultivo marginal. La producción se inició en zonas donde la climatología era más adecuada y los suelos no demasiado desfavorables, ya que la propagación se hacia por semilla y la especie *P. persica* no tolera los suelos pesados y calizos. Posteriormente, su expansión en zonas con características edafológicas más limitantes, se produjo gracias a la utilización de patrones de distintas especies de *Prunus* adaptados a dichos suelos. Así, el grupo de patrones ciruelo permitió la expansión del cultivo en suelos más arcillosos, compactos o de peor drenaje, y la utilización de híbridos de melocotonero x almendro permitió el cultivo en suelos calizos, inductores de problemas de clorosis por deficiencia de hierro (Moreno, 2004). Desde el punto de vista varietal, la mayor presión de selección de los fruticultores y la propagación vegetativa mediante injerto, facilitó la consolidación de variedades-población autóctonas a principios del siglo XX (Badenes, 2000).

Las variedades modernas de melocotonero tienen una base genética reducida, debido al limitado número de genotipos utilizados como progenitores en los programas de mejora más importantes a nivel internacional. En consecuencia, la diversidad se ha reducido drásticamente por el uso de un número limitado de variedades modernas que comparten genitores comunes (Aranzana et al., 2003). Hacia mitad del siglo XX, en el sector productivo español predominaban los melocotones de color amarillo, con carne firme no fundente y de hueso adherido, provenientes principalmente de las poblaciones nativas adaptadas a las áreas de cultivo españolas y propagadas por semilla (Herrero,

1953). La sustitución de las variedades tradicionales en España por otras de carne fundente, en su mayoría procedentes de América del Norte, fue debida a la preferencia del mercado europeo por este tipo de melocotones (Badenes et al., 1998; Cambra, 1988) y a la tendencia de la producción española hacia la exportación a dicho mercado. Las variedades norteamericanas, se utilizaron extensivamente en los programas de mejora de EE.UU., pero también en Europa como variedades comerciales o como progenitores en programas de mejora (Bouhadida et al., 2011; Scorza et al., 1985).

1.2. IMPORTANCIA ECONÓMICA DEL CULTIVO

1.2.1. Interés económico

Actualmente, el melocotonero es la especie frutal con mayor número de variedades comercializadas, que proceden de los programas de selección y mejora sobre todo de EE.UU. El melocotonero es la tercera especie frutal de mayor producción a nivel mundial después del manzano y del peral (Arús et al., 2012). En los últimos 15 años, la producción de melocotonero se ha duplicado como consecuencia del uso de técnicas de cultivo más eficientes y de la introducción de nuevas variedades y patrones mejor adaptados (Llácer, 2005), pasando de 11,4 millones de toneladas en 1995 a más de 20,2 millones de toneladas en 2010 (Figura 1.1) (FAOSTAT, 2012). Entre otras zonas, este incremento ha sido más acusado en algunas áreas de inviernos suaves y con pocas horas de frío, como Chile, Argentina, Méjico, sur de Estados Unidos y sur de Italia (Byrne et al., 2012).

La superficie mundial total cultivada de melocotonero en 2010 estuvo en torno a 1,54 millones de ha (Figura 1.1). Solamente en China se alcanzó casi la mitad de esta superficie cultivada (731.100 ha), seguida de Italia (90.200 ha), España (73.100 ha) y EE.UU. (59.400 ha). Otros países con una superficie cultivada significativa a nivel mundial son Irán (45.100 ha), Méjico (41.600 ha) e India (39.100 ha) (FAOSTAT, 2012). A nivel de continentes, las principales áreas de producción se encuentran en Asia (China), con una producción total de 12,9 millones de t, sur de Europa (4 millones de t) y América (2 millones de t).

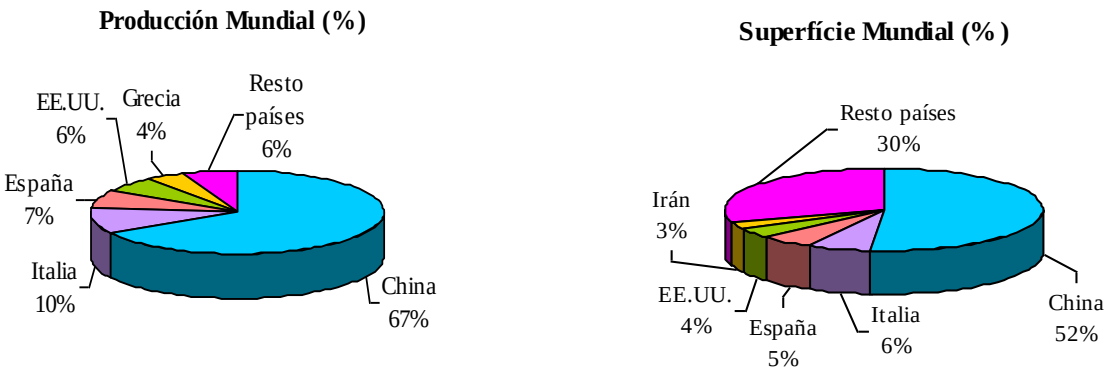

Figura 1.1. Distribución (%) de la producción y superficie mundial de melocotonero por países en 2010 (FAOSTAT, 2012).

Dentro de estas áreas, el máximo país productor es China, con una producción de 10,72 millones de toneladas en 2010, representando un 67% de la producción mundial (FAOSTAT, 2012). A continuación, le sigue Italia (1,59 millones de t), España (1,13 millones de t) y Estados Unidos (1,04 millones de t). Otros países con una producción significativa a nivel mundial son Grecia (639.400 t), Turquía (534.900 t), Irán (500.000 t) y Chile (357.000 t).

1.2.2. Producción nacional y superficie cultivada

Actualmente, España es el tercer productor de melocotonero a nivel mundial y el segundo de la unión Europea. Las diferencias de las condiciones climáticas entre las regiones productoras, con grandes variaciones en la disponibilidad de horas frío, dan lugar a importantes diferencias en las variedades cultivadas y en su época de maduración. Estas diferencias dan como resultado un amplio calendario de cosecha, desde finales de abril (Andalucía, Extremadura) hasta finales de octubre (Valle del Ebro). La producción de melocotonero se obtiene mayoritariamente en las regiones de clima templado seco y caluroso (Valle del Ebro y Región de Murcia) por la menor incidencia de enfermedades y heladas de primavera. Entre los frutales caducifolios, la producción de melocotonero es la más importante, seguida de la del manzano y del peral (Iglesias y Casals, 2011). La producción total de melocotonero y nectarina en España, de 1,13 millones de toneladas en 2010, supuso un incremento de casi el doble con respecto a la de 1995 (661.200 t), debido fundamentalmente a la renovación de las plantaciones, al aumento de la superficie en regadío y a la mejora en las técnicas de cultivo.

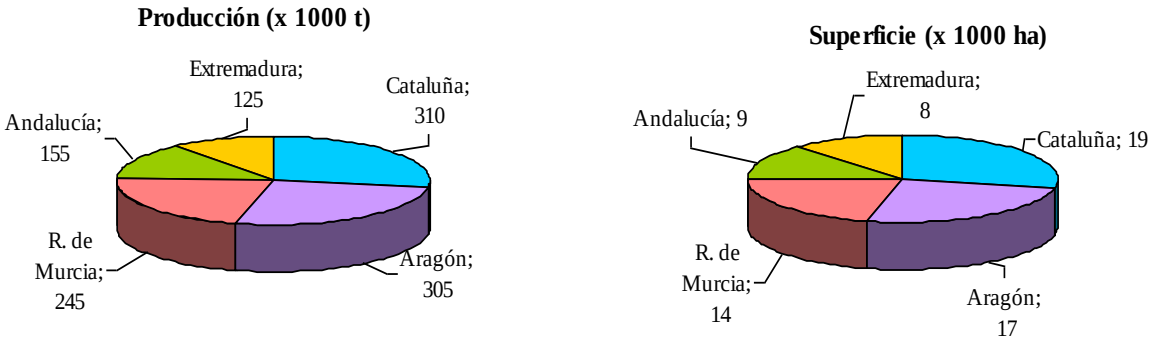

Figura 1.2. Producción y superficie cultivada en las comunidades autónoma s españolas en 2011 (MAGRAMA, 2012).

El cultivo del melocotonero se localiza principalmente en las regiones del arco mediterráneo, siendo las comunidades de Cataluña (405.900 t) y Aragón (370.500 t) las que presentan la mayor contribución a la producción española, seguidas por la Región de Murcia (241.800 t). En conjunto, representaron el 75% de la producción total española en el año 2011 (MAGRAMA, 2012). La superficie total de cultivo en España fue de 77.917 ha en 2011 (Figura 1.2), ocupando el segundo lugar en superficie cultivada por detrás del almendro, y por delante del manzano, del peral y del cerezo (MAGRAMA, 2012).

Desde 1999 hasta 2009 las exportaciones españolas, tanto intra como extracomunitarias, pasaron de 185.000 a 574.400 t (FAOSTAT, 2012), lo que supuso un incremento anual de más del 30%, convirtiendo a España en el primer exportador de Europa desde el año 2006. En 2010 dicha cantidad descendió ligeramente a 530.000 t por la menor producción extra-temprana y temprana principalmente. La cantidad media anual de melocotón exportado (incluidos los paraguayos) en el período 2004-2009 alcanzó las 215.000 t siendo Francia, Alemania, Italia y Portugal los principales países importadores. En el caso de la nectarina, las exportaciones en el período 2004-2009 alcanzaron las 269.000 t, superando por tanto a las del tipo de fruto melocotón. En este caso, los destinos más importantes fueron Francia, Alemania, Italia, Reino Unido y los Países Bajos. Hay que destacar también el incremento de las exportaciones ocurrido en 2010 hacia Hungría, Rusia y Finlandia, así como el retroceso de Italia, Reino Unido y Bélgica (MAGRAMA, 2012).

1.3. DIVERSIDAD GENÉTICA DEL MELOCOTONERO

1.3.1. Estructura varietal y programas de mejora

Debido a las diferentes mutaciones que se han ido originando a lo largo de la historia evolutiva de la especie *P. persica*, sus frutos se suelen diferenciar con distintos nombres según su forma, firmeza, color de la pulpa, adherencia al hueso y vellosidad de la piel. Estos nombres varían según países, y también según las distintas regiones en España (Cambra, 1984). Los melocotones (o duraznos) son los frutos de carne blanca o amarilla, de hueso libre o adherente, forma más o menos redondeada y piel vellosa. Las nectarinas se diferencian de los melocotones en que tienen la piel glabra (no vellosa) y los paraguayos se caracterizan fundamentalmente por su forma chata o aplanada y pueden ser de piel vellosa (tipo melocotón) o glabra (tipo nectarina). Entre las variedades de melocotón más cultivadas en España, están las de carne amarilla dura 'Sudanell', 'Miraflores', 'Tardíos de Calanda', 'Jerónimo', 'San Lorenzo' y 'Campiel', de origen español, y las de procedencia norteamericana, como 'Catherina', la serie 'Babygold', 'Andross' y 'Carson' (Llácer, 2005). Dentro de las variedades de melocotón de carne blanda, más producidas en España pero de origen americano, destacan 'Springcrest', 'Maycrest', grupo 'Florida', 'Royal Glory', 'Sunred', y 'Spring Lady'. Entre las nectarinas de carne amarilla más cultivadas están: 'Big Top', 'Fantasia' y 'Fairlane', y las de carne blanca: 'Snow Queen', 'Caldesi' y 'Flavor Giant', también de origen norteamericano. Finalmente, los paraguayos 'Sweet Cap', 'UFO 3' y 'UFO 4' son los predominantes (Llácer, 2005). En el Levante y en el sur de España, se tiende a la producción de variedades de carne blanda y/o nectarinas, más precoces y con menores necesidades en horas frío. Por el contrario, en el Valle del Ebro, hay una tendencia a la producción de variedades de carne dura y hueso adherente, tardías o de media estación y con mayores necesidades en horas frío (Llácer, 2005). A nivel internacional, por ejemplo en Asia, la preferencia del consumo se dirige a variedades sub-ácidas, con baja acidez, elevado contenido en azúcares y de carne blanda. Sin embargo, en Europa del Este se buscan variedades tolerantes al frío, y con mayores valores de acidez.

A partir de 1989 se observó una tendencia a la disminución del consumo del melocotón en España, probablemente debida a la falta de calidad de la fruta, por una excesiva recolección anticipada, por desconocimiento del momento óptimo de cosecha en variedades de epidermis muy roja y para evitar pérdidas en el proceso de

comercialización y envío a mercados más alejados (Iglesias et al., 2005). La mayor innovación en los últimos años se ha concentrado en la mejora de la calidad externa de los frutos, sobre todo en coloración y calibre. Sin embargo, muchas variedades ampliamente difundidas por la buena calidad de los frutos, tienen un manejo más difícil y una producción más irregular que la mayoría de las variedades cultivadas tradicionales, ya que son de vigor elevado y con una menor rusticidad. Las recolecciones muy anticipadas también han llevado a una gran pérdida de la calidad interna (sabor, aroma, textura y jugosidad) (Iglesias et al., 2005). Las variedades autóctonas tradicionales han sido sustituidas en parte por variedades de carne dura extranjeras, que ofrecen una mayor productividad, y por variedades de carne blanda y maduración precoz que se destinan principalmente a la exportación (Llácer, 2005). Además, en los últimos años se ha observado la tendencia hacia la producción de variedades sub-ácidas, principalmente en nectarinas de carne amarilla o blanca, como 'Big Bang', 'Big Top', 'Nectareine', 'Luciana', y 'Nectaperla', y en melocotón rojo como 'Royal Glory'.

Estas variedades tienen su origen en más de 70 programas de mejora existentes en el mundo, liderados por los EE.UU. de donde proceden el 50% de las nuevas variedades, seguido por algunos países europeos (principalmente Francia e Italia), y otros de Sudáfrica, Australia, China, Japón, Méjico y Brasil (Byrne et al., 2012). A finales de los años 80 y principios de los 90, se iniciaron en España varios programas de mejora genética del melocotonero, con el objetivo de evitar la dependencia de los programas extranjeros y conseguir variedades mejor adaptadas a las condiciones agroclimáticas de nuestras regiones productoras. Así, a finales de 2007 existían en España al menos 14 programas activos de mejora, distribuidos entre Andalucía, Aragón, Comunidad Valenciana, Cataluña, Murcia y Extremadura. Entre los principales objetivos de dichos programas cabe citar la adaptación climática y ampliación del calendario de comercialización, la independencia de las obtenciones extranjeras para evitar el pago de royaltis, el incremento de la calidad nutricional y sensorial, la resistencia a plagas y enfermedades y la disminución de los costes de producción. En Aragón y más concretamente en la Estación Experimental de Aula Dei-CSIC (Zaragoza) hay que destacar la selección de la variedad 'Sudanell' por el investigador M. Cambra (1979), dando lugar a tres clones, con maduración escalonada y que todavía se cultivan. Posteriormente, se seleccionaron otras variedades locales como 'Montaced'

y ‘Montejota’ por el Dr. M. Carrera del Centro de Investigación y Tecnología Agroalimentaria del Gobierno de Aragón, así como tres clones del melocotonero ‘Tardío de Calanda’ (‘Jesca’, ‘Calante’ y ‘Evaisa’) seleccionados por el investigador J.L. Espada del Centro de Técnicas Agrarias de la DGA (Moreno, 2005). Además, en la EEAD se inició el estudio y selección clonal de la variedad ‘Miraflores’, originaria de la comarca de Valdejalón (Zaragoza) (Bouhadida et al., 2007; Moreno y Casas, 2002). También se inició un programa de selección a partir de 15 familias de cruzamientos entre variedades comerciales como ‘Andross’, ‘Babygold 9’, ‘Big Top’, ‘Calante’, ‘Crown Princess’, ‘O’Henry’, ‘Orion’, ‘Red Top’, ‘Rich Lady’ y ‘Venus’. Según la diferente tipología del fruto, se evaluaron los caracteres agronómicos más importantes como fecha de cosecha y producción, y diversos parámetros de calidad del fruto y de postcosecha (Abidi et al., 2011; Cantín et al., 2009a, 2009b; Cantín et al., 2010a, 2010b) continuando actualmente los trabajos de selección. El consumidor exige cada vez más, la presencia de nuevos productos en el mercado que presenten una buena calidad organoléptica, dada la deficiencia de muchas variedades que fueron obtenidas en el pasado con criterios casi exclusivos de productividad, tamaño y aspecto externo del fruto. Para ello, se requiere la utilización de unos buenos progenitores en los programas de cruzamientos caracterizados por sus buenas propiedades organolépticas. En la figura 1.3, se puede observar una fotografía de la colección de melocotonero y nectarina existente en la EEAD, utilizada en la presente tesis doctoral.

Figura 1.3. Plantación de la colección de melocotonero y nectarina de la Estación Experimental de Aula Dei (CSIC) en Zaragoza, con los árboles en el momento de floración.

1.3.2. Influencia del patrón sobre la variedad injertada

Entre los problemas más frecuentes del cultivo del melocotonero en España cabe destacar la abundancia de suelos calizos con pH y caliza activa elevados, suelos compactos y pesados, la presencia de nematodos, así como los problemas de replantación en zonas tradicionales de cultivo. El patrón aporta la adaptación al tipo de suelo, eficiencia en la absorción de agua y nutrientes, resistencia a patógenos, etc., pero también influye significativamente sobre la calidad, aspecto y tamaño de la fruta (Albás et al., 2004; Caruso et al., 1996; Jiménez et al., 2011; Scalzo et al., 2005; Tsipouridis y Thomidis, 2005).

La importancia del patrón sobre el comportamiento vegetativo y productivo de la variedad injertada es bien conocida (Byrne, 1988). Uno de los principales factores sobre los que influye el patrón es el vigor del árbol (Font i Forcada et al., 2012a; Giorgi et al., 2005), ya que ejerce un mayor efecto en el tamaño del árbol de lo que lo hace la propia variedad (Wertheim y Webster, 2005). Según el vigor inducido, los patrones pueden clasificarse en distintas escalas de vigor, desde los enanizantes hasta los muy vigorosos (Wertheim y Webster, 2005). Otro de los factores agronómicos en los que el patrón influye significativamente es en la productividad del árbol. Numerosos estudios muestran que los patrones menos vigorosos suelen inducir una producción menor que los de vigores intermedios o los más vigorosos, pero su productividad puede ser mayor (Font i Forcada et al., 2012a; Moreno et al., 1995; Zarrouk et al., 2005). El patrón también puede modificar la fecha del inicio de floración por su efecto sobre las necesidades de horas frío de la variedad (Tabuenca y Gracia, 1971), por la tolerancia al frío de las flores (Durner, 1990) o por los cambios en la densidad de las yemas florales (Chun et al., 2002). Igualmente, determinados patrones son capaces de inducir una mayor precocidad en la maduración de los frutos (Felipe, 1989). Además, el patrón ejerce una gran influencia en la nutrición mineral de la planta (Moreno et al., 2001; Zarrouk et al., 2005). También se sabe la influencia que determinados patrones ejercen sobre los parámetros básicos de la calidad del fruto, como sólidos solubles, acidez, tamaño o firmeza (Albás et al., 2004; Font i Forcada et al., 2012a; Moreno et al., 2001). Sin embargo, es mucho más reducido el conocimiento sobre la influencia del patrón en la calidad bioquímica del fruto (azucares solubles, vitamina C, capacidad antioxidante o compuestos fenólicos) (Albás et al., 2004; Orazem et al., 2011a, 2011b; Remorini et al., 2008).

1.3.3. Patrones más utilizados para el melocotonero

Hay dos tipos básicos de patrones que pueden clasificarse en patrones francos y patrones clonales. Los primeros provienen de semillas de la misma especie que la variedad injertada (Howard, 1987). Sin embargo, en la actualidad, la propagación de los patrones frutales se hace generalmente de forma clonal (vegetativa), lo que asegura una uniformidad en el desarrollo vegetativo y en la producción del material utilizado (Felipe, 1989). Entre los distintos grupos mayoritarios para el cultivo del melocotonero, cabe citar los patrones francos de melocotonero, los patrones clonales de ciruelo y de híbridos interespecíficos, principalmente de melocotonero x almendro.

Entre ellos, los más utilizados para el cultivo del melocotonero en suelos calizos, son los híbridos almendro-melocotonero (*P. persica* x *P. dulcis*) ya que toleran la clorosis férrica (Socias i Company et al., 1995). El patrón GF 677 es el más utilizado en las condiciones edafoclimáticas mediterráneas por su buen comportamiento en suelos calizos (Socias i Company et al., 1995). Otros patrones también muy tolerantes a la clorosis férrica son Adafuel (Cambra, 1990), Adarcias (Moreno y Cambra, 1994) y los híbridos Felinem, Garnem y Monegro, estos últimos seleccionados además por su resistencia a nematodos (Felipe et al., 1997), pero más sensibles a la asfixia de raíces (Font i Forcada et al., 2012a; Zarrouk et al., 2005).

Figura 1.4. Parcela de un ensayo de patrones híbridos almendro x melocotonero, injertados con la variedad de melocotonero 'Tebana' y de nectarina 'Queen Giant' en la Estación Experimental de Aula Dei (CSIC) en Zaragoza. Árboles en fase de maduración del fruto.

os híbridos interespecíficos Barrier y Cadaman (*P. persica* x *P. davidiana*) también son resistentes a nematodos (Pinochet et al., 1999), pero más sensibles a la clorosis férrica en suelos calizos (Jiménez et al., 2008). Otros híbridos más recientemente seleccionados son los patrones Greenpac y Purplepac (Pinochet, 2009), de gran vigor y resistentes a nematodos. En la figura 1.4 se puede observar el ensayo de patrones híbridos almendro x melocotonero utilizado en la presente tesis doctoral.

Los patrones ciruelo más utilizados comprenden las especies *P. insititia* y *P. domestica*. Estos patrones son tolerantes a la asfixia de raíces provocada por los suelos pesados, compactos y con problemas de drenaje (Felipe et al., 1997). En España, el Pollizo de Murcia tiene gran relevancia además por su tolerancia a la clorosis férrica, a la asfixia y a la salinidad (Moreno, 2004), destacando especialmente el patrón Adesoto, muy utilizado por su resistencia a nematodos y buena compatibilidad con melocotonero y otras especies frutales de hueso (Moreno et al., 1995). Montizo y Monpol son otros dos Pollizos clonales (Felipe et al., 1997) con una sensibilidad media a la clorosis férrica. Finalmente, hay que destacar el patrón ciruelo Constantí 1, el cual ha mostrado un buen comportamiento en trabajos experimentales llevados a cabo en la EEAD-CSIC (Moreno, 2004).

Figura 1.5. Parcela de patrones ciruelo injertados con la variedad de melocotonero ‘Catherina’ en la Estación Experimental de Aula Dei (CSIC) en Zaragoza. Árboles en fase de floración.

Dentro de los patrones ciruelo es bien conocida la marcada incompatibilidad de melocotonero sobre los patrones Mirobolán *P. cerasifera* y Mariana (Moreno et al., 1993). Sin embargo, existen clones seleccionados con una mejor compatibilidad con melocotonero y que han sido utilizados en cruzamientos interespecíficos con melocotonero y almendro (Moreno, 2004; Pinochet, 2010). En la figura 1.5 se puede observar el ensayo de distintos patrones ciruelo utilizado en la presente tesis doctoral.

1.4. CALIDAD DEL FRUTO EN MELOCOTONERO

1.4.1. Concepto de calidad

A lo largo del tiempo, la calidad ha sido definida por distintos autores como «conjunto de características que diferencian las unidades individuales del producto y determinan el grado de aceptabilidad de estas unidades por el usuario o consumidor» (Kramer y Twigg, 1966) o como «la aptitud para el consumo». La palabra «calidad» proviene del latín ***qualitas***, que significa atributo, propiedad o naturaleza básica de un objeto. Sin embargo, en la actualidad y en sentido abstracto su significado es «grado de excelencia o superioridad» (Kader, 1985). Aceptando esta definición, se puede decir que un producto es de mejor calidad cuando es superior en uno o varios atributos que son valorados objetiva o subjetivamente.

En términos del servicio o satisfacción que produce a los consumidores, podríamos también definirla como el «grado de cumplimiento de un número de condiciones que determinan su aceptación por el consumidor». La calidad de un producto puede dividirse en cuatro componentes: calidad organoléptica, nutricional, post-cosecha y sanitaria. En este trabajo solamente se han abordado los componentes de la calidad organoléptica y nutricional del fruto. Los aspectos de tamaño, color, forma, firmeza, aroma, sabor, acidez o sólidos solubles, formarían parte de la calidad organoléptica, y los componentes del tipo: azúcares solubles, vitaminas, fenoles, flavonoides, antocianinas o capacidad antioxidante, se incluirían en la calidad nutricional.

1.4.2. Maduración del fruto y cosecha

Se pueden distinguir dos tipos de maduración en el melocotón: la madurez fisiológica y la comercial. La primera es el estado en el cual el fruto ha alcanzado un

estado de desarrollo suficiente para que después de la cosecha su calidad sea la mínima aceptable para el consumidor, es decir, que el fruto alcance su máxima calidad organoléptica y nutricional. La segunda es el momento óptimo de recolección para cada fruto y cultivar, y también es determinante para conseguir una buena calidad final para el consumidor. La madurez comercial influye en la calidad organoléptica, nutricional, post-cosecha y duración de la vida útil del fruto (Kader et al., 1982). Comúnmente guarda escasa relación con la madurez fisiológica y puede ocurrir en cualquier fase del desarrollo o envejecimiento. Si el melocotón se cosecha antes de que su estado fisiológico sea el adecuado, no podrá completar su evolución climatérica durante su conservación y su calidad será deficiente, el fruto perderá firmeza y no tendrá ni aroma ni sabor (Lill et al., 1989). Sin embargo, si se recolecta tardíamente, su vida útil se acortará y será más susceptible a los daños mecánicos y a desarrollar problemas de harinosidad. Por el comportamiento de su respiración y por la capacidad de desarrollar consistencia, jugosidad, aroma, color y sabor atractivos después de haber sido cosechado, el melocotón es catalogado como un fruto climatérico (Tromp et al., 2005). Entre los índices de madurez más utilizados para indicar el momento óptimo de cosecha están: los cronológicos, como la determinación de los días tras la plena floración; los físicos, como el color, tamaño, forma o firmeza; los químicos, como el contenido en sólidos solubles (SS), acidez o índice de maduración (relación SS/acidez), y los fisiológicos, como la actividad respiratoria (emisión de etileno) (Crisosto, 1994).

1.4.3. Percepción de la calidad

La mayoría de los programas de mejora van dirigidos a conseguir características específicas, mejorando la calidad del fruto en cuanto al aspecto y a las características organolépticas. Las variedades europeas se desarrollaron a partir de las introducciones iniciales que se realizaron de Asia Central y China adaptándose a los gustos del consumidor europeo, con frutos de gran calibre y más ácidos que los asiáticos (Byrne, 2003). No obstante, en los programas de mejora actuales se van introduciendo nuevos caracteres, que dependen también de las necesidades concretas de las zonas de cultivo y de las exigencias de los consumidores.

Hoy en día, el componente de la calidad de la fruta es uno de los factores principales en un programa de mejora. La calidad es una percepción compleja de muchos atributos que son evaluados simultáneamente en forma objetiva o subjetiva por

el consumidor. La noción de calidad es a menudo apreciada por el consumidor a través de la calidad gustativa, que se expresa y se mide por una serie de criterios que incluyen la firmeza, la concentración de sólidos solubles y la acidez. No obstante, hay otros componentes que juegan un papel importante en la calidad del fruto como son el carácter jugoso del fruto, el color, los aromas, etc. La calidad interna de los frutos depende sobre todo de la firmeza y del sabor. Una firmeza adecuada es esencial para el buen manejo y comercialización de los frutos (Crisosto et al., 2001). La falta de firmeza de algunas variedades obliga a recolectar los frutos demasiado verdes, para que resistan los procesos de manipulación y post-cosecha, con la consiguiente pérdida de sabor. Una firmeza mayor de las variedades permite recolectar los frutos más cerca de la madurez fisiológica, y por lo tanto, con una notable mejora de la calidad. El sabor de los frutos es más difícil de evaluar, ya que es una medida subjetiva y depende tanto de la cantidad de sólidos solubles presentes en el fruto, como de las cantidades relativas de los diferentes azúcares (sacarosa, glucosa, fructosa y sorbitol) y de la acidez (Colaric et al., 2005). Por otra parte, una de las líneas de la mejora actual es la obtención de frutas con un alto contenido en principios bioactivos beneficiosos para prevenir los procesos de envejecimiento celular, enfermedades cardiovasculares y los distintos tipos de cáncer (Prior y Cao, 2000).

En los últimos años, existe una preocupación generalizada hacia un mayor consumo de frutas y hortalizas, motivado fundamentalmente por un creciente interés por una dieta más equilibrada, con menor proporción de carbohidratos, grasas y aceites y con una mayor participación de la fibra dietaria, vitaminas y minerales. Además, existe una creciente demanda por una calidad superior, tanto externa como interna de los frutos. Los aspectos externos (presentación, apariencia, uniformidad, madurez, frescura) son los componentes principales de la decisión de compra. Sin embargo, la calidad interna (sabor, aroma, textura, valor nutritivo) está vinculada a aspectos generalmente no perceptibles aunque no menos importantes para los consumidores.

1.4.4. Calidad organoléptica

La *forma* es uno de los componentes más fácilmente perceptibles aunque, en general, no es un carácter decisivo de la calidad organoléptica, salvo que existan deformaciones o defectos morfológicos. No obstante, este parámetro tiene un impacto importante en la aceptación del consumidor y éxito en el mercado, ya que en general se

prefieren las formas globulares sin protuberancias ni irregularidades (Badenes et al., 2006). La ausencia de defectos, en general, es uno de los principales requisitos que se exige por parte de los consumidores e influye en la decisión de compra del fruto. El tamaño y la forma son características que se alcanzan durante el desarrollo del fruto, mientras que la coloración y los parámetros organolépticos se alcanzan durante la maduración. Además del color de la epidermis, cuando la sutura y los hombros de los melocotones están bien desarrollados se consideran maduros (Kader, 1999).

El *color* es otro de los principales parámetros para estimar el grado de madurez de un fruto, ya que es el aspecto externo más fácil de evaluar por el consumidor. Cuando el fruto cambia de color en el árbol, normalmente de verde a amarillo o a rojo, se puede decir que ha llegado la fecha de cosecha. Este parámetro tiene un impacto importante en la aceptación del consumidor y en su comercialización (Badenes et al., 2006). Para la medición del color, el método CIELAB es el más usado. Las tres coordenadas de color, L*, a* y b*, son representadas tri-dimensionalmente en el diagrama de Hunter. L* representa la luminosidad y sus valores oscilan de 0 (color negro) a 100 (color blanco). La coordenada a* representa la desviación hacia la claridad (a*>0 hacia el rojo y a*<0 hacia el verde). Finalmente, b* representa la desviación hacia el amarillo (b*>0) o hacia el azul (b*<0).

La *firmeza* de la pulpa es otro de los indicadores más utilizados para determinar que los melocotones han alcanzado el grado de maduración ideal para ser cosechados. Sin embargo, no es siempre el más adecuado ya que ésta varia según otros factores, como el tamaño del fruto, las condiciones climatológicas o las prácticas culturales (Crisosto, 1994). Existen métodos destructivos y no destructivos para medir la firmeza. Entre los destructivos, se utiliza la penetromía, y entre los no destructivos, los durómetros o la espectroscopía de reflectancia en el infrarrojo cercano (Bureau et al., 2009; Sudebi y Walsh, 2009). Las características de firmeza dividen las variedades de melocotón en dos tipos: de carne blanda (‘melting flesh’) y de carne dura (‘non-melting flesh’). Los frutos de carne dura tienen una mejor aptitud para el manejo y el transporte, y suelen utilizarse en la industria conservera, aunque en España, sur de Italia y algunos países latinoamericanos también son muy apreciados para su consumo en fresco (Byrne et al., 2012).

El *sabor* se expresa normalmente en términos de la combinación de principios dulces y ácidos, ya que es un indicador de la madurez y de la calidad gustativa. El

contenido de sólidos solubles (SS) es una buena estimación del contenido de azúcares totales y muchos frutos deben tener un contenido mínimo de SS para ser cosechados. La *acidez valorable* es otro de los componentes principales del sabor y del aroma de los frutos (Colaric et al., 2005). Los ácidos orgánicos (cítrico, málico, oxálico, tartárico, quínico) son un componente importante del sabor (Moing et al., 1998), y tienden a disminuir a medida que el fruto madura, por lo que la relación SS/acidez tiende a aumentar. El amargor o la astringencia dependen de otros componentes como los flavonoides, taninos y otros compuestos fenólicos. La relación sólidos solubles/acidez valorable se denomina *índice de madurez* y se usa frecuentemente en frutos como melocotón, manzana o cítricos. Resulta especialmente útil en frutas como el melocotón en las que el equilibrio dulce-ácido es clave para su aceptación (Ferrer et al., 2005). Sin embargo, la acidez no depende solamente de la variedad sino de otros factores precosecha, por lo que no suele usarse solo como índice de madurez (Lill et al., 1989).

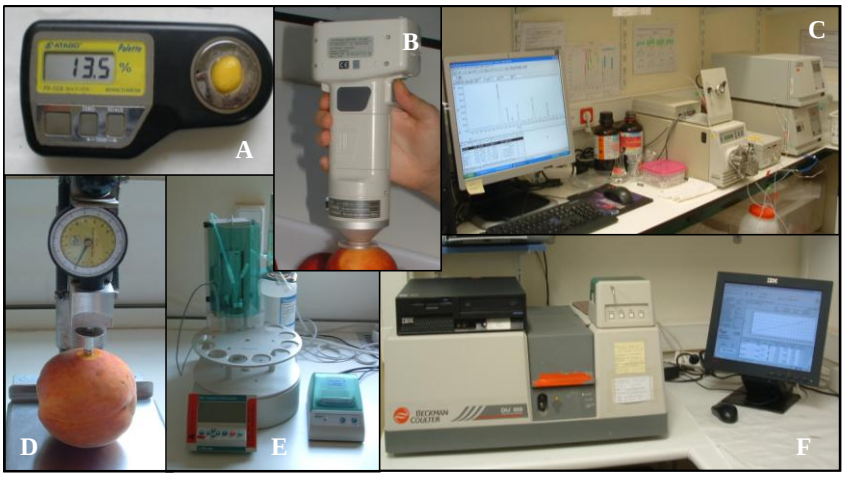

Figura 1.6. Aparatos utilizados para medir la calidad de los frutos de melocotón y nectarina: (A) refractómetro digital (sólidos solubles), (B) colorímetro MINOLTA (coordenadas L*, a* y b*), (C) HPLC (azúcares solubles), (D) penetrómetro (firmeza), (E) valorador automático (acidez valorable) y (F) espectrofotómetro (compuestos antioxidantes).

En este trabajo, se han tenido en cuenta varios índices para determinar la fecha de cosecha. Se ha considerado que el fruto estaba listo para cosechar cuando su tamaño deja de aumentar, el color de fondo vira de verde a amarillo o rojo, la firmeza disminuye (Crisosto, 1994) y el fruto se separa fácilmente del árbol (Crisosto et al., 1995). En la figura 1.6 se pueden observar algunos de los aparatos utilizados en esta tesis doctoral para determinar la calidad del fruto.

A fin de determinar la calidad organoléptica del fruto de una manera objetiva, se pueden llevar a cabo correlaciones entre atributos sensoriales y medidas de firmeza, color u otros parámetros nutricionales susceptibles de ser medidos (Colaric et al., 2005; Ruiz y Egea, 2008).

1.4.5. Calidad nutricional

Además de las características agronómicas y morfológicas de las variedades, la composición química del fruto, relacionada con factores nutricionales, se va introduciendo como un requisito cada vez más valorado en los programas de mejora genética. El creciente interés por los alimentos funcionales y nutraceúticos, ha convertido a las frutas y verduras en la gran expectativa de la nutrición humana (Prior y Cao, 2000). Desde el punto de vista nutritivo, las frutas poseen un alto contenido de agua y carbohidratos, proteínas y lípidos, y son, en general, una buena fuente de minerales y vitaminas (Knee, 2002). Las frutas son particularmente ricas en fitoquímicos como los terpenos, fenoles, lignanos y tioles, y las vitaminas A y C (Tabla 1.2). Entre los compuestos bioquímicos más importantes, se pueden destacar la vitamina C, la capacidad antioxidante o los componentes fenólicos. Los carbohidratos también juegan un papel importante en la calidad el fruto. Todos estos compuestos tienen gran importancia porque reducen el riesgo de determinadas enfermedades, debido a sus efectos beneficiosos en la prevención de enfermedades crónicas como las cardiovasculares o algunos tipos de cáncer. Son neutralizantes de los radicales libres, reducen el colesterol y la hipertensión y previenen la trombosis, entre otros efectos beneficiosos (Prior y Cao, 2000; Rice-Evans et al., 1997).

Otros compuestos beneficiosos son los *carbohidratos*. Además, influyen en la textura del fruto, ya que tienen un papel importante en el ablandamiento de la pulpa debido a la solubilización y pérdida de azúcares neutros de las cadenas laterales (Seymour y Gross, 1996). Conforme el fruto madura, el contenido en azúcares totales aumenta. Por otra parte, el contenido en sólidos solubles (SS) es una medida muy utilizada para la estimación del contenido en azúcares, debido a la correlación existente con el contenido en azúcares totales (Dirlewanger et al., 1999). Entre los azúcares, los más abundantes en el melocotón son la sacarosa, glucosa, fructosa y sorbitol. Mediante análisis por HPLC (Figura 1.7) se observa que el azúcar predominante es la sacarosa (~40%), seguido por la glucosa (~13%) y fructosa (~13%), y finalmente el sorbitol

(~10%) (Cantín et al., 2009a). Además, la fructosa tiene mayor capacidad endulzante que la sacarosa (1,75-1,8 veces más) (Doty, 1976). Se ha descrito una alta correlación entre la sacarosa y el sabor y aroma del melocotón, observándose mayor cantidad de este disacárido en aquellos frutos con más aroma (Colaric et al., 2005).

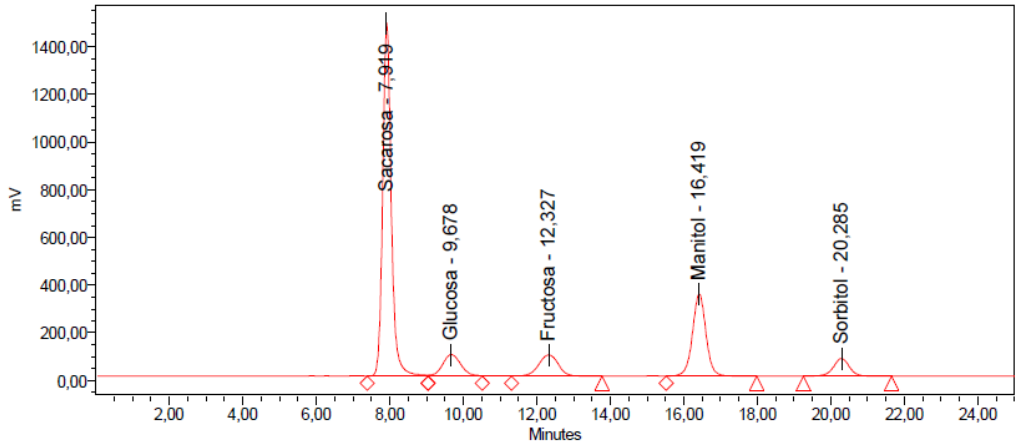

Figura 1.7. Perfil de azúcares de una muestra de pulpa de melocotón analizada por HPLC. El manitol es utilizado como un control interno.

También se ha demostrado que los azúcares mayoritarios presentes en el melocotón (sacarosa y glucosa) son importantes edulcorantes y fuentes de energía antioxidante en los sabores de la frutas (Moriguchi et al., 1990). Otros azúcares como la fructosa tienen efectos beneficiosos en la salud gastrointestinal (Muir et al., 2009) y el sorbitol juega un papel importante en la translocación de fotosintatos y puede usarse como sustituto de la glucosa en los pacientes con diabetes (Moriguchi et al., 1990). Todos estos compuestos dependen sobre todo del genotipo de la variedad cultivada (Cantín et al., 2009a) pero también pueden verse muy influidos por el patrón utilizado (Albás et al., 2004; Font i Forcada et al., 2012a; Orazem et al., 2011a, 2011b) y por las condiciones climáticas, especialmente la temperatura ambiental (Gil et al., 1995; Scalzo et al., 2005).

La acidez del fruto viene determinada por los *ácidos orgánicos*, que junto con los azúcares intervienen en la percepción del dulzor y del aroma del melocotón (Colaric et al., 2005). El ácido orgánico mayoritario en el melocotón es el málico, seguido del cítrico, quínico y succínico. La acidez alcanza un máximo durante el desarrollo del melocotón y luego disminuye con la maduración y con el periodo de cosecha del fruto.

Tabla 1.2. Composición nutricional del melocotón (Rodríguez et al., 1999).

Componente	Valor por 100g de porción comestible
Agua (g)	88,87
Energía (kcal)	39
Proteínas (g)	0,91
Grasa total (g)	0,25
Carbohidratos totales (g)	9,54
Fibra dietética (g)	1,5
Calcio (mg)	6
Hierro (mg)	0,25
Magnesio (mg)	9
Fósforo (mg)	20
Potasio (mg)	190
Sodio (mg)	0
Vitamina C (ácido ascórbico) (mg)	6,6
Niacina (mg)	0,806
Vitamina B6 (mg)	0,025
Ácido fólico (mg)	4
Vitamina E (alfa-tocopherol) (mg)	0,73

Los *antioxidantes* pueden anular los efectos perjudiciales de los radicales libres en las células. Con una dieta de frutas y verduras, ricas en polifenoles y antocianinas, disminuye el riesgo de contraer cáncer, enfermedades cardíacas y algunas enfermedades neurológicas (Stanner et al., 2004). También se ha sugerido que estos compuestos pudieran prevenir enfermedades tales como la degeneración macular, inmunidad suprimida debido a una nutrición pobre (Stanner et al., 2004), y la neurodegeneración, todas ellas causadas por el estrés oxidativo.

Los *compuestos fenólicos* juegan un papel importante en la calidad de los alimentos, además de contribuir al aroma o al sabor del fruto, y se ha descrito que el mayor contenido de fenoles en un fruto suele ir asociado con una mejora en su sabor (Kim et al., 2003) aunque también pueden tener un efecto negativo aumentando el daño por pardeamiento del fruto (Lurie y Crisosto, 2005). Muchos de los compuestos fenólicos han mostrado tener una importante actividad fisiológica en humanos como agentes antioxidantes y anticancerígenos (Kim et al., 2003). La concentración de fenoles, en general, varía con la maduración del fruto (Serrano et al., 2005).

La *vitamina C* (ácido ascórbico) es un importante micronutriente en la dieta humana y uno de los factores más importantes de la calidad nutricional de las frutas. Participa en la formación de colágeno y en la absorción del hierro, reduce el nivel de

colesterol, potencia el sistema inmunitario y tiene un papel importante como agente antioxidante (Proteggente et al., 2002).

1.5. CARACTERIZACIÓN MORFOLÓGICA, MOLECULAR Y ESTUDIOS DE ASOCIACIÓN EN EL MELOCOTONERO

1.5.1. Identificación morfológica del melocotonero

Tradicionalmente las variedades se han identificado por sus características morfológicas y pomológicas (Figuras 1.8 y 1.9) y/o agronómicas, siguiendo las directrices de los distintos organismos internacionales como IPGRI (*The International Plant Genetic Resources Institute*), UPOV (*International Union for the Protection of New Varieties of Plants*) y CPVO (*Community Plant Variety Office*). Dichas directrices incluyen observaciones sobre el vigor del árbol, la precocidad, la producción, la aptitud a la propagación vegetativa, la susceptibilidad a ciertas plagas y enfermedades, tamaño del fruto, prominencia y/o profundidad de la cavidad pistilar y peduncular, color de fondo, color de la chapa, firmeza de la pulpa, dulzor y acidez del fruto, adherencia del hueso a la carne, tipo de hueso, fecha de inicio, plena y final de floración, y época de madurez, entre otras, como se describe en el capítulo 3 de esta tesis doctoral. El procedimiento de caracterización debe de ser a la vez fiable, rápido y económicamente rentable.

Los caracteres morfológicos establecidos para la caracterización de especies del género *Prunus* están estandarizados principalmente por la UPOV a partir de una serie de características fenotípicas recogidas para la especie considerada (UPOV, 2010). La UPOV establece unas directrices para la realización de las observaciones necesarias para determinar las características de las variedades de cada especie.

Sin embargo, los distintos criterios utilizados por los mejoradores a la hora de valorar los diferentes caracteres, así como la influencia medioambiental, han dado lugar a muchas confusiones. Además, esta identificación requiere largos períodos de tiempo, con observaciones en períodos vegetativos y productivos de varios años. Por ello, la aplicación de los análisis moleculares de ADN, están siendo utilizadas, en combinación con las morfológicas, como una herramienta complementaria, tanto en los programas de mejora como en la identificación varietal.

Figura 1.8. Caracteres morfológicos y pomológicos del fruto en variedades de la colección de melocotonero y nectarina de la EEAD: color de piel y de pulpa (blanca/amarilla/naranja), adherencia del hueso (libre/adherido), tamaño y forma del fruto (pequeño/grande, redondo/alargado/ovalado).

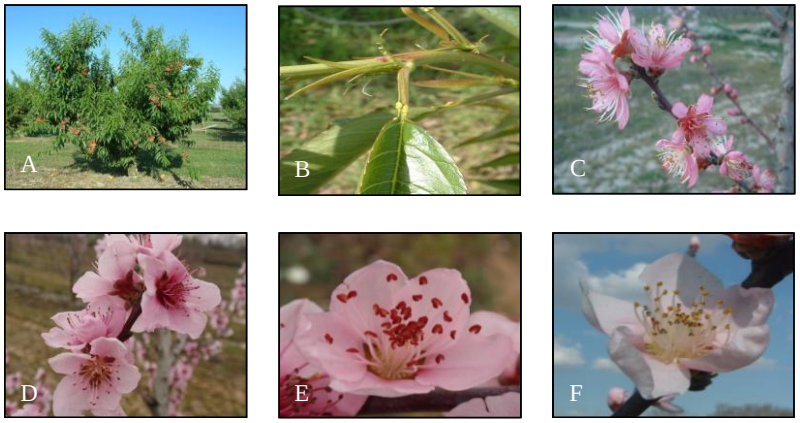

Figura 1.9. Caracteres morfológicos del árbol, de la hoja y de la flor en la colección de melocotonero y nectarina de la EEAD: árbol de porte erguido (A); glándulas reniformes de la hoja (B); floración del tipo campanulácea (C); floración del tipo rosácea (D); y colores de los pétalos, estambres y de las anteras (E, F).

1.5.2. Identificación molecular en melocotonero

La aplicación de la tecnología de marcadores moleculares en los programas de mejora y bancos de germoplasma es cada vez más utilizada para una caracterización más eficiente del material vegetal y del análisis de la diversidad. La caracterización genotípica complementa a la caracterización morfológica, permitiendo la identificación en ausencia de rasgos fenotípicos, en un estado temprano del desarrollo y en cualquier fase fenológica del árbol. Los diferentes marcadores moleculares desarrollados tienen

ventajas y desventajas y, dependiendo del objetivo a alcanzar, las características de cada uno de ellos han de ser tenidas en cuenta. Hoy en día, los parámetros agronómicos, morfológicos y moleculares se utilizan conjuntamente para conseguir una evaluación más completa de los recursos genéticos disponibles.

Los microsatélites (SSRs, *Simple Sequence Repeats*) han sido los marcadores más utilizados en los últimos años. Los SSRs son secuencias de ADN repetidas en tándem, de 1 a 6 nucleótidos. Son altamente polimórficos, multi-alélicos, codominantes y reproducibles entre laboratorios (Cipriani et al., 1999; Testolin et al., 2000). Su elevado nivel de polimorfismo los convierte en una técnica muy útil, sobre todo en especies con niveles bajos de variabilidad genética como es el caso del melocotonero. Los alelos amplificados pueden separarse mediante electroforesis en geles de agarosa, acrilamida o electroforesis con marcaje fluorescente. En la figura 1.10 se puede observar los perfiles de distintas muestras de melocotonero analizadas con SSRs mediante electroforesis capilar con marcaje fluorescente.

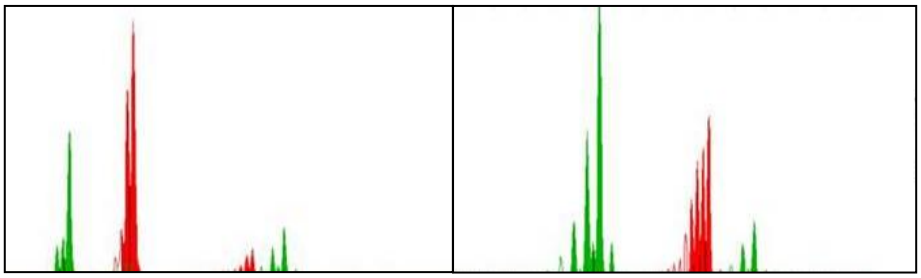

Figura 1.10. Diferentes perfiles de identificación varietal obtenidos con los SSRs.

Más recientemente, los polimorfismos en un único nucleótido (*Single Nucleotide Polymorphisms*, SNPs), están adquiriendo mayor protagonismo, debido a su menor tasa de mutación, abundancia en los genomas vegetales y a la robustez del análisis automatizable. Se basan en la sustitución de un solo nucleótido por otro en una región específica del material genético. En muchos casos, el polimorfismo puede resultar en un cambio de aminoácido, una cadena polipeptídica con características diferentes a la cadena original, y a su vez, presentará un posible cambio en el fenotipo (Vignal et al., 2002). Este hecho hace que sean unos excelentes marcadores para estudios de caracteres genéticos complejos y para algunos estudios de poblaciones (Jehan y Lakhanpaul, 2006). A pesar de ser una de las tecnologías económicamente más costosa, en los últimos años la identificación y diseño de SNPs se está viendo simplificada gracias a los

avances en secuenciación de genomas. La figura 1.11 muestra los diferentes materiales utilizados para la realización del genotipado mediante SNPs en esta tesis.

Figura 1.11. Chips utilizados para el genotipado mediante SNPs (A, B), plataforma de 'Illumina Infinium® BeadArray Technology' (C, D).

Tanto los SSR como SNPs desarrollados en melocotonero se han empleado para el estudio genético de esta especie, más concretamente para la construcción de mapas genéticos (Chágne et al., 2008; Fernández i Marti et al., 2012; Martínez-García et al., 2012; Wu et al., 2008), la identificación y localización de genes o caracteres de interés (Aranzana et al., 2003; Bouhadida et al., 2007, 2011; Downey e Iezzoni, 2000; Fan et al., 2010; Fernández i Marti et al., 2011; Font i Forcada et al., 2012c; Sosinski et al., 2000) o la genética de asociación (Flint-García et al., 2005; Font i Forcada et al., 2012b). Además de los estudios de melocotonero, estos marcadores se han utilizado en otras especies *Prunus* como almendro (Bliss et al., 2002; Fernández i Marti et al., 2009), albaricoquero (Hurtado et al., 2002), cerezo (Olmstead et al., 2008) y ciruelo (Bouhadida et al., 2009).

Una de las metodologías usadas en esta tesis ha sido el genotipado masivo de SNPs. Existen varias plataformas que han sido desarrolladas para el genotipado rápido de cientos de miles de estos marcadores. Esta técnica de alto rendimiento, llamada la plataforma de 'Illumina Infinium® BeadArray Technology' (Fan et al., 2006), permite la detección de hasta 2,5 millones de SNPs por muestra de ADN. El genotipado con múltiples SNPs permite una mayor rentabilidad en los estudios de selección asistida por marcadores o en los de asociación del genoma (GWAS, Genome-Wide Association Studies). Este método ha sido utilizado recientemente en melocotonero (Ahmad et al.,

2011; Martínez-García et al., 2012; Verde et al., 2012), en cerezo (Cabrera et al., 2012) y también en diferentes especies como el maíz (Yan et al., 2010) o la cebada (Rostoks et al., 2006).

En este trabajo se han utilizado estos dos tipos de marcadores moleculares, SSRs y SNPs, para estudiar la variabilidad molecular en la colección de melocotonero y nectarina de la EEAD y analizar la posibilidad de aplicar métodos de mapeo por asociación en esta especie.

1.5.3. Una nueva alternativa al mapeo convencional: el mapeo por asociación o desequilibrio de ligamiento (DL)

En el mapeo convencional, se estudia la segregación del gen en una población de individuos, descendientes de un cruzamiento conocido. Sin embargo, en frutales, trabajar con un número elevado de individuos supone una gran limitación, debido al tamaño del árbol y al periodo de juvenilidad característica de cada especie, que puede oscilar entre 3 y 7 años. A esto se añade el hecho de que cada población segrega para un número limitado de caracteres morfológicos o fenotípicos, por lo que la evaluación es siempre parcial. Aunque ha dado buenos resultados en la mejora genética vegetal, sus limitaciones han motivado la búsqueda de un método complementario para mapear genes.

Una alternativa a este tipo de mapeo se encuentra en la genética de asociación o desequilibrio de ligamiento (DL), basada en el ligamiento entre marcadores moleculares y los caracteres de interés en un conjunto diverso de germoplasma, individuos "no relacionados" entre sí y que no proceden de un cruzamiento dirigido (Rafalski, 2010; Zhu et al., 2008). Es decir, se trata de localizar mediante marcadores una región de cromosoma que se asocie al carácter de interés y así establecer una asociación entre el marcador (genotipo) y el fenotipo. En definitiva, el DL consiste en la asociación no aleatoria de alelos de dos o más loci presentes en individuos de una población. En el mapeo por asociación se utiliza como material vegetal variedades autóctonas y/o variedades comerciales ubicadas en bancos de germoplasma, frente al mapeo convencional que utiliza para el análisis de ligamiento cruces biparentales. El mapeo por asociación permitirá una mayor precisión en la localización de genes de interés, ya que el número de alelos en estudio es superior a los dos aportados en un cruzamiento biparental (Mackay y Powell, 2007).

En el caso del melocotonero, se ha mantenido un elevado grado de DL en comparación con otras especies hortícolas, debido sobre todo a la reproducción vegetativa predominante en las especies frutales y al inicio tardío de la mejora moderna de la especie (Scorza et al., 1985). Hay factores que influyen de manera positiva o negativa en el mantenimiento de este DL. Por ejemplo, los genes que se sitúan sobre el mismo cromosoma tienden a heredarse juntos. Sin embargo, con la recombinación esta tendencia desaparece debido a que aumenta la distancia entre loci. No obstante, si la recombinación es reducida, ésta será poco efectiva y no generará nueva variabilidad, por lo que se generarán haplotipos idénticos a los ya existentes. La tasa estimada de alogamia en el melocotonero es aproximadamente del 15% (Miller et al., 1989), siendo una especie autofértil y con elevados niveles de consanguinidad. Aparte de la recombinación, otros factores que favorecen la ruptura del desequilibrio de ligamiento son la mutación y la fecundación cruzada (Gupta et al., 2005). La selección afecta al DL, ya que si una mutación en un gen confiere una característica interesante a la planta y se produce una selección positiva a favor de un alelo, éste tiende a aumentar su frecuencia en la población. Así, el DL disminuirá y aumentará la distancia entre loci. Sin embargo, hay otros factores que favorecen que los alelos en desequilibrio se mantengan ligados y por lo tanto mantengan un alto DL. Estos factores son la autofecundación, la consanguinidad, el tamaño de la población, una baja recombinación y la estructura poblacional (Pritchard et al., 2000).

La estructura poblacional influye enormemente en el DL. Cuando las frecuencias alélicas del genoma se distribuyen en subpoblaciones decimos que la muestra está estructurada o que tiene estructura poblacional. Es decir, la probabilidad de encontrar un alelo en una población dependerá de la subpoblación muestreada. La compleja historia genética de muchos cultivos y el limitado flujo de genes en la mayoría de las especies silvestres han creado una estratificación compleja del germoplasma que podría complicar los estudios de asociación (Sharbel et al., 2000). Sin embargo, los estudios del análisis de la estructura de la población, las mezclas y los subgrupos del germoplasma ayudan a confiar en los estudios de genética de asociaciones (Ganopoulos et al., 2011; Mariette et al., 2010).

El objetivo fundamental de la genética de asociación es localizar mediante marcadores una región del genoma que se asocie al carácter de interés. La resolución genética en este tipo de estudios depende sobre todo de la tasa de recombinación de la

población estudiada, lo que se mide con los niveles de desequilibrio de ligamiento (DL) existentes y de la posible estructura poblacional. Por ello, antes de iniciar los estudios de asociación deben conocerse las características de la población de estudio, es decir, la variabilidad genética, la estructura poblacional y la extensión del DL. Para ello se pueden emplear diferentes métodos estadísticos. Uno de ellos es el programa estadístico *Structure*, el cual estudia la estructura poblacional (Q) presente en la colección de variedades y es un método de agrupación de cada individuo a partir de su frecuencia alélica. Otro programa es *Tassel*, que permite calcular el grado de DL en la población (Flint-Garcia et al., 2003) y al mismo tiempo las asociaciones significativas entre los marcadores y los fenotipos. A fin de corregir los posibles falsos positivos que se pudieran encontrar en estos estudios de asociación se tienen que realizar varias correcciones. Según Yu y Buckler (2006) se cree que las falsas asociaciones se pueden corregir utilizando los valores de Q generados por el programa *Structure* como covarianza para el estudio de asociación. También conviene aplicar el test de múltiple comparación de *Bonferroni* considerando significantes los marcadores con una $p<0.05$ (Schulze y McMahon, 2002) con el objetivo de eliminar estos falsos positivos. Además, los marcadores que contengan alelos de baja frecuencia, menor del 5%, deben ser eliminados por su tendencia a dar falsos positivos en los análisis de asociación.

Una de las metodologías propuestas para los análisis de asociación es el '*Genome Wide Association* (GWA)', el cual consiste en genotipar suficientes marcadores de un extremo a otro del genoma para que los alelos funcionales puedan estar en DL con al menos uno de los marcadores genotipados (Myles et al., 2009). Esta estrategia ha sido la utilizada en esta tesis doctoral como ya se ha mencionado en el apartado anterior.

Hasta la fecha, son pocos los trabajos publicados en genética de asociación en plantas y más concretamente en frutales. Entre las especies más estudiadas en ensayos de asociación, se encuentran el maíz (Remington et al., 2001), la cebada (Kraaman et al., 2004) y *Arabidopsis thaliana* (Hagenblad y Nordborg, 2002). En frutales, más concretamente en melocotonero, algunos de los trabajos publicados estudian el DL o la estructura poblacional, como es el caso del trabajo realizado por Aranzana et al. (2010) y Font i Forcada et al. (2012b). Además, Peace et al. (2005) identificaron el gen que controla la adherencia del hueso a la pulpa y la firmeza del fruto (F) y Eduardo et al. (2011) asociaron marcadores al tipo de floración, ambos trabajos también en

melocotonero. En cerezo, recientemente se publicaron los trabajos que estudian el DL o la estructura poblacional (Ganopoulos et al., 2011; Mariette et al., 2010), así como otros trabajos en la familia de las Rosáceas que estudian la identificación de marcadores ligados a caracteres pomológicos, concretamente en manzano (Cevik et al., 2010) y en peral (Oraguzie et al., 2010).

En estos últimos años, la genética de asociación se está convirtiendo en un campo de investigación muy activo, principalmente en humanos, donde se está aplicando para determinar genes relacionados con enfermedades, como por ejemplo los responsables de la enfermedad del Parkinson (Paisán-Ruiz et al., 2006). Por tanto, la aplicación más directa de encontrar una asociación entre el marcador o loci y el carácter de interés o fenotipo es la selección asistida por marcadores (SAM). De esta forma, la selección es independiente de la interacción fenotipo-ambiente, y hace posible la selección en estados muy precoces (Oraguzie et al., 2007).

Estos estudios han mostrado que el mapeo por asociación es una herramienta útil y válida para determinar las asociaciones entre un marcador y un fenotipo, detectando nuevos genes de interés agronómico y desarrollando nuevas técnicas para el estudio de la variabilidad genética en todo el genoma.

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Capítulo 2

Objetivos

El objetivo general de esta tesis consiste en la caracterización pomológica y molecular de variedades de melocotonero y nectarina de la colección existente en la Estación Experimental de Aula Dei (EEAD-CSIC). Se pretende profundizar en el conocimiento de los factores asociados con el control genético de la calidad organoléptica del fruto. Además, se pretende determinar la influencia de otros factores sobre la calidad del fruto, como el efecto de diferentes patrones *Prunus* con distinta base genética.

Los objetivos secundarios son:

1) Evaluación del comportamiento agronómico, caracterización morfológica, análisis de parámetros básicos de calidad y bioquímicos del fruto en 94 variedades de la colección de melocotonero **(Capítulo 3)**.
2) Estudio de la estructura poblacional, desequilibrio de ligamiento y mapeo por asociación en 94 variedades de la colección de melocotonero con el fin de identificar y localizar genes asociados a los parámetros agronómicos y organolépticos de calidad del fruto, mediante marcadores moleculares del tipo SSRs **(Capítulo 4).**
3) Aplicación de la técnica de mapeo por asociación o 'Genome Wide Association (GWA)' en la colección de melocotonero con el fin de identificar y localizar genes asociados a los parámetros agronómicos y organolépticos de calidad del fruto, mediante marcadores del tipo SNPs **(Capítulo 5).**
4) Influencia en el comportamiento agronómico y calidad del fruto de las variedades de nectarina 'Queen Giant' y de melocotonero 'Tebana' en un ensayo de patrones híbridos almendro x melocotonero y melocotonero x *P. davidiana* **(Capítulo 6)**.
5) Influencia en el comportamiento nutricional del fruto de las variedades de nectarina 'Queen Giant' y de melocotonero 'Tebana' en un ensayo de patrones híbridos almendro x melocotonero y melocotonero x *P. davidiana* **(Capítulo 7)**.
6) Influencia de siete patrones ciruelo (*P. insititia*, *P. domestica*) sobre el comportamiento agronómico, parámetros básicos de calidad y características nutricionales del fruto en la variedad de melocotonero 'Catherina' **(Capítulo 8)**.

Capítulo 3

Phenotypic diversity among local Spanish and modern peach and nectarine [*Prunus persica* (L.) Batsch] cultivars

3.1. ABSTRACT

Phenotypic data for tree and fruit characteristics was collected over three consecutive years from a diverse selection of 94 peach and nectarine cultivars. Genotypes were selected which represented both traditional Spanish as well as modern cultivars with widespread global plantings. All selections were grown at the 'Experimental Station of Aula Dei' (CSIC) located in the Ebro Valley (northern Spain, Zaragoza) under a Mediterranean climate.

Tree traits evaluated included bloom and harvest date, vigor, yield, yield efficiency and flower and leaf characteristics. Fruit traits included fresh weight, firmness, soluble solids concentration, titratable acidity and levels of individual soluble sugars (sucrose, glucose, fructose and sorbitol), vitamin C, total phenolics, flavonoids, anthocyanins, as well as relative antioxidant capacity and ripening index.

Extensive variability was observed for most qualitative and quantitative traits with significant correlations identified between many traits. While the traditional Spanish cultivars demonstrated good adaptability to the northern Spain evaluation site, opportunities for continued improvement in tree and fruit quality traits were demonstrated by an extensive phenotypic variability within the evaluation group.

Keywords: trait correlations, yield, fruit quality, sugars, antioxidant activity, vitamin C

3.2. INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is one of the most economically important fruit tree species worldwide. Within the economically important *Rosaceae,* it ranks behind only apples and pears. Peach is also the fruit species with the largest number of commercial varieties, representing a diverse international germplasm. In recent years, peach production has doubled due to the introduction of new varieties and rootstocks, along with more efficient growing and irrigation techniques. World production has increased from 11.4 million tons in 1995 to more than 20.2 million tons in 2010 (FAOSTAT, 2012). The largest producer is China, followed by Italy, Spain, and United States.

Many peach breeding programs are currently pursuing improved fruit quality and productivity for locally adapted cultivars (Byrne et al., 2012; Monet and Bassi, 2008). Initial breeding goals have been improvements in external fruit quality, postharvest life, and disease/pest resistance, as well as a greater range of fruit maturities and types (Byrne, 2005). More recently, increased fruit eating quality, including improved nutritional composition, has also been targeted. Early results indicate that important tree and fruit quality parameters may not be independent of each other (Abidi et al., 2011; Cantín et al., 2010; Font i Forcada et al., 2012a) as might be anticipated owing to their complex genetic control. Genetic control of traits affecting plant growth and architecture, yield, blooming and harvesting time are usually quantitative (Dirlewanger et al., 1999). Fruit size is reported to be a polygenic trait with a low to moderate heritability (Souza et al., 1998) because of the large influence that environmental conditions, plant nutrition, and cultural practices have on its expression. Potential fruit firmness is largely determined by the multi-allelic *F* locus (Lester et al., 1996). Both color and acidity levels in peach fruit are reportedly controlled by qualitative genes (Souza et al., 1998). Total SSC has a moderate heritability, which may be sufficient to allow steady improvement of fruit sugar levels in spite of the variations caused by environmental, maturity and production differences among regions and years (Cantín et al., 2009a).

More recently, the biochemical components of peach as well as several other fruits have received greater attention because of their potential health benefits (Prior and Cao, 2000). The major soluble sugars in peach are sucrose followed by glucose and

fructose, with lower contents of sorbitol (Brooks et al., 1993). In ripe fruit, these sugars comprise about 60% of the soluble solids concentration (SSC) (Cantín et al., 2009a). Glucose and fructose concentrations show a continuous increase during fruit development, while sucrose mostly accumulates during maturation (Hancock, 1999). Both sucrose and fructose have been shown to have beneficial effects on gastrointestinal health (Muir et al., 2009) while sorbitol can be used as a glucose substitute for diabetics (Forni et al., 1992). Fructose is perceived to be between 1.75-1.8 times sweeter than sucrose (Doty, 1976) while glucose is reported to be perceived as less sweet than sucrose (Yamaguchi et al., 1970). Because most previous breeding efforts targeted improve yield and vigor (Byrne et al., 2012), the fruit nutrient composition as well as variability among cultivars remains poorly understood.

Peach fruits are also a rich source for antioxidant compounds (Tomás-Barberán and Robins, 1997). The phenolic substances are a major source of potential antioxidants (Gil et al., 2002) and appear to be under strong genetic control (Gil et al., 2002; Cevallos-Casals et al., 2006). Phenolics have also been found to be natural antimicrobial agents for increasing the shelf life of fresh fruit while inhibiting the growth of pathogenic microorganisms (Bowles and Juneja, 1998). Flavonoids and anthocyanins also show strong antioxidant capacity (Wang et al., 1997). Antioxidant capacity to neutralize free radicals appears important for protection against certain diseases, such as heart or vascular diseases and cancer. While peach has a lower antioxidant capacity compared with other fruit types such as strawberry, kiwifruit, orange or apple, it is one of the few tree fruits available during spring and summer and so becomes an important contributer to human diets during this period (Besco et al., 2007).

The Spanish peach industry was traditionally based on non-melting, clingstone and yellow flesh peach cultivars. Recently, the supplementation of traditional varieties with internationally developed cultivars, has introduced the melting and freestone peach and nectarine types (Badenes et al., 1998). Unfortunately, peach diversity has been shown to be relatively low within the modern cultivars since most share a common ancestry (Aranzana et al., 2003).

In the present work, a diverse peach germplasm is evaluated, representing both traditional Spanish cultivars as well as more recent cultivars with a more global commercial importance. Tree and fruit quality characteristics for these 94 peach and

nectarine cultivars have been determined as have various associations between individual traits.

3.3. MATERIALS AND METHODS

3.3.1. Plant material and field trial

A collection of 94 peach and nectarine cultivars obtained from the peach germplasm collection at the 'Experimental Station of Aula Dei' (CSIC) have been evaluated (Table 3.1). This set included 43 native local Spanish cultivars and 51 modern cultivars mostly from the U.S. programs, but also from France, Italy, New Zealand and South Africa. All cultivars were budded on the 'Pollizo' plum rootstock 'Adesoto' (Moreno et al., 1995) and established in an experimental orchard (three trees per genotype). Most accessions are non-melting, clingstone and yellow flesh peach cultivars. Among the accessions, only 7 out of 94 cultivars were nectarines, 4 had white flesh, 10 had melting flesh and 5 were freestone. Harvest season ranges from June to October.

The orchard was located in the Ebro Valley (Northeast Spain, Zaragoza), and grown under a Mediterranean climate, on a heavy and calcareous soil, with 27% total calcium carbonate, 8% active lime, water pH 8.3, and a clay-loam texture. Trees were grown under standard conditions of fertilization, irrigation, pest and disease control, spring thinning and winter pruning. Trees were hand-thinned at 45–50 days after full bloom (DAFB) leaving approximately 20 cm between fruits. Open vase trees were pruned to strengthen existing scaffold branches and eliminate vigorous shoots, inside and outside the vase, that would compete with selected scaffolds or shade fruiting wood. The plot was level-basin irrigated every 12 days during the summer. Most vegetative and fruit quality traits have been evaluated over four consecutive years (2008-2011).

3.3.2. Pomological and fruit quality characterization

Blooming date was recorded for each cultivar according to Fleckinger (1945). The average date for bloom beginning (E stage), full bloom (F stage) and bloom end (G stage) was scored in each cultivar. The mean harvesting data was also calculated for each cultivar. Harvesting date ranged from late-June to late-October.

Table 3.1. Cultivar name, accession number, classification, origin and main fruit characteristics of the 94 cultivars studied.

Cultivar	Accession number	Cultivar classification	Origin	Harvest date (JD)	Fruit type	Flesh colour	Flesh type	Skin colour	Shape type	Stone type	Gland type	Bloom type
(1) Adriatica	3323 AD	Modern	Italy	188	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(2) Alcañiz 1	3097 AD	Local	Teruel, SP	274	Peach	Orange	Non-melting	Orange	Round	Clingstone	Reniform	Showy
(3) Alcañiz 2	3098 AD	Local	Teruel, SP	246	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Showy
(4) Alejandro Dumas	351 AD	Local	La Rioja, SP	245	Peach	Orange	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(5) Amarillo Calanda	131 AD	Local	Huesca, SP	256	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Reniform	Non-showy
(6) Amarillo Calanda	2400 AD	Local	Huesca, SP	266	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Reniform	Showy
(7) Amarillo Gallur	2361 AD	Local	Zaragoza, SP	244	Peach	Orange	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(8) Andora	2273 AD	Modern	USA	223	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(9) Andross	3253 AD	Modern	USA	213	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Globose	Non-showy
(10) Baby Gold 5	2562 AD	Modern	USA	205	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(11) Baby Gold 6	2563 AD	Modern	USA	203	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(12) Baby Gold 7	2564 AD	Modern	USA	210	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(13) Baby Gold 8	2565 AD	Modern	USA	205	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(14) Baby Gold 9	2566 AD	Modern	USA	203	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(15) Baladin	3209 AD	Modern	France	188	Peach	Orange	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(16) Benasque	3135 AD	Local	Huesca, SP	235	Peach	White	Melting	White	Ovate	Freestone	Reniform	Showy
(17) Big Top	3656 AD	Modern	USA	184	Nectarine	Yellow	Melting	Red	Round	Clingstone	Reniform	Showy
(18) Bonet I	2831 AD	Local	Lérida, SP	231	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(19) Bonet II	2832 AD	Local	Lérida, SP	232	Peach	Orange	Non-melting	Orange	Round	Clingstone	Reniform	Non-showy
(20) Bonet III	2833 AD	Local	Lérida, SP	261	Peach	Orange	Non-melting	Orange	Round	Clingstone	Reniform	Non-showy
(21) Bonet IV	2834 AD	Local	Lérida, SP	258	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(22) Bonet V	2835 AD	Local	Lérida, SP	272	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(23) Borracho de Jarque	3185 AD	Local	Zaragoza, SP	255	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(24) Brasileño	2184 AD	Local	Murcia, SP	193	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Non-showy
(25) Calabacero	2247 AD	Local	Murcia, SP	221	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Globose	Non-showy
(26) Calanda San Miguel	2383 AD	Local	Teruel, SP	251	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Reniform	Showy
(27) Calanda Tardío	1920 AD	Local	Teruel, SP	273	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Showy
(28) Campiel	3139 AD	Local	Huesca, SP	242	Peach	Orange	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(29) Campiel Rojo	3142 AD	Local	USA	231	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Globose	Non-showy

(continue)

Cultivar	Accession number	Cultivar classification	Origin	Harvest date (JD)	Fruit type	Flesh colour	Flesh type	Skin colour	Shape type	Stone type	Gland type	Bloom type
(30) Carolyn	2274 AD	Modern	USA	199	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(31) Carson	2957 AD	Modern	USA	194	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(32) Catherina	3137 AD	Modern	Zaragoza, SP	190	Peach	Orange	Non-melting	Orange	Round	Clingstone	Reniform	Non-showy
(33) Del Gorro	2830 AD	Local	Teruel, SP	245	Peach	Yellow	Non-melting	Yellow	Round	clingstone	Reniform	Showy
(34) Diamante Amarillo	2581 AD	Local	USA	199	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Globose	Non-showy
(35) Dixon	2278 AD	Modern	USA	231	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Non-showy
(36) Everts	3060 AD	Modern	USA	210	Peach	Yellow	Non-melting	Orange	Ovate	Clingstone	Reniform	Showy
(37) Fantasia	2971 AD	Modern	USA	237	Nectarine	Yellow	Melting	Red	Round	Freestone	Reniform	Showy
(38) Flamekist	2970 AD	Modern	USA	202	Nectarine	Yellow	Melting	Red	Ovate	Clingstone	Reniform	Showy
(39) Flavortop	2969 AD	Modern	USA	196	Nectarine	Yellow	Melting	Red	Round	Freestone	Reniform	Non-showy
(40) Fortuna	2279 AD	Modern	Huesca, SP	243	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(41) GF3	3045 AD	Modern	France	204	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(42) Goiri	3035 AD	Local	Bilbao, SP	208	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Reniform	Non-showy
(43) Golden Queen	2282 AD	Modern	NZL	253	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Globose	Showy
(44) Gomes	3063 AD	Modern	USA	248	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Globose	Non-showy
(45) Halford	3059 AD	Modern	USA	239	Peach	Orange	Non-melting	Bicolour	Round	Clingstone	Globose	Non-showy
(46) Infanta Isabel	1068 AD	Local	Castellón, SP	216	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(47) Jerónimo de Alfaro	3010 AD	Local	Murcia, SP	226	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Globose	Showy
(48) Jungerman	2959 AD	Modern	USA	216	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Globose	Showy
(49) Kakamas	2801 AD	Modern	South Africa	241	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Reniform	Showy
(50) Keimoes	3245 AD	Modern	South Africa	235	Peach	Orange	Non-melting	Yellow	Round	Clingstone	Reniform	Showy
(51) Klamt	3144 AD	Modern	USA	228	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Globose	Non-showy
(52) Loadel	2802 AD	Modern	USA	197	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Globose	Non-showy
(53) Lovell	3046 AD	Modern	USA	252	Peach	Yellow	Melting	Bicolour	Round	Freestone	Reniform	Non-showy
(54) Maluenda	2375 AD	Local	Zaragoza, SP	246	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Showy
(55) Maria Serena	3320 AD	Modern	Italy	181	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Showy
(56) Maruja	2261 AD	Local	Murcia, SP	196	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Non-showy
(57) Maruja Porvenir	2955 AD	Local	Murcia, SP	196	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Non-showy
(58) Miraflores	2844 AD	Local	Zaragoza, SP	250	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Globose	Showy
(59) Mountaingold	3254 AD	Modern	USA	205	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(60) Nectar del Jalón	561 AD	Local	Aragón, SP	218	Peach	Orange	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy

(continue)

Cultivar	Accession number	Cultivar classification	Origin	Harvest date (JD)	Fruit type	Flesh colour	Flesh type	Skin colour	Shape type	Stone type	Gland type	Bloom type
(61) NJC 97	3422 AD	Modern	USA	183	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(62) Nuevo	2803 AD	Modern	USA	235	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(63) Oropel	2582 AD	Local	SP	258	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Reniform	Showy
(64) Paloro A	3057 AD	Modern	USA	248	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(65) Paloro B	3058 AD	Modern	USA	246	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(66) Queen Giant	3639 AD	Modern	USA	188	Nectarine	White	Melting	Red	Round	Clingstone	Globose	Non-showy
(67) Redhaven	3640 AD	Modern	USA	186	Peach	Yellow	Melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(68) Rojo del Rito	3189 AD	Local	Lérida, SP	251	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(69) San Jaime	2355 AD	Local	Lérida, SP	199	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(70) San Lorenzo	2358 AD	Local	Huesca, SP	218	Peach	Orange	Non-melting	Orange	Round	Clingstone	Reniform	Non-showy
(71) Sarell	3246 AD	Local	Zaragoza, SP	207	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Showy
(72) Selma	255 AD	Modern	USA	220	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(73) Shasta	2286 AD	Modern	USA	198	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(74) Stanford	2033 AD	Modern	USA	237	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Globose	Non-showy
(75) Starn	3062 AD	Modern	USA	244	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(76) Sudanell 1	2211 AD	Local	Lérida, SP	226	Peach	Orange	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(77) Sudanell 2	2212 AD	Local	Lérida, SP	231	Peach	Orange	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(78) Sudanell 3	2213 AD	Local	Lérida, SP	233	Peach	Orange	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(79) Sudanell Blanco	3099 AD	Local	Zaragoza, SP	231	Peach	White	Non-melting	White	Round	Clingstone	Globose	Non-showy
(80) Sudanell GF	2804 AD	Modern	France	227	Peach	Orange	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(81) Sudanell GF	2972 AD	Modern	France	224	Peach	Orange	Non-melting	Orange	Round	Clingstone	Globose	Non-showy
(82) Suncling	2805 AD	Modern	USA	210	Peach	Yellow	Non-melting	Bicolour	Ovate	Clingstone	Reniform	Non-showy
(83) Super Crimson Gold	3657 AD	Modern	USA	181	Nectarine	White	Melting	Red	Round	Clingstone	Globose	Showy
(84) Tebana	3249 AD	Modern	Italy	188	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(85) Tempranillo de Aytona	3138 AD	Local	Huesca, SP	186	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Globose	Non-showy
(86) Tipo Campiel	2921 AD	Local	Zaragoza, SP	242	Peach	Yellow	Non-melting	Yellow	Round	Clingstone	Reniform	Non-showy
(87) Venus	3660 AD	Modern	Italy	221	Nectarine	Yellow	Melting		Round	Freestone	Reniform	-
(88) Vesuvio	2288 AD	Modern	Italy	191	Peach	Yellow	Non-melting	Red	Round	Clingstone	Reniform	Showy
(89) Vivian	2289 AD	Modern	USA	249	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(90) Walgant	3247 AD	Modern	South Africa	234	Peach	Yellow	Non-melting	Orange	Round	Clingstone	Reniform	Showy
(91) Wiser	3064 AD	Modern	USA	243	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(92) Zaragozano	553 AD	Local	Zaragoza, SP	259	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Non-showy
(93) Zaragozano Amarillo	2857 AD	Local	Zaragoza, SP	253	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy
(94) Zaragozano Rojo	2858 AD	Local	Zaragoza, SP	246	Peach	Yellow	Non-melting	Bicolour	Round	Clingstone	Reniform	Showy

Data from this table were partially presented Font i Forcada et al. (2012b).

Agronomical traits like tree vigor (trunk cross-sectional area, TCSA), yield, annual yield efficiency and fruit weight were evaluated. Trunk girths were measured during the dormant season 20 cm above the graft union, and TCSA was calculated. At harvest, all fruits from each tree were counted and weighed to determine total yield per tree (Kg/tree) and mean fruit weight. The data for two years (2010-2011) were recorded for cumulative yield per tree and annual yield efficiency (cumulative yield in kilograms per tree per final TCSA) of each cultivar were computed from the harvest data.

Other pomological traits such as leaf gland type (reniform/globose) and bloom type (showy/non-showy) were measured directly in the field, while others such as fruit type (peach/nectarine), flesh colour (yellow/orange), flesh type (melting/non-melting), skin colour (bicolour/orange/yellow/white/red) and stone type (clingstone/freestone) were determined in the laboratory immediately after harvest.

From 2008 to 2011, twenty mature fruits were yearly harvested from each cultivar at commercial maturity. Fruit samples were randomly harvested by a single person to keep consistency of maturity grade. Then, basic quality traits such as skin colour, flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA) and ripening index (RI) were evaluated. In the entire fruits, values of L* (brightness or lightness), a* (-a* = greenness, +a* = redness), b* (-b* = blueness, +b* = yellowness), C* (chroma) and H (lightness's angle) were measured using a colorimeter (Chroma Meter, CR-400 Konica Minolta, Japan). Flesh firmness was measured using a penetrometer (Model FT-327) on both sides of each fruit after removing a 1 mm thick disk of skin, with an 8 mm diameter probe. SSC was measured with a digital refractometer (Atago PR-101, Tokyo, Japan). TA and pH were determined using an automatic titration system (Metrohm Ion analysis, 807 Dosing Unit, Switzerland) with NaOH titrated to pH end-point of 8.1. RI was calculated based on SSC/TA ratio.

Phytochemical characters like sugars, phenolics, flavonoids, anthocyanins, relative antioxidant capacity (RAC) and vitamin C were evaluated for three consecutive years (2009-2011). Fruits were peeled and cut longitudinally into two halves and a portion of the mesocarp was removed from each half and cut into small pieces. For each compound to be analyzed a composite sample of 5 g was built by mixing all pieces from the selected fruits. This sample was frozen in liquid nitrogen and kept at -20ºC until analysed.

For analysis of sugars content, samples were homogenized with 10 mL of extraction solution consisting of 800 mL/L ethanol/Milli-Q water, using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington). A sample of 250 µL of the homogenized extract was incubated at 80°C for 20 min in 200 µL of 800 mL/L ethanol/water, with 5 g/L manitol added as an internal standard. Samples were purified using ion exchange resins (Bio-Rad Barcelona, Spain) as reported by Moing et al. (1992). Samples were then vacuum concentrated and then re-suspended to 1 mL of Milli-Q water, before High Performance Liquid Chromatography (HPLC) analysis. Then, sucrose, glucose, fructose and sorbitol were analyzed by HPLC (Aminex HPX-87C column, 300 mm x 7.8 mm; Bio-Rad, Barcelona, Spain) with a refractive index detector (Waters 2410) as previously reported by (Cantín et al., 2009a). PC Millenium 3.2 software (Waters) was used to perform sugar quantification. A standard calibration curves were used to quantify each different sugar and the concentrations were expressed as g per kg of fresh weight (FW).

Samples for vitamin C determination were kept at -20ºC in metaphosphoric solution (5% HPO_3) until analysis for preservation of oxidation. For analysis of antioxidant compounds, samples were homogenized with 10 mL of extraction solution consisting of 0.5 N HCl in methanol/Mili-Q water (80% v/v). Vitamin C and antioxidant compounds were analyzed using a spectrophotometer photodiode array detector DU 800 (Beckman Coulter, Inc., Fullerton, CA) as described by Cantín et al. (2009b). Absorbance for vitamin C was determined at 525 nm and the results were expressed as mg of ascorbic acid (AsA) per 100 g of FW. The Folin-Ciocalteau reagent at 0.25 N was used to determine the total phenolics content, and the absorbance was measured at 725 nm and the results were expressed as mg of Gallic acid equivalents (GAE) per 100 g FW. The flavonoid content absorbance was measured at 510 nm and the results were expressed as mg of catechin equivalents per 100 g of FW. For determining anthocyanin content, spectrophotometric readings at 535 nm were taken subtracting absorbance at 700 nm (due to turbidity) and the results were expressed as mg of cyaniding 3-glucoside equivalents (C3GE) per kg of FW (using a molecular weight of 494 and a molar extinction absorptivity coefficient ε = 25,965/cm M). The relative antioxidant capacity (RAC) was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and the absorbance was measured at 515 nm and the results were expressed as µg of Trolox equivalents per g of FW.

3.3.3. Statistical analysis

All statistical analyses were performed with the program SPSS 19.0 (SPSS, Inc, Chicago, USA). Differences between cultivars for each trait when comparing more than two fruit types (yellow, orange and white flesh; and bicolour, orange, yellow, red and white skin) were analyzed by Duncan's multiple range test ($P \leq 0.05$). When comparing two different fruit types (peach or nectarine, non-melting or melting flesh, clingstone or freestone flesh, reniform or globose leaf, showy or non-showy flower) or cultivars origin (local or modern) *t* test ($P \leq 0.05$) was used. Correlations between traits to reveal possible associations was calculated with raw data based on the average of three trees per cultivar over the three years, using Pearson correlation coefficient at $P \leq 0.05$. Principal components analysis (PCA) was used to study correlations among traits. A 2D PCA plot was designed using combined data from three years of the study using the program SPSS version 19.0 (SPSS, Inc, Chicago, USA).

3.4. RESULTS AND DISCUSSION

3.4.1. Cultivar influence and phenotypic evaluation

A broad phenotypic variation was found for all the parameters studied in the 94 peach and nectarine cultivars. Range and means for the Pomological and fruit quality traits are shown in Table 3.2. Mean values for each cultivar are shown in supplementary material in annexes (Tables 11.1.1, 11.1.2 and 11.1.3).

Table 3.2 shows factors affecting Pomological and fruit quality parameters in cultivars. ANOVA results showed that cultivars influenced SSC, TA, RI, glucose, fructose, sorbitol and RAC. It has been shown that levels of some quality traits in peach fruit differ among rootstocks or cultivars (Colaric et al., 2005; Orazem et al., 2011).

Early blooming is a desirable character in Mediterranean areas to obtain earliest yields (George and Nissen, 1992) even though spring frosts may reduce production in some years. In this study, full bloom date was mainly recorded in the second half of March (from 79 to 87 Julian days). The earliest cultivars to bloom were the nectarines 'Big Top' and 'Fantasia', and the peach cultivars 'Shasta' and 'Stanford' (≈80 JD), among others. The latest cultivars to reach full bloom were 'Amarillo de Calanda' (131 AD) and 'Oropel' (≈87 JD), among others. Harvest dates were not stable from one year to other mainly due to annual variation on temperature. The earliest cultivars to be

harvested (181 JD, late June) were 'Maria Serena' 'and 'Super Crimson Gold'. In contrast, the traditional Spanish cultivars from the Ebro Valley (Northeast Spain) 'Alcañíz 1', 'Bonet V' and 'Calanda Tardío' were harvested on late October (≈272-274 JD). Blooming and harvest traits have been established as quantitatively inherited in peach and other *Prunus* species (Dirlewanger et al., 1999). The peach fruit development period is highly dependent on cultivar (Mounzer et al., 2008). Nevertheless, blooming and harvest date may change every year depending on the environmental conditions, especially temperature (Mounzer et al., 2008).

Vigor of trees was estimated based on TCSA (cm^2). Among cultivars, 'Bonet III' and 'Paloro B' had the highest values of TCSA (≈280 cm^2), while 'Fortuna' (48±9.2 cm^2) or 'Shasta' (44±12 cm^2) showed the lower ones. The mean value for yield considering all the different cultivars was 14.2 kg/tree, but a high variability was found among them. Among cultivars, 'Lovell' (47±3.2)', 'Sudanell GF 2804 AD' (43±5.6) and 'GF3' (32±3.5) showed the highest yields. Yield on trees depends on the genetic background of the cultivar (density of flower buds and flowers, fruit set, fruit size) and on agronomic and environmental factors (Milatović et al., 2010). Mean value for annual yield efficiency was 0.30 kg/cm^2, with 'Lovell' having the highest values (1.31±0.08), and 'Sudanell GF' (0.69±0.02) or 'Suncling' (0.66±0.03) showing intermediate values. Fruit weight greatly varied among cultivars showing a range of 64 to 315 g. Among cultivars, 'Alejandro Dumas' (315±15) and 'Baby Gold 6' (312±18.5) showed the higher fruit weight values, 'Klamt' (233±15) and 'Lovell' (223±15) had intermediate values, while 'Benasque' (64±15), 'Diamante Amarillo' (102±12), 'Nectar del Jalón (114±10) and 'Super Crimson Gold' (129±13) presented the lower ones. Yield and fruit weight are also major quantitative inhered factors (Dirlewanger et al., 1999).

Table 3.2. Units, minimum, maximum and mean values for the traits evaluated, and ANOVA analysis of the effect of the 94 peach and nectarine cultivars for the average of the all years of study.

					Source of variation[1]		
Trait	Units	Minimum	Maximum	Mean ± SE	Cultivar (C)	Year (Y)	Y x C
Bloom beginning	Julian days	72	83	78 ± 0.19	ns	ns	ns
Full Bloom	Julian days	79	87	82 ± 0.15	ns	ns	ns
Harvest date	Julian days	185	275	224 ± 2.5	ns	ns	ns
TCSA	cm^2	44	280	92 ± 3.9	ns	ns	ns
Yield	Kg/tree	1.0	46.5	13.4 ± 1.9	ns	ns	ns
Yield efficiency	Kg/cm^2	0.11	1.31	0.30 ± 0.02	ns	ns	ns
Fruit weight (FW)	Grams	64	315	178 ± 2.8	ns	ns	ns
Soluble Solids Content (SSC)	ºBrix	12	18	15 ± 0.13	***	ns	ns
Flesh firmness (FF)	Newtons (kg/cm^2)	9	61	38 ± 0.9	ns	ns	ns
Titratable acidity (TA)	g malic acid/100 g FW	0.4	0.9	0.6 ± 0.01	***	ns	ns
Ripening index (RI)	SSC/TA	15	67	25 ± 0.43	***	ns	ns
L*	Lightness	10.6	76.8	61.9 ± 9.0	ns	ns	ns
a*	Greenness/redness	-1.18	60.8	22.4 ± 5.2	ns	ns	ns
b*	Blueness/yellowness	8.9	69.1	52.0 ± 11.5	ns	ns	ns
C*	Chroma	25.3	80.6	58.9 ± 9.1	ns	ns	ns
h*	Lightness's angle	16.9	91.4	62.7 ± 14.0	ns	ns	ns
Sucrose	g/kg FW	35	98	75 ± 0.9	ns	ns	ns
Glucose	g/kg FW	4	15	10 ± 0.19	*	ns	ns
Fructose	g/kg FW	2	14	11 ± 0.18	***	ns	ns
Sorbitol	g/kg FW	2	35	13 ± 0.76	***	ns	ns
Total sugars (TS)	g/kg FW	63	136	110 ± 1.35	ns	ns	ns
Vitamin C	mg AsA/100 g FW	3	28	13 ± 0.41	ns	ns	ns
Total phenolics	mg GAE/100 g FW	18	62	44 ± 0.65	ns	ns	ns
Flavonoids	mg CE/100 g FW	3	63	24 ± 1.49	ns	ns	ns
Anthocyanins	mg C3GE/kg FW	0.7	12	2.5 ± 0.21	ns	ns	ns
Relative Antioxidant Capacity (RAC)	mg TE/g FW	186	1184	840 ± 19.0	*	ns	ns

AsA ascorbic acid, *GAE* gallic acid equivalents, *CE* catechin equivalents, *C3GE* cyanidin-3-glucoside equivalents, *TE* trolox equivalents. [1]Data were evaluated by two-way variance (ANOVA); ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns, not significant.v Data from this table were partially presented in Font i Forcada et al. (2012b).

Firmness, soluble solids content (SSC), TA and RI, greatly varied among cultivars in the range of 9 to 61 N (the maximum level of fruit firmness for marketing fresh peaches and nectarines is 63.7 N; Commission Regulation EC, No.1861/2004 of 28 October 2004), 12 to 18 ºBrix (the minimum value of SSC for consumer acceptance is over 10 ºBrix; Kader, 1999), 0.4 to 0.9 g malic acid/ 100 g FW and 15 to 67 SSC/TA, respectively. These mean values are in the same range reported by other authors in peach studies (Abidi et al., 2011; Cantín et al., 2010). The non-melting native Spanish peaches 'Borracho de Jarque' (61±1.5), 'Amarillo Calanda' (2400 AD) (58±0.5), 'Bonet III' (56±3.2), 'Calanda Tardío' (55±2.5) and 'Sudanell 1' (52±4.3), as well as the commercial cultivars 'Keimoes' (54±1.2), 'Lovell' (52±1.3) and 'Vivian' (52±1.3) presented the highest fruit firmness. In contrast, the white flesh peach 'Benasque' (17±1.5) (a peach seedling rootstock), and the nectarines 'Fantasia' (9.1±1.2) and 'Super Crimson Gold' (17.4±1) showed the lowest firmness.

Regarding SSC (ºBrix), the native non-melting peaches 'Bonet I', 'Bonet III', 'Borracho de Jarque', 'Rojo del Rito', and 'Sudanell 1' presented the higher contents (≈18 ºBrix), as well as the commercial cultivars 'Nuevo' (≈18) and 'Golden Queen', 'Halford', 'Paloro A', 'Oropel' and 'Vivian' (≈17 ºBrix). In contrast, the melting nectarine 'Queen Giant' and the melting peach 'Redhaven' showed the lower values (≈12 ºBrix). For acidity of fruits, 'Maria Serena', and 'Tebana' showed the lowest acidity (≈0.4 g malic acid/ 100 g FW) based on TA, followed by the native non-melting clingstone Spanish peaches 'Alcañíz 2', 'Borracho de Jarque', 'Calabacero', 'Calanda San Miguel', 'Fraga', 'Goiri', Jerónimo de Alfaro', and 'Zaragozano Rojo', and the commercial yellow peaches 'Andross', 'Babygold 6', 'Babygold 9', 'Carson', 'Dixon', 'Stanford', and 'Suncling' (≈0.5), among others. In contrast, 'Andora', 'Calanda Tardío' and 'Paloro B' presented the highest content of TA (≈0.9). Ripening index (RI) is also a major organoleptic quality trait of the mature fruit in peaches (Bassi and Selli, 1990) and depends on the SSC and TA ratio. Among cultivars, the native local cultivar 'Borracho de Jarque' (67±2.3) showed the higher values. Intermediate values were observed on 'Maria Serena' (≈36), 'Alcañíz 2', 'Nuevo' and 'Tebana' (≈33), 'Dixon' (≈32), and 'Andross', 'Bonet I' and 'Rojo del Rito' (≈31), among others. In contrast, 'Andora' (15±0.5) and 'Queen Giant' (17±0.8) showed the lower RI values.

Concerning individual sugars, sucrose was the major sugar present in peach fruit, followed by glucose, fructose and lower amounts of sorbitol. These sugars play an

important role in peach flavor quality (Robertson et al., 1988). Also, sorbitol is the most related to peach aroma and taste among carbohydrates and organic acids (Colaric et al., 2005). Values for sucrose, glucose, fructose, sorbitol, and total sugars are within the range reported by other authors (Abidi et al., 2011; Cantín et al., 2009a; Yoshida, 1970). Total sugars varied in the range of 63 to 136 g/kg FW. Among cultivars, the local Spanish cultivars 'Bonet III' (136±5.6), 'Calabacero' and 'Calanda San Miguel' (≈134) showed the higher contents. In contrast, the nectarines 'Super Crimson Gold' (80.9±10.9) and 'Venus' (71.5±12.5), as well as the peaches 'Alcañíz 1' (75.5±10.2) and 'Amarillo Calanda' (131 AD) (63±15.3) showed lower content on total sugars. Regarding individual sugars, sucrose content values varied from 35 to 98 g/kg FW with 'Calabacero' (98±9.1), 'Jungerman' (93±5.3) and 'Diamante Amarillo' (90±2.4) showing the highest contents. For glucose content, the values varied from 4 to 15 g/kg FW, with 'Babygold 9' and 'Bonet (IV)' (≈15) and 'Calabacero' and 'Fantasia' (≈14) presenting the higher contents. For fructose content, the values varied from 2 to 14 g/kg FW, with 'Amarillo Calanda' (2400 AD), 'Babygold 9', 'Bonet IV', 'Calabacero', 'Fantasia', 'Infanta Isabel', and 'Venus' showing the higher fructose contents (≈14). Finally, for sorbitol content, the values varied from 2 to 35 g/kg FW and two native cultivars, 'Bonet III' (35±5.3) and 'Rojo del Rito' (31±4.8), followed by the commercial cultivar 'Vivian' (27.4±2.5) presented the higher contents.

The phytochemical compounds also showed a wide range of variability (Table 3.2). The vitamin C varied in the range of 3 to 28 mg ASA/100 g FW, with 'Shasta' showing the higher value (27.8±1.9), followed by native Spanish peaches 'Alcañíz 2' (≈20) and 'Goiri' (≈19). Others studies showed similar values for the quantification of ascorbic acid (Gil et al., 2002). These results suggest that the peach fruit is a good source of vitamin C, thus the ascorbic acid is an important part of the evaluation of peach quality. Total phenolics, as determined by the Folin-Ciocalteau assay, varied among cultivars with values in the range of 18 to 62 mg of GAE /100 g of FW, with the native peach 'Alcañíz 1' having the higher values for phenolic contents (62±2.8), followed by other Spanish peaches as 'Amarillo Calanda' (131 AD), 'Calanda San Miguel' and 'Miraflores' (≈52) and the commercial cultivars 'Golden Queen', 'Nuevo', 'Paloro B' and 'Vivian' (≈49). These values are within or close to the range reported for peach cultivars in the literature, namely, 14 to 50 mg of GAE per 100 g of FW (Abidi et al., 2011; Cantín et al., 2009b; Tavarini et al., 2008). Flavonoids content ranged from 3

to 63 mg of CE per 100 g of FW in agreement with others studies in peach (Abidi et al., 2011; Gil et al., 2002; Tomás-Barberán et al., 2001). Selecting fruits rich in flavonoids is very interesting from the point of health perspective (Vauzour et al., 2008). Among cultivars, 'Nuevo' (63±5.6), 'Alcañíz 2' (60±2.5), 'Amarillo Calanda' (131 AD) (57±2.5) and 'Zaragozano Amarillo' (56±1.6) showed higher values for flavonoid contents. Total anthocyanins greatly varied among cultivars (0.7 to 12 mg of cyaniding 3-glucoside equivalents (C3GE) per kg of FW) depending on the percentage of red pigmentation of the flesh. Cultivars with staining reddish endocarp flesh as 'Flavortop' (12±0.9), 'Rojo del Rito' (10±4), 'Amarillo de Gallur', 'Brasileño' and 'Vivian' (≈8) and 'Borracho de Jarque' and 'Fantasia' (≈7) had higher anthocyanins content than cultivars with pure yellow flesh as 'Andora' (0.7±0.06), 'Goiri' (0.8±0.01) and 'Maria Serena' (0.9±0.01). Their values are within the range reported by other authors (Abidi et al., 2011; Gil et al., 2002). Relative antioxidant capacity (RAC) varied from 186 to 1184 mg TE/g FW among cultivars, with the native Spanish cultivars 'Alcañíz 2', 'Amarillo Calanda' (131 AD), 'Bonet III' and 'Zaragozano Amarillo' showing the higher values (between 1130 and 1184 mg TE/g FW), followed by other native peaches 'Amarillo de Gallur', 'Benasque', 'Bonet IV', 'Calanda Tardío', 'Fraga', 'Sudanell 1', 'Sudanell Blanco', 'Tipo Campiel' and the commercial cultivars 'Golden Queen', 'Gomes', 'Halford', 'Kakamas', 'Nuevo', 'Paloro A', 'Paloro B' and 'Vivian' (between 1000 and 1130). In contrast, 'Big Top', 'Maria Serena' and 'Venus' showed lower content on RAC (between 180 and 400 mg TE/g FW). This high variability could be explained because the content of RAC in fruits varied in relation to the RAC molecules present in the genotypes of a single species (Gil et al., 2002). Values in a similar range were obtained in other studies with peach cultivars or genotypes (Abidi et al., 2011; Cevallos-Casals et al., 2006; Gil et al., 2002).

3.4.2. Influence of Pomological traits on several fruit quality traits

Significant differences were found among cultivars with different pomological traits for the basic fruit quality and phytochemical traits (Tables 3.3, 3.4, 3.5).

Modern cultivars showed later full bloom and harvest date in average. In contrast, nectarine and melting flesh cultivars presented the earliest harvest date, although they were smaller groups compared to peaches and non-melting cultivars. Modern and nectarine cultivars showed lower yield and annual yield efficiency than

local and peach cultivars. No significant differences were found among the other pomological traits evaluated (flesh color, flesh type, skin color and stone type) (Table 3.3), according to other studies in peach (Cantín et al., 2010). Local cultivars, yellow and orange flesh and bicolor, orange, red and yellow skin had higher fruit weight than modern white flesh and white skin cultivars. Firmness was lower for melting flesh cultivars compared to the non-melting ones, as well as for white flesh compared to yellow and orange flesh, in agreement with Crisosto et al. (2001) and Cantín et al. (2010). Firmness was also higher for yellow skin cultivars than red and white skin ones, although all of them did not differ significantly from bicolor and orange skin cultivars. The content of soluble solids (SSC) was higher on modern cultivars, peaches, and yellow and white skin compared to local cultivars, nectarines and bicolor, orange and red skin, respectively. Significantly higher TA was observed for nectarine, white and melting flesh and freestone fruits. On the other hand, peaches showed higher RI than nectarines due to their reported higher SSC.

About parameters of color, the most significant results were that modern and peach cultivars had higher contents of lightness, yellowness and blueness and lightness's angle than local and nectarine cultivars (Table 3.4). Also, other significantly differences between flesh and skin color were found.

In general, peach and modern cultivars had significantly higher content on sorbitol, total sugars, vitamin C, phenolics, flavonoids and RAC than local cultivars and nectarines (Table 3.5). Also, clingstone cultivars had higher content on sucrose and total sugars than freestone cultivars. In contrast, Cantín et al. (2010) reported that nectarine-white flesh fruits and freestone cultivars had higher content on sucrose, glucose and fructose than peach-yellow flesh fruits. These differences were probably due to the smaller number of cultivars with nectarine fruits or cultivars with white flesh and white skin in our study. Fructose content and yellow/white flesh are co-localising in the same QTL in LG1, and these might explain the linked segregation of these two traits (Bliss et al., 2002; Quilot et al., 2004). Moreover, LG4 is involved in sucrose and glucose contents, near the physical trait controlling adhesion to the stone (Quilot et al., 2004).

Table 3.3. Full bloom (BD), harvest date (HD), yield and annual yield efficiency (AYE), fruit weight (FW), flesh firmness (FF), soluble solids content (SSC), titratable acidity (TA) and ripening index (RI) with qualitative traits in peach and nectarine cultivars.

Trait	n	BD	HD	Yield	AYE	FW	FF	SSC	TA	RI
Local	43	81*	209*	17.3*	0.37*	185*	36	15*	0.62	25
Modern	51	82*	229*	10.6*	0.23*	170*	40	16*	0.63	26
Peach	87	82	220*	14.6*	0.32*	178	39	15*	0.62*	25*
Nectarine	7	81	194*	9.1*	0.27*	158	31	14*	0.68*	22*
Yellow flesh	72	82	218	14.1	0.30	181 b	38 b	15	0.61 a	26 b
Orange flesh	17	82	224	15.5	0.34	172 b	41 b	16	0.63 a	25 b
White flesh	4	82	201	10.7	0.26	148 a	26 a	14	0.73 b	20 a
Non-melting	84	82	220*	13.8	0.30	179	39*	16	0.61*	26*
Melting	10	81	202*	17.7	0.32	175	32*	15	0.70*	21*
Bicolour skin	44	82 ab	217 ab	17.5	0.36	189 b	39 ab	15 ab	0.61	25
Orange skin	27	82 ab	219 ab	12.1	0.26	167 b	36 ab	15 ab	0.61	26
Yellow skin	13	83 b	234 b	9.6	0.24	169 b	43 b	16 b	0.63	26
Red skin	7	81 a	193 a	9.5	0.23	180 b	30 a	14 a	0.66	22
White skin	2	83 b	222 b	13.1	0.31	125 a	29 a	16 b	0.76	21
Clingstone	89	82	218	13.6	0.29	179	38	15	0.62*	26*
Freestone	5	81	224	18.0	0.44	177	35	15	0.72*	21*

The number of observed cultivars (*n*) is shown for each fruit type. Data are means over the three years of study. In each trait column, means with * are significantly different according to t test ($P \leq 0.05$) and values with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$).

Table 3.4. Chromatic parameters (L*= lightness; a*= redness and greenness; and b*= yellowness and blueness; C*= chroma; H= lightness's angle) with qualitative traits in peach and nectarine cultivars.

Trait	n	L*	a*	b*	C*	h*
Local	43	60*	25*	49*	58	60*
Modern	51	64*	20*	51*	60	66*
Peach	87	63*	21*	53*	59*	64*
Nectarine	7	42*	35*	23*	43*	30*
Yellow flesh	72	61 b	23	52 b	59 b	61
Orange flesh	17	65 b	20	56 b	61 b	68
White flesh	4	56 a	22	32 a	46 a	55
Bicolour skin	44	45 a	25 c	50 ab	57 ab	59 ab
Orange skin	27	67 ab	18 b	59 b	62 b	71 ab
Yellow skin	13	66 ab	19 b	58 b	65 b	67 ab
Red skin	7	45 a	34 d	26 a	45 a	34 a
White skin	2	76 b	3 a	45 ab	46 a	87 b

The number of observed cultivars (*n*) is shown for each fruit type. Data are means over the three years of study. In each trait column, means with * are significantly different according to t test ($P \leq 0.05$) and values with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$).

Table 3.5. SSC, sucrose, glucose, fructose, sorbitol, total sugars (TS), phenolics, flavonoids, anthocyanins, vitamin C and RAC (relative antioxidant capacity) with qualitative traits in peach and nectarine cultivars.

Trait	n	SSC	Sucrose	Fructose	Sorbitol	TS[a]	Vitamin C	Phenolics	Flavonoids	Anthocyanins	RAC
Local	43	15*	75	11	11*	107*	12*	42*	19*	2.2	771*
Modern	51	16*	75	11	16*	113*	14*	47*	29*	2.8	926*
Peach	87	15*	75*	10	14*	110*	13*	45	24*	2.3*	861*
Nectarine	7	14*	65*	11	7*	94*	7*	35	8*	4.1*	606*
Yellow flesh	72	15	76 b	11 b	13	110 b	12	44	23	2.5	830
Orange flesh	17	16	74 b	11 b	16	112 b	13	46	28	2.2	899
White flesh	4	14	66 a	8 a	13	98 a	10	42	21	3.6	821
Bicolour skin	44	15 ab	76	11 b	13 ab	110 ab	13 b	44 ab	20 ab	2.7	820 ab
Orange skin	27	15 ab	75	11 b	13 ab	110 ab	13 b	46 ab	26 ab	2.0	881 ab
Yellow skin	13	16 b	77	11 b	16 ab	116 b	13 b	47 b	35 b	1.8	946 ab
Red skin	7	14 a	69	10 b	7 a	97 a	8 a	39 a	10 a	4.0	639 a
White skin	2	16 b	68	6 a	22 b	109 ab	15 b	48 b	38 b	2.9	1056 b
Clingstone	89	15	76*	11	13	110*	13	46	24	2.5	848
Freestone	5	15	62*	10	13	95*	11	39	18	2.2	742

The number of observed cultivars (*n*) is shown for each fruit type. Data are means over the three years of study. In each trait column, means with * are significantly different according to t test ($P \leq 0.005$) and values with the same letter are not significantly different according to Duncan's test ($P \leq 0.005$). [a] Sum of sucrose, glucose, fructose and sorbitol.

3.4.3. Correlations among traits

The Pearson's correlation coefficients between pairs of traits are shown in Table 3.6. Significant correlations were found between agronomic, basic fruit quality traits, individual soluble sugars and antioxidants compounds.

Harvesting date showed significant and positive correlations with bloom date, fruit weight, SSC, sucrose, fructose, sorbitol, total sugars, phenolics, flavonoids and RAC contents. When fruits are harvested late, they are in general larger and sweeter (SSC). Contrary, harvest date was negatively correlated with flesh firmness. These results are in agreement with previous reports where positive correlations between harvesting date, fruit weight and SSC have been reported (Cantín et al., 2010; Dirlewanger et al., 1999).

Yield was significantly and positively correlated with TCSA, annual yield efficiency and fruit weight, and negatively correlated with TA, fructose and sorbitol. Annual yield efficiency was also positively correlated with fruit weight and negatively correlated with fructose. These results suggest that yield increases with fruit weight but several sugars decreases as consequence of higher crop loads inducing lower fruit total sugar content owing to sink competition among fruits, as showed by Morandi (2008).

Significant positive correlations were also found between fruit weight and SSC, TA, glucose, fructose and total sugars. These results were expected, since the amount of translocated carbohydrates determines the fruit growth rate, as demonstrated by Morandi (2008). Also, significant positive correlations were found between fruit weight and phenolics, flavonoids and RAC. Similar results were showed in other species such as plums (Díaz-Mula et al., 2008), apricots (Bureau et al., 2009) and sweet cherries (Serrano et al., 2005). Also, significant positive correlations were found between SSC and phenolics, flavonoids and RAC in agreement with Abidi et al. (2011) and Cantín et al. (2009b). The relationship of fruit weight with bioactive compounds could be explained by the well-known influence of the sink size on the ability to attract photosynthates from the plant sources, because a sufficient accumulation of sugars in or near the fruit is essential for phenolics compounds synthesis during fruit growth (DeJong, 1999).

Table 3.6. Pearson's correlation coefficients between pairs of traits studied.

Trait	Full bloom	TCSA	Yield	YE	FW	SSC	FF	TA	RI	Sucrose	Glucose	Fructose	Sorbitol	TS	Vitamin C	Phenolics	Flavonoids	RAC
Full bloom	-	ns	ns	ns	ns	0.20**	ns	ns	ns	ns	ns	ns	0.20**	ns	ns	ns	0.18**	0.19**
HD	0.18*	ns	ns	ns	0.63**	0.63**	-0.52*	ns	ns	0.62**	ns	0.21*	0.78**	0.66**	ns	0.65**	0.79**	0.72**
TCSA		-	0.22*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Yield			-	0.82*	0.28**	ns	ns	-0.21*	ns	ns	ns	-0.29*	-0.27*	ns	ns	ns	ns	ns
YE				-	0.30**	ns	ns	ns	ns	ns	ns	-0.24**	ns	ns	ns	ns	ns	ns
FW					-	0.56**	ns	0.15*	ns	ns	0.36**	0.39*	ns	0.25*	ns	0.53**	0.21*	0.34*
SSC						-	0.49**	0.26**	ns	0.29**	0.27**	0.36*	0.77**	0.49**	ns	0.56**	0.60**	0.61**
FF							-	0.40**	-0.57*	-0.50**	-0.64**	-0.49**	-0.42*	-0.59*	ns	-0.52**	-0.26*	ns
TA								-	ns	ns	0.41**	ns	0.40**	ns	0.46**	ns	0.35**	ns
RI									-	0.42**	0.24*	0.35*	0.41**	0.27**	-0.21*	ns	ns	ns
Sucrose										-	0.57**	0.63**	0.48**	0.95**	ns	0.43**	0.47**	ns
Glucose											-	0.83**	0.44**	0.81**	ns	0.42**	0.44**	0.52**
Fructose												-	0.49**	0.83**	ns	ns	0.24**	ns
Sorbitol													-	0.56**	0.37**	0.52**	0.47**	0.64**
TS														-	0.42**	0.58**	0.61**	0.64**
Vitamin C															-	ns	ns	0.25*
Phenolics																-	0.68**	0.79**
Flavonoids																	-	0.87**
RAC																		-

*$p \leq 0.05$, **$p \leq 0.01$ represent significant values, *ns* not significant. [a] Data from this table were partially presented Font i Forcada et al. (2012b).

The positive correlations between SSC, TA and individual and total sugars, suggests a dependent genetic control (Cantín et al., 2009a; Dirlewanger et al. 1999). The location of QTLs for sucrose, fructose and sorbitol (Dirlewanger et al., 1999) with possible pleiotropic effect, could partly explain this result.

A significant negative correlation was found between flesh firmness and ripening index, sucrose, glucose, fructose, sorbitol, total sugars, phenolics and flavonoides. However, a significant positive correlation was found between flesh firmness and TA and SSC. This suggests that softer fruit is linked to lower acidity fruits in agreement with Byrne et al. (1991) and Cantín et al. (2010). A positive relationship between firmness and SSC has also been reported in sweet cherry (Jiménez et al., 2004). This result suggests that, at the same level of ripening, firmer fruits show a tendency to have higher SSC.

High and significant correlations were found between individual and total sugars in agreement with other studies (Abidi et al., 2011; Cantín et al., 2009a; Dirlewanger et al., 1999). Among individual sugars, the highest correlation was found between glucose and fructose as previously reported (Cantín et al., 2009a; Dirlewanger et al., 1999). Moreover, individual and total sugars showed positive significant correlations with phytochemical compounds. Pirie and Mullins (1977) reported a good correlation in grapes between sugar content in berries and levels of phenolic substances, due to the role of sugars in the regulation of phenolic biosynthesis.

Finally, other important positive and significant correlations were found between vitamin C and RAC, between phenolics and both flavonoids and RAC, as well as between flavonoids and RAC. These results show that they are important bioactive compounds for the antioxidant activity of peaches, in accordance with Cantín et al. (2009a) and Abidi et al. (2011).

3.4.4. Principal components analysis

The principal components analysis (PCA) was performed to analyze the data for the 25 agronomical and fruit quality traits obtained from the 94 peach and nectarine cultivars (Table 3.7, Figure 3.1 and 3.2). The PCA analysis showed that more than 55% of the observed variance could be explained by the first three components. The PC1,

PC2 and PC3 axes explained 25.4%, 20.6% and 9.8% of total variability, respectively. Cultivars displayed a great variability (Figure 3.2).

Table 3.7. Eigenvectors of the three principal component (PC) axes of the 25 agronomic, basic fruit quality traits, sugars and phytochemical compounds evaluated on 94 peach and nectarine cultivars.

	Component loading		
	PC1 (25.4%)	PC2 (20.6%)	PC3 (9.8%)
Full Bloom	-0.008	0.186	**-0.397**
Harvest date	0.584	**0.614**	0.199
Trunk cross-sectional area (TCSA)	0.205	-0.015	**0.389**
Yield	-0.045	-0.080	**0.821**
Annual yield efficiency	-0.045	-0.207	**0.788**
Fruit weight (FW)	-0.247	0.173	**0.584**
Soluble solid content (SSC)	**0.621**	0.429	-0.043
Flesh firmness (FF)	**0.448**	0.228	0.368
Titratabe acidity (TA)	**-0.519**	-0.292	-0.344
Ripening index (RI)	**0.676**	-0.018	0.240
L*	**0.668**	-0.598	-0.062
a*	**-0.703**	0.470	0.083
b*	**0.698**	-0.325	0.030
C*	0.533	**-0.639**	-0.049
h*	**0.624**	-0.219	-0.018
Sucrose	0.360	**-0.643**	-0.025
Glucose	0.234	**0.739**	-0.023
Fructose	0.305	**0.670**	0.070
Sorbitol	0.593	**0.643**	-0.001
Total sugars	**0.712**	0.330	-0.008
Vitamin C	0.181	**-0.387**	-0.214
Phenolics	**0.327**	0.260	0.147
Flavonoids	**0.777**	0.384	-0.080
Anthocyanins	-0.127	**0.616**	-0.216
Relative Antioxidant Capacity (RAC)	**0.667**	0.334	-0.119

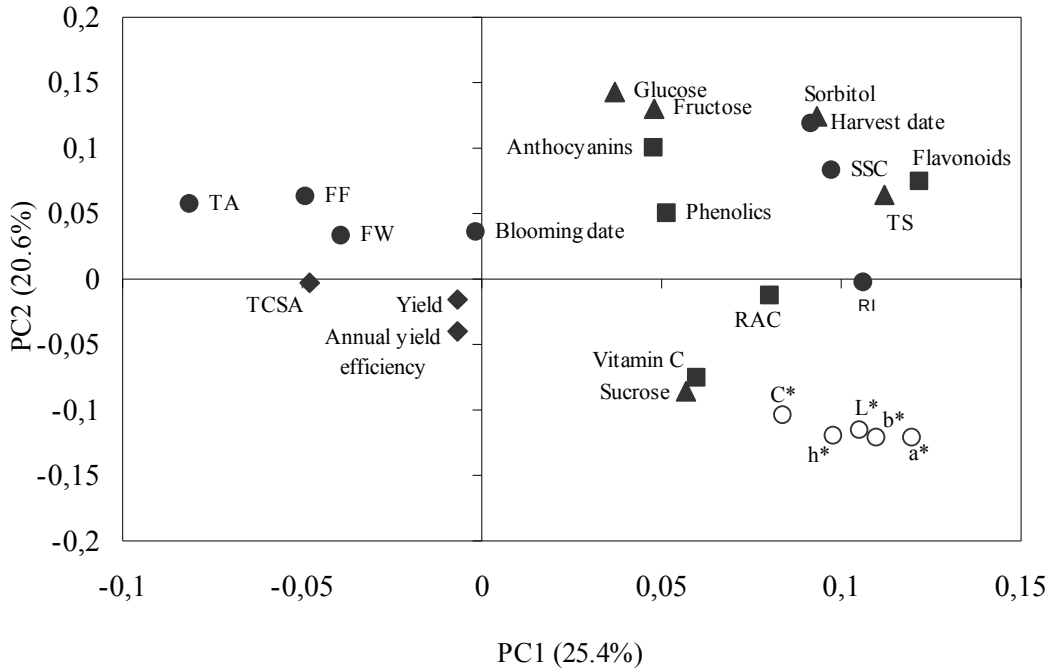

Figure 3.1. Principal components analysis axes of the 25 agronomic, basic traits, sugars and phytochemical compounds evaluated on 94 peach and nectarine cultivars. Symbols: (♦) agronomical traits, (●) basic fruit quality traits, (O) colour parameters, (▲) sugars and (■) phytochemical compounds.

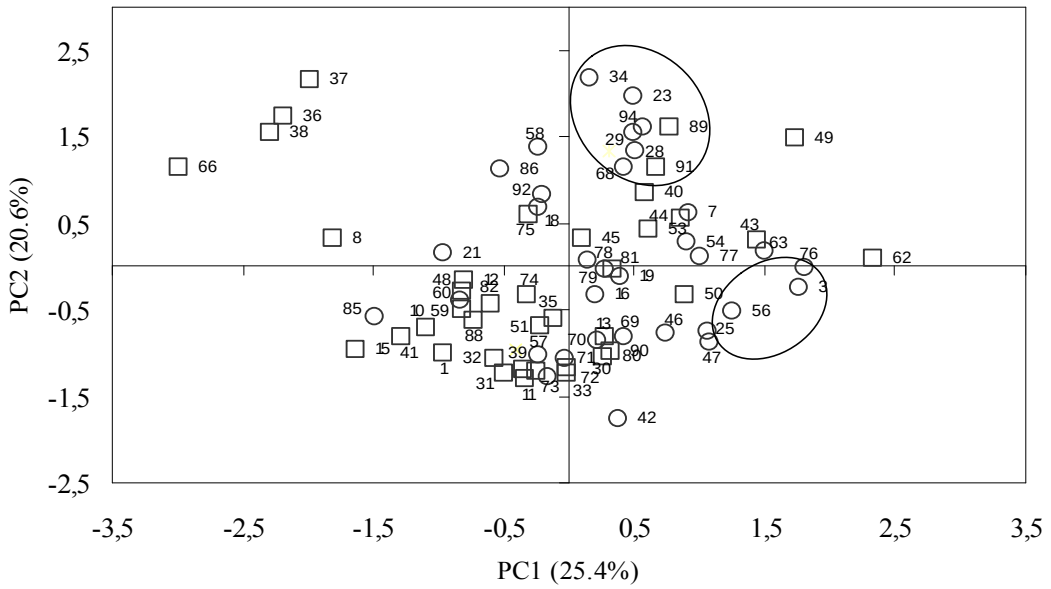

Figure 3.2. Principal components analysis axes of the 94 peach and nectarine cultivars evaluated. Symbols: (□) modern cultivars and (O) local cultivars. Numbers are used to name cultivars according to Table 3.1.

PC1 represents mainly SSC, firmness, TA, RI, colour parameters, total sugars, phenolics, flavonoids and RAC. PC2 explains mainly harvest date, sucrose, glucose, fructose, sorbitol, vitamin C and anthocyanins, and PC3 mainly contributes to full bloom, TCSA, yield, annual yield efficiency and FW.

An examination of PC1 loadings suggested that cultivars in the positive side were in general less acid, showed less firmness, and accumulated more sugars and less anthocyanins than cultivars on the negative side. Cultivars on the PC2 loadings suggested that separation on this component was mainly due to agronomical traits (yield, TCSA, annual yield efficiency) and to some basic fruit quality parameters such as fruit weight and FF (Figure 3.1).

Analysis confirmed the higher contents in phenolics, flavonoids, anthocyanins and RAC and the lower content on yield for some cultivars, especially for the local ones 'Alcañíz 2' and 'Rojo del Rito' and the foreign 'Vivian'. Also, 'Alcañíz 2' cultivar presents higher content on vitamin C and RAC. Other cultivars such as 'Kakamas' and 'Calabacero' are placed on the positive side of PC1 and presented higher values on sugars content. We can see that most of modern cultivars were placed on the negative side of PC2 and most of local cultivars were placed on the positive side of PC1 (Figure 3.2).

3.5. CONCLUSIONS

Considerable variation has been found in this peach and nectarine germplasm collection for agronomical, pomological, sugar profile and phytochemical traits. This wide variability in the agronomical and fruit quality traits among the studied cultivars indicates their genetic diversity and their diverse genetic origins. The genetic diversity in peach and nectarine cultivars shows that the cultivars and genotypes play a key role in determining these fruit characteristics. In addition, these results show the importance of the traditional Spanish and the old modern peach cultivars to be considered in future breeding programs searching for high quality fruits.

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Capítulo 4

Population structure and marker-trait associations for pomological traits in peach and nectarine cultivars using SSR markers

4.1. ABSTRACT

Marker-trait associations based on populations from controlled crosses have been established in peach using markers mapped on the peach consensus map. In this study, we explored the utility of unstructured populations for association mapping to determine useful marker-trait associations in peach/nectarine cultivars. We used 94 peach cultivars representing local Spanish and modern cultivars from international breeding programs that are maintained at the Experimental Station of Aula Dei, Spain. This collection was characterized for pomological traits and was screened with 40 SSR markers that span the peach genome.

Population structure analysis using STRUCTURE software identified two subpopulations, the local and modern cultivars, with admixture within both groups. The local Spanish cultivars were somewhat less diverse than modern cultivars. Marker-trait associations were determined in TASSEL with and without modelling coefficient of membership (Q) values as covariates. The results showed significant associations with pomological traits. We chose three markers on LG4 because of their proximity to the endoPG locus (freestone-melting flesh) that strongly affects pomological traits. Two genotypes of BPPCT015 marker showed significant associations with harvest date, flavonoids and sorbitol. Also, two genotypes of CPPCT028 showed associations with harvest date, total phenolics, RAC and total sugars.

Finally, two genotypes of endoPG1 showed associations with flesh firmness and total sugars. The analysis of linkage disequilibrium (LD) revealed a high level of LD up to 20 cM, and decay at farther distances. Therefore, association mapping could be a powerful tool for identifying marker-trait associations and would be useful for marker-assisted selection (MAS) in peach breeding.

Keywords: *Prunus persica*, germplasm, population genetics, linkage disequilibrium, simple sequence repeats

4.2. INTRODUCTION

Peach (*Prunus persica* L.) is the third most important temperate fruit crop worldwide, after apple and pear. The main producer countries are China, Italy, Spain, and the United States (FAOSTAT, 2011; http://faostat.fao.org). Peach is native to China and spread to the Mediterranean through Persia (Hedrick, 1917). Later, peaches were brought by Spanish explorers to America and disseminated among the Aztecs in Mexico. From Mexico, peaches spread to New Mexico, Arizona, and California (Hedrick, 1917). Early peach culture was based on seed propagation and for centuries, peach has been cultivated and selected for different agronomic characters, leading to locally adapted populations (Hedrick, 1917). Modern peach cultivars have a narrow genetic base due to the limited number of genotypes used as parents in breeding programs (Myles et al., 2009). Consequently, peach diversity has been drastically reduced by the use of modern cultivars that share a few common ancestors (Aranzana et al., 2003). The Spanish peach industry was based on yellow, non-melting fleshed and clingstone types, but the replacement of the Spanish traditional varieties by introduced ones, mostly from North America, has induced the domain of the melting flesh cultivars (Badenes et al., 1998). The local germplasm collection at the Experimental Station of Aula Dei (Zaragoza, Spain) have been previously evaluated, regarding harvest season from June to October and horticultural traits like flesh and skin color (yellow/orange/white), depth of stalk cavity (deep/shallow), stone adherence (clingstone/freestone), and size and shape of fruit (small/large and round/ovate) (Bouhadida et al., 2011).

One of the most practical applications of DNA-based markers in breeding is the ability to select phenotypic traits using markers tightly linked to genes controlling these traits. Economically valuable fruit traits cannot be evaluated until the trees mature and produce ripe fruit. Once markers have been identified, marker assisted selection (MAS) can increase economic returns, as the larger selection gains compensate for the higher costs of MAS (Bus et al., 2009) since higher selection gains compared with phenotypic selection (Moreau et al., 2000) will accelerate the breeding process (Yousef and Juvik, 2001). The MAS application during the juvenile phase has been proposed to speed selection or reduce progeny sizes and the cost of carrying individuals to maturity in the field. The endoPG marker plays a vital role in fruit texture and cell wall degradation in peach. It has been used in peach breeding programs to distinguish between freestone

and clingstone melting flesh and clingstone non-melting flesh progeny at the seedling stage (Peace et al., 2005). Potential benefits of MAS for fruit breeding programs in *Prunus* are many, including estimation of haplotype frequencies and haplotype-phenotype associations (Bielenberg et al., 2009; Pozzi and Vecchietti, 2009). Peach is one of the best genetically characterized *Prunus* species, with known genes controlling important traits that display Mendelian inheritance patterns such as flesh color, flesh adherence to the stone, or acidity (Dirlewanger and Arús, 2004; Monet et al. 1996). The conventional approach for analysis of marker-trait association in *Prunus* uses mapping populations which segregate for the characters of interest. In peach, several candidate genes and QTLs controlling important traits, such as blooming and harvest date, soluble solids content, titratable acidity, sugars, and other fruit quality traits, have previously been mapped and many have been located on the *Prunus* reference map (Arús et al., 2012 and references therein; Illa et al., 2011; Ogundiwin et al., 2009). To our knowledge, few of these molecular markers associated with fruit traits are being used in practical peach breeding programs.

Association mapping, also known as linkage disequilibrium (LD) mapping, is an approach that detects and locates genes relative to an existing map of genetic markers (Mackay and Powell, 2007). In plants, it can be done using a case-control design or unstructured populations (i.e., populations without progenies that are also non-pedigree linked) (Oraguzie et al., 2007). A few studies have been carried out in the Rosaceae family members, including apple (Cevik et al., 2010) and pear (Oraguzie et al., 2010). These studies demonstrated that association mapping is a valuable tool for determining marker-trait association, detecting novel genes for important agronomic traits, and developing tools for genome-wide variability surveys. The complex breeding history of many important crops and the limited gene flow in most wild plant species have created complex stratification within the germplasm, which could complicate association studies (Sharbel et al., 2000). Analysis of population structure and accounting for admixture or subgroups within unrelated germplasm (Ganopoulos et al., 2011; Mariette et al., 2010) increases confidence in association studies.

Our study was designed 1) to analyze population structure within the peach/nectarine germplasm located at the Experimental Station of Aula Dei (CSIC), Spain, and 2) to explore the utility of association mapping for detecting marker-trait association in fruit quality traits for potential application in breeding programs.

4.3. MATERIALS AND METHODS

4.3.1. Plant material

A collection of 94 peach and nectarine [*Prunus persica* (L.) Batsch] cultivars encompassing a wide range of geographic origins were used in this study (Table 4.1). This set included 43 native local Spanish cultivars and 51 modern cultivars mostly from the U.S., but also from France, Italy, New Zealand, and South Africa. The presumed parentage of most of these cultivars is also included. The genotypes were grown under Mediterranean soil conditions at the Experimental Station of Aula Dei (CSIC) located at Zaragoza in the Ebro Valley (northern Spain).

4.3.2. Fruit Sampling

Twenty fruits were randomly harvested from each cultivar at commercial maturity. Fruits were peeled and cut longitudinally into two halves and a portion of the mesocarp was removed from each half and cut into small pieces. A composite sample of 5 g was built by mixing all pieces from the selected fruits. This was frozen in liquid nitrogen and kept at -20ºC until analyses. Samples for vitamin C determination were kept at -20ºC in metaphosphoric solution (5% HPO_3) until analysis for preservation of oxidation. For analysis of sugars content, samples were homogenized with 10 mL of extraction solution consisting of 800 mL/L ethanol/Milli-Q water. For analysis of antioxidant compounds, samples were homogenized with 10 mL of extraction solution consisting of 0.5 N HCl in methanol/Mili-Q water (80% v/v) and, to determine vitamin C, samples were homogenized with 5% HPO_3. Samples were homogenized using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington) and extracts were centrifuged at 20,000 g for 20 min at 4ºC, and the supernatant was collected and stored at -20ºC.

Table 4.1. Cultivar name, classification, origin, main fruit characteristics and pedigree of the cultivars studied.

Cultivar	Classification	Origin	Flesh colour	Fruit type	Flesh type	Stone type	Reported parentage
Adriatica	Modern cultivars	Italy	Yellow	Peach	Non-melting	Clingstone	-
Alcañiz 1	Local cultivars	Teruel, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Alcañiz 2	Local cultivars	Teruel, Spain	Yellow	Peach	Non-melting	Clingstone	op
Alejandro Dumas (351 AD)	Local cultivars	La Rioja, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Amarillo Calanda (131 AD)	Local cultivars	Huesca, Spain	Yellow	Peach	Non-melting	Clingstone	op
Amarillo Calanda (2400 AD)	Local cultivars	Huesca, Spain	Yellow	Peach	Non-melting	Clingstone	op
Amarillo Gallur	Local cultivars	Zaragoza, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Andora	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Libbee x Lovell
Andross	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Dix 5A-1 x Fortuna
Baby Gold 5	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	PI35201 x NJ196
Baby Gold 6	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	NJ13232 x NJ196
Baby Gold 7	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	(Lemon Free x PI35201) x NJ196
Baby Gold 8	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	PI35201 x Ambergem
Baby Gold 9	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	PI35201 x PI43137
Baladin	Modern cultivars	France	Yellow/Orange	Peach	Non-melting	Clingstone	-
Benasque (3135 AD)	Local cultivars	Huesca, Spain	White	Peach	Melting	Freestone	op
Big Top	Modern cultivars	USA	Yellow	Nectarine	Melting	Clingstone	-
Bonet I	Local cultivars	Lérida, Spain	Yellow	Peach	Non-melting	Clingstone	op
Bonet II	Local cultivars	Lérida, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Bonet III	Local cultivars	Lérida, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Bonet IV	Local cultivars	Lérida, Spain	Yellow	Peach	Non-melting	Clingstone	op
Bonet V	Local cultivars	Lérida, Spain	Yellow	Peach	Non-melting	Clingstone	op
Borracho de Jarque	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	Clingstone	op
Brasileño	Local cultivars	Murcia, Spain	Yellow	Peach	Non-melting	Clingstone	op
Calabacero (2247 AD)	Local cultivars	Murcia, Spain	Yellow	Peach	Non-melting	Clingstone	op
Calanda San Miguel	Local cultivars	Teruel, Spain	Yellow	Peach	Non-melting	Clingstone	op
Calanda Tardío	Local cultivars	Teruel, Spain	Yellow	Peach	Non-melting	Clingstone	op
Campiel (3139 AD)	Local cultivars	Huesca, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Campiel Rojo	Local cultivars	Huesca, Spain	Yellow	Peach	Non-melting	Clingstone	op
Carolyn	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Libbee x Lovell

(continue)

Cultivar	Classification	Origin	Flesh colour	Fruit type	Flesh type	Stone type	Reported parentage
Carson	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Maxine x Leader
Catherina	Modern cultivars	USA	Yellow/Orange	Peach	Non-melting	Clingstone	NJC95 x D42-13W
Del Gorro	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	clingstone	op
Diamante Amarillo	Local cultivars	Teruel, Spain	Yellow	Peach	Non-melting	Clingstone	op
Dixon	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Orange Cling x Australian Muir
Everts	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Dix 5A-1 x Dix 22A-5
Fantasia	Modern cultivars	USA	Yellow	Nectarine	Melting	Freestone	Gold King x P101-24
Flamekist	Modern cultivars	USA	Yellow	Nectarine	Melting	Clingstone	Gold King self
Flavortop	Modern cultivars	USA	Yellow	Nectarine	Melting	Freestone	Fairtime op
Fortuna	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Leader sdlg x (Tuscan x Paloro)
GF3	Modern cultivars	France	Yellow	Peach	Non-melting	Clingstone	-
Goiri	Local cultivars	Bilbao, Spain	Yellow	Peach	Non-melting	Clingstone	op
Golden Queen	Modern cultivars	New Zealand	Yellow	Peach	Non-melting	Clingstone	-
Gomes	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	unknown (originated in California)
Halford	Modern cultivars	USA	Yellow/Orange	Peach	Non-melting	Clingstone	chance sdlg in Phillips Cling orchard
Infanta Isabel (1068 AD)	Local cultivars	Castellón, Spain	Yellow	Peach	Non-melting	Clingstone	op
Jerónimo de Alfaro	Local cultivars	Murcia, Spain	Yellow	Peach	Non-melting	Clingstone	op
Jungerman	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Dix 22A-5 x Dixon 1
Kakamas	Modern cultivars	South Africa	Yellow	Peach	Non-melting	Clingstone	St. Helena op
Keimoes	Modern cultivars	South Africa	Yellow/Orange	Peach	Non-melting	Clingstone	Transvaal op
Klamt	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Dixon 1 x Wiser
Loadel	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Lovell op?
Lovell	Modern cultivars	USA	Yellow	Peach	Melting	Freestone	chance sdlg
Maluenda	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	Clingstone	op
Maria Serena	Modern cultivars	Italy	Yellow	Peach	Non-melting	Clingstone	-
Maruja	Local cultivars	Murcia, Spain	Yellow	Peach	Non-melting	Clingstone	op
Maruja Porvenir	Local cultivars	Murcia, Spain	Yellow	Peach	Non-melting	Clingstone	op
Miraflores (2844 AD)	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	Clingstone	op
Mountaingold	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	PI35201 x NJ196
Nectar del Jalón	Local cultivars	Zaragoza, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
NJC 97	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	-

(continue)

Cultivar	Classification	Origin	Flesh colour	Fruit type	Flesh type	Stone type	Reported parentage
Nuevo (2803 AD)	Modern cultivars	France	Yellow	Peach	Non-melting	Clingstone	Includes PI32374, Peak, Elberta, Peen-To
Oropel	Local cultivars	Teruel, Spain	Yellow	Peach	Non-melting	Clingstone	op
Paloro A	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	-
Paloro B	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	-
Queen Giant	Modern cultivars	USA	White	Nectarine	Melting	Clingstone	-
Redhaven	Modern cultivars	USA	Yellow	Peach	Melting	Semi-clingstone	Halehaven x Kalhaven
Rojo del Rito	Local cultivars	Lérida, Spain	Yellow	Peach	Non-melting	Clingstone	op
San Jaime	Local cultivars	Lérida, Spain	Yellow	Peach	Non-melting	Clingstone	op
San Lorenzo	Local cultivars	Huesca, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Sarell	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	Clingstone	op
Selma	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	-
Shasta	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Leader sdlg x (Tuscan x Paloro)
Stanford	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Hauss x Phillips
Starn	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	chance sdlg in Paloro orchard
Sudanell 1	Local cultivars	Lérida, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Sudanell 2	Local cultivars	Lérida, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Sudanell 3	Local cultivars	Lérida, Spain	Yellow/Orange	Peach	Non-melting	Clingstone	op
Sudanell Blanco	Local cultivars	Zaragoza, Spain	White	Peach	Non-melting	Clingstone	op
Sudanell GF (2804 AD)	Modern cultivars	France	Yellow/Orange	Peach	Non-melting	Clingstone	-
Sudanell GF (2972 AD)	Modern cultivars	France	Yellow/Orange	Peach	Non-melting	Clingstone	-
Suncling	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	PI35201 x NJ196
Super Crimson Gold	Modern cultivars	USA	White	Nectarine	Melting	Clingstone	-
Tebana	Modern cultivars	Italy	Yellow	Peach	Non-melting	Clingstone	-
Tempranillo de Aytona	Local cultivars	Huesca, Spain	Yellow	Peach	Non-melting	Clingstone	op
Tipo Campiel (2921 AD)	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	Clingstone	op
Venus	Modern cultivars	Italy	Yellow	Nectarine	Melting	Freestone	-
Vesuvio	Modern cultivars	Italy	Yellow	Peach	Non-melting	Clingstone	-
Vivian	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	(Marine x Leader) x [(Tuscan x Paloro) x (Paloro x Pratt Low)]
Walgant	Modern cultivars	South Africa	Yellow	Peach	Non-melting	Clingstone	Kakamas selfed
Wiser	Modern cultivars	USA	Yellow	Peach	Non-melting	Clingstone	Lovell x Sims
Zaragozano (553 AD)	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	Clingstone	op
Zaragozano Amarillo (2857 AD)	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	Clingstone	op
Zaragozano Rojo (2858 AD)	Local cultivars	Zaragoza, Spain	Yellow	Peach	Non-melting	Clingstone	op

op open-pollinated, *sdlg* seedlings, *NJ* New Jersey, *self* self-pollinated.

4.3.3. Evaluation of pomological traits

The germplasm was evaluated for morphology of flowers, leaves, and fruits. Bloom and harvest dates were recorded in Julian days. Flower and leaf traits were measured directly in the field while some of the fruit traits were measured in the laboratory immediately after harvest. Phenotypic evaluations were made in 2008, 2009, and 2010. The eleven pomological traits of flowers and leaves evaluated include anther color (red-brown, red-yellow), bloom type (showy, non-showy), flower density (high, medium, few), flower size (small, big), flesh color (yellow, white), flesh type (melting, non-melting), fruit type (peach, nectarine), gland type (globose, reniforme), petal color (pink-salmon, pink), shape type (round, ovate), and stone type (clingstone, freestone). Moreover, other fifteen parameters were analyzed including fruit weight (g), flesh firmness (N), soluble solids content (SSC) (ºBrix), titratable acidity (TA) (g malic acid/100 g FW), ripening index (RI) (SSC/TA), and concentrations of vitamin C (mg AsA/100 g FW), anthocyanins (mg C3GE/kg FW), total phenolics (mg GAE/100 g FW), flavonoids (mg CE/100 g FW), relative antioxidant capacity (mg TE/g FW), and sugars (g/kg FW). Soluble solids content (SSC) measures total juice dissolved solids, including sugars (sucrose, glucose, fructose, and sorbitol), salts, proteins, and acids, while total sugars is the sum of sucrose, glucose, fructose, and sorbitol after fixation and separation by HPLC.

The fruit weight was calculated considering the total number of fruits and the total yield per tree, as previously reported (Font i Forcada et al., 2012). Flesh firmness was measured using a penetrometer (Model FT-327) on both sides of each fruit after removing a 1 mm thick disk of skin. Soluble solids content (SSC) was measured with a digital refractometer (Atago PR-101, Tokyo, Japan). Titratable acidity and pH were determined using an automatic titration system with NaOH titrated to pH end-point of 8.1 (Metrohm Ion analysis, 807 Dosing Unit, Switzerland). Ripening index was calculated based on SSC/TA ratio. Details for all methods were described by Abidi et al. (2011) and Cantín et al. (2009a).

Phytochemical analyses were performed as described by Cantín et al. (2009b) with minor modifications based on Abidi et al. (2011) using a spectrophotometer (Beckman Coulter DU 800). Spectrophotometric determination of vitamin C (ascorbic acid) was as described in Zaharieva and Abadía (2003). Total phenolics were

determined by the Folin-Ciocalteau method as described in Singleton and Rossi (1965), while measurement of total flavonoids was according to Zhishen et al. (1999). The determination of total anthocyanins was based on Fuleki and Francis (1968) while determination of antioxidant capacity was according to Brand-Williams et al. (1995). Total sugars were purified and analyzed by HPLC (Waters 515, Milford, MA, USA) using a 300 x 7.8 mm column (Aminex® HPX-87C, CA, USA) and manual injection (20 µL injection volume) interfaced with a PC Millenium^{32} software.

4.3.4. Microsatellite loci analysis and genotyping

For DNA extraction, one young leaf was collected from each tree, frozen immediately in liquid nitrogen, and stored at -20ºC. DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Dusseldorf, Germany) following the manufacturer's instructions. Forty-two markers previously described in *Prunus* were tested in our population (Table 4.2). These markers were selected for their polymorphism in peach (Bouhadida et al., 2011) (dinucleotide or complex repeats) and their location on the *Prunus* reference map of 'Texas' x 'Earlygold' (Dirlewanger et al., 2004, http://www.rosaceae.org). Twenty-nine SSRs were separated using polyacrylamide gels, eleven markers were separated using an ABI PRISM 3130 Genetic Analyzer and two were analyzed using an ABI PRISM 310 Genetic Analyzer as it is shown in Table 4.2. Forward SSR primers were labelled with 5'-fluorescence dyes including PET, NED, VIC, and 6-FAM and the size standard was Gene Scan^{TM} 500 Liz$^{®}$ (Applied Biosystems) for the ABI PRISM 3130 and ROX (Applied Biosystems) for the ABI PRISM 310. For primers that were separated by polyacrylamide gels, the polymerase chain reaction (PCR) was performed in a 15 µL volume (Bouhadida et al., 2011) and the reaction mixture contained 1x PCR buffer (Biotools, Madrid, Spain), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.15 µM of each primer, 0.5 units Taq DNA Polymerase (Biotools, Madrid, Spain), and 10 ng genomic DNA. PCR was performed in a 16 µL volume and the reaction mixture contained 1x PCR buffer (Biotools, Madrid, Spain), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM of each primer, 0.5 units Taq DNA Polymerase (Biotools, Madrid, Spain), and 30 ng genomic DNA. Both amplifications were conducted in a Gene Amp 2700 (Applied Biosystems) programmed as follows: one cycle of 3 min at 95° C, followed by 35 cycles of 1 min at 94° C, 45 s at the annealing temperature indicated in Table 4.2 for each primer, and 1 min at 72° C, followed by a final incubation of 7 min at 72° C and an infinite hold at 4° C.

Table 4.2. Names and characteristics of the SSR markers used for genotyping the 94 peach/nectarine cultivars.

SSR	Species of origin	Position on LG in the 'TxE' reference map (cM from the top)	AT (ºC)	References	SSRs analysis
		Position on LG 1			
UDP96-005	Peach	29.2	57	Cipriani et al. 1999	Polyacrylamide gels
pchgms3	Peach	37.5	60	Sosinski et al. 2000	Polyacrylamide gels
CPPCT029	Peach	65.1	55	Aranzana et al. 2002	Polyacrylamide gels
BPPCT028	Peach	77.4	57	Dirlewanger et al. 2002	ABI PRISM 3130 Genetic Analyzer
		Position on LG 2			
CPPCT044	Peach	7.4	58	not published (origin IRTA)	Polyacrylamide gels
UDP98-025	Peach	9.6	57	Testolin et al. 2000	Polyacrylamide gels
BPPCT001	Peach	20.9	57	Dirlewanger et al. 2002	Polyacrylamide gels
UDP96-013	Peach	27.8	57	Cipriani et al. 1999	ABI PRISM 3130 Genetic Analyzer
BPPCT024	Peach	36.3	57	Dirlewanger et al. 2002	Polyacrylamide gels
UDP98-410	Peach	38	57	Testolin et al. 2000	Polyacrylamide gels
PceGA34	Sour cherry	43.9	50	Downey and Iezzoni 2000	Polyacrylamide gels
		Position on LG 3			
BPPCT007	Peach	11.2	57	Dirlewanger et al. 2002	ABI PRISM 3130 Genetic Analyzer
CPPCT002	Peach	31.9	52	Aranzana et al. 2002	Polyacrylamide gels
UDP96-008	Peach	36.4	57	Cipriani et al. 1999	Polyacrylamide gels
		Position on LG 4			
BPPCT010	Peach	2.1	57	Dirlewanger et al. 2002	ABI PRISM 3130 Genetic Analyzer
CPPCT028	Peach	11	50	Aranzana et al. 2002	Polyacrylamide gels
pchgms5	Peach	24.1	55	Sosinski et al. 2000	Polyacrylamide gels
UDP96-003	Peach	28.3	55	Cipriani et al. 1999	ABI PRISM 3130 Genetic Analyzer
BPPCT015	Peach	44.0	62	Dirlewanger et al. 2002	ABI PRISM 310 Genetic Analyzer
endoPG1	Peach	47.8	60	Peace et al. 2005	ABI PRISM 310 Genetic Analyzer
CPSCT005	Plum	53.8	62	Mnejja et al. 2004	Polyacrylamide gels
		Position on LG 5			
UDP97-401	Peach	11	57	Cipriani et al. 1999	ABI PRISM 3130 Genetic Analyzer
BPPCT017	Peach	20.1	57	Dirlewanger et al. 2002	ABI PRISM 3130 Genetic Analyzer

(continue)

SSR	Species of origin	Position on LG in the 'TxE' reference map (cM from the top)	AT (ºC)	References	SSRs analysis
pchgms4	Peach	26.7	52	Sosinski et al. 2000	Polyacrylamide gels
BPPCT038	Peach	32.9	57	Dirlewanger et al. 2002	ABI PRISM 3130 Genetic Analyzer
BPPCT014	Peach	44	57	Dirlewanger et al. 2002	Polyacrylamide gels
		Position on LG 6			
UDP96-001	Peach	17.5	57	Cipriani et al. 1999	Polyacrylamide gels
BPPCT008	Peach	30.1	57	Dirlewanger et al. 2002	Polyacrylamide gels
CPPCT023	Peach	41.5	55	Aranzana et al. 2002	Polyacrylamide gels
BPPCT025	Peach	56.4	57	Dirlewanger et al. 2002	Polyacrylamide gels
UDP98-412	Peach	72	57	Testolin et al. 2000	Polyacrylamide gels
CPPCT030	Peach	80.2	50	Aranzana et al. 2002	Polyacrylamide gels
		Position on LG 7			
CPPCT022	Peach	18.7	50	Aranzana et al. 2002	ABI PRISM 3130 Genetic Analyzer
UDP98-408	Peach	23.7	57	Cipriani et al. 1999	Polyacrylamide gels
CPPCT033	Peach	38.9	50	Aranzana et al. 2002	ABI PRISM 3130 Genetic Analyzer
CPPCT017	Peach	61.8	60	Aranzana et al. 2002	Polyacrylamide gels
		Position on LG 8			
BPPCT006	Peach	14.1	57	Dirlewanger et al. 2002	Polyacrylamide gels
BPPCT033	Peach	18.8	57	Dirlewanger et al. 2002	Polyacrylamide gels
CPPCT006	Peach	24.8	59	Aranzana et al. 2002	ABI PRISM 3130 Genetic Analyzer
UDP98-409	Peach	44.5	57	Cipriani et al. 1999	Polyacrylamide gels
CPDCT013	Almond	**Multiloci: LG 3, 6 and 7**	62	Mnejja et al. 2005	Polyacrylamide gels
CPPCT004	Peach	**Multiloci: LG 1 and 5**	52	Aranzana et al. 2002	Polyacrylamide gels

LG linkage group location of the 42 SSR markers, *AT* annealing temperature used.

The gels were silver-stained as described in Bassam et al. (1983). Fragment sizes were estimated with the 30-330 bp AFLP ladder DNA sizing markers (Invitrogen, Carlsbad, CA) and analyzed using the Quantity One program (Bio Rad, Hercules, CA).

For automatic sequencing analysis, PCR products were multiplexed according to their size and primer labelling and separated on the platform of PCTAD (Parque Científico y Tecnológico de Aula Dei, Zaragoza, Spain, in an ABI PRISM 3130 Genetic Analyzer). Amplified fragments were sized using GeneMapper and PeakScanner software (Applied Biosystems). Additionally, fragment analyses for multiplexed primers in an ABI PRISM 310 Genetic Analyzer were performed following published protocols (Peace et al., 2005) at the Washington State University Irrigated Agriculture Research and Extension Center (WSU-IAREC), Prosser, USA.

4.3.5. Data analysis

4.3.5.1. Genetic variability

Several genetic parameters were calculated for all 40 SSRs and between local and modern cultivars (Table 4.2). Two multilocus markers (CPDCT013 and CPPCT004) were not included in this analysis because they are multiloci. The number of observed alleles per locus (A), effective number of alleles per locus (Kimura and Crow 1964) (A_e), observed heterozygosity (H_o = number of heterozygous individuals/ number of individuals scored), expected heterozygosity ($H_e = 1-\sum \rho i^2$, where ρi is the frequency of the ith allele) (Nei, 1973), Wright's fixation index ($F_{is} = 1-H_o/H_e$), Shannon's information index (I) (Lewontin, 1972) and power of discrimination (PD) (Kloosterman et al., 1993) were calculated using PopGene 1.31 software (Yeh et al., 1997, http://www.ualberta.ca). The marker data was used to generate a 0/1 matrix (presence/absence of allele in heterozygosity or homozygosity at the marker locus) that was used to estimate the genetic distance between cultivars. Genetic similarities (GS) were calculated using the Dice coefficient (Nei and Li, 1979) and a dendrogram depicting relationships of the germplasm was built from the GD matrix based on the un-weighted pair group method average (UPGMA) cluster analysis in NTSYS-pc version 2.1 (Rohlf, 2000).

4.3.5.2. Analysis of population structure

STRUCTURE analysis was performed on the whole dataset to test whether peach local cultivars and modern cultivars can be separated. The program STRUCTURE (version 2.3) implements a model-based clustering criterion for inferring population structure using genotypic data from unlinked markers (Pritchard et al., 2000). We fitted all kinds of models including both 'ancestry' and 'allele frequency' models with the option of admixture/no admixture and allele frequency correlated/allele frequency independent, respectively. We used the statistic, ΔK, (where K specifies the number of subpopulations or clusters) based on the rate of change in the log probability of the data (Evanno et al., 2005) to select the number of K (in our case, varying from two to six under the admixture model). We also performed 10 independent runs per K value starting with 10,000 burn-in period and 100,000 MCMC replications. A burn-in of 20,000 and 250,000 Markov Chain Monte Carlo (MCMC) replications seemed to be the best fit for our data at K=3. This cluster showed a very clear peak with the highest height which gave us an indication of the strength of the signal detected by STRUCTURE.

4.3.5.3. Linkage disequilibrium

The analysis of LD was calculated using the TASSEL (Trait Analysis by Association, Evolution and Linkage) version 3 software (http://www.maizegenetics.net). Alleles with frequency below 5% (MAF) were removed. LD between pairs of multiallelic loci was calculated using the r^2 coefficient, separately for loci on the same or on different linkage group (LG). We chose the statistical r^2 as a measure of linkage disequilibrium instead of "D'' which measures only recombination whereas r^2 gives an indication of both recombination and mutation (Flint-Garcia et al., 2003). The significance level of LD between loci was examined using a permutation test implemented in TASSEL software for multiallelic loci, using the ''rapid permutation'' option.

4.3.5.4. Association mapping

We used TASSEL with the General Linear Model (GLM) option (Yu and Buckler 2006) to examine association between the phenotypic traits and DNA markers. We focused the association mapping on LG4 on the *Prunus* reference map of 'Texas' x

'Earlygold' because the *endoPG* gene, involved in softening of peach fruit, is located on this linkage group (Peace et al., 2005), as well as BPPCT015 and CPPCT028. Moreover, these markers showed the highest discrimination power estimation in our study. It is believed (Yu and Buckler, 2006) that a structured association approach could correct for false associations using a Q-matrix of population membership estimates. Therefore, the population membership estimates obtained from STRUCTURE analyses were fitted as a covariate in a GLM where, phenotype=population structure + marker effect + residual. A standard correction for multiple testing, such as Bonferroni procedure (Schulze and McMahon, 2002), was applied. Significant markers were declared using the Bonferroni procedure at the $p<0.00125$ experimental-wide threshold. Alleles with minor frequency (MAF) lower than 5% were removed (Wilson et al., 2004). A minimal number of individuals (<10%) were excluded in the less frequent class of pomological traits.

4.4. RESULTS

4.4.1. Phenotypic evaluation and correlations

A broad phenotypic variation was found for most of the parameters studied in the 94 peach/nectarine cultivars. Range and means for the pomological traits, bioactive compounds content and total antioxidant activity are shown in Table 4.3. Harvest time was earlier almost one week every year. The earliest cultivars to be harvested 185 Julian days (late June) belonged to 'Maria Serena' and 'Super Crimson Gold' whereas the 'Alcañiz 1' and 'Calanda Tardío' latest were harvested with 275 Julian days (late October). Mean values of flesh firmness, vitamin C, phenolics, flavonoids, RAC and total sugars were 38 N, 13 mg AsA/100 g FW, 44 mg GAE/100 g FW, 24 mg CE/100 g FW, 842 mg TE/g FW and 110 g/kg FW, respectively. The Pearson's correlation coefficients between pairs of traits are shown in 4.4. High and significant correlations were found between harvest date, fruit weight, and concentrations of soluble solids, antioxidants, and sugars. These results show that when fruits are harvested late, they are sweeter, larger, and have high total phenolics, flavonoids, RAC, sucrose, sorbitol, and total sugars concentrations.

Table 4.3. Units, minimum, maximum and mean values for the pomological traits evaluated.

Trait	Units	Minimum	Maximum	Mean
Bloom date*	Julian days	79	87	82
Harvest date	Julian days	185	275	224
Fruit weight (FW)*	Grams	64	315	178
Soluble Solids Content (SSC)*	ºBrix	12	18	15
Flesh firmness (FF)	Newtons (kg/cm^2)	9	61	38
Titratable acidity (TA)*	g malic acid/100 g FW	0.4	0.9	0.62
Ripening index (RI)	SSC/TA	15	67	25
Vitamin C	mg AsA/100 g FW	3	28	13
Total phenolics	mg GAE/100 g FW	18	62	44
Flavonoids	mg CE/100 g FW	3	63	24
Anthocyanins	mg C3GE/kg FW	0.7	12	3
Relative Antioxidant Capacity (RAC)	mg TE/g FW	186	1184	842
Sucrose	g/kg FW	35	97	75
Glucose*	g/kg FW	4	15	10
Fructose*	g/kg FW	2	14	10
Sorbitol	g/kg FW	2	35	13
Total sugars (TS)	g/kg FW	63	136	110

AsA ascorbic acid, *GAE* gallic acid equivalents, *CE* catechin equivalents, *C3GE* cyanidin-3-glucoside equivalents, *TE* trolox equivalents. *Association analysis was performed with these traits but no association was found.

A significant negative correlation was found between harvest date and flesh firmness and between ripening index, flesh firmness, and concentrations of flavonoids, total phenolics, sucrose, glucose, fructose, sorbitol, and total sugars. This suggests that softer fruit is linked to late harvest date and higher concentrations of sugars and health-benefiting compounds.

High and significant correlations were found between total sugars and sucrose, glucose, fructose, and sorbitol, and between SSC and flavonoids, total phenolics, RAC, and sorbitol (Table 4.4). Other important positive and significant correlations were found between RAC and fruit weight, SSC, vitamin C, flavonoids, and total phenolics and between total phenolics and fruit weight, SSC, and flavonoids. Flavonoids also correlated with fruit weight, SSC, and TA.

Table 4.4. Pearson's correlation coefficients between pairs of pomological traits studied.

Trait	FW	SSC	FF	TA	RI	Vitamin C	Total phenolics	Flavonoids	RAC	Sucrose	Glucose	Fructose	Sorbitol	TS
Harvest date (Julian days)	0.63**	0.63**	-0.52**	ns	ns	ns	0.65**	0.79**	0.72**	0.62**	ns	0.21*	0.78**	0.66**
Fruit weight (g)	ns	0.56**	ns	0.15*	ns	ns	0.53**	0.21*	0.34*	ns	0.36**	0.39*	ns	0.25*
SSC (ºBrix)		-	0.49**	0.26**	ns	ns	0.56**	0.60**	0.61**	0.29**	0.27**	0.36*	0.77**	0.49**
Flesh firmness (N)			-	0.40**	-0.57*	ns	-0.52**	-0.26*	ns	-0.50**	-0.64**	-0.49**	-0.42*	-0.59**
TA (g malic acid/100 g FW)				ns	ns	0.46**	ns	0.35**	ns	ns	0.41**	ns	0.40**	ns
RI (SSC/TA)					ns	-0.21*	ns	ns	ns	0.42**	0.24*	0.35*	0.41**	0.27**
Vitamin C (mg AsA/100 g FW)						ns	ns	ns	0.25*	ns	ns	ns	0.37**	0.42**
Total phenolics (mg GAE/100 g FW)							ns	0.68**	0.79**	0.43**	0.42**	ns	0.52**	0.58**
Flavonoids (mg CE/100 g FW)								ns	0.87**	0.47**	0.44**	0.24*	0.47**	0.61**
RAC (mg TE/g FW)									ns	ns	0.52**	ns	0.64**	0.64**
Sucrose (g/kg FW)										ns	0.57**	0.63**	0.48**	0.95**
Glucose (g/kg FW)											ns	0.83**	0.44**	0.81**
Fructose (g/kg FW)												ns	0.49**	0.83**
Sorbitol (g/kg FW)													ns	0.56**
Total sugars (g/kg FW)														ns

*$p \leq 0.05$, **$p \leq 0.01$ represent significant values, *ns* not significant. See Table 4.3 for abbreviations.

4.4.2. Allelic variation, fixation index and heterozygosity measures

Forty-two SSR markers amplified successfully in the 94 peach/nectarine accessions. To avoid potential error in estimating genetic parameters, markers CPPCT004 and CPDCT13, which amplified more than one locus, were excluded from the analysis. The average estimates of allelic variation, heterozygosity measures, Wright's fixation index, Shannon's information index, and power of discrimination for the remaining 40 SSRs are shown (see supplementary material in annexes 11.2.1). All primers pairs but two produced a maximum of two bands per genotype in accordance with the diploid level of this species. The mean value found in this study was of 5.10 alleles per locus. Microsatellite BPPCT025 detected the highest number of alleles (11) among the 94 genotypes analyzed, followed by BPPCT015 with 10 different alleles. BPPCT014, CPPCT023, CPPCT033, CPSCT005, pchgms4, pchgms5, UDP96-005, and UDP97-401 detected the lowest number of alleles, only two. Amplification with the others 30 SSRs were variable, ranging between 3 and 9 (see supplementary material in annexes 11.2.1). H_o values ranged from 0.06 (BPPCT014) to 0.98 (BPPCT033, UDP98-025 and UDP98-409), and the values for H_e ranged between 0.06 (BPPCT014) to 0.81 (BPPCT015), with an average of 0.48 and 0.49, respectively. F_{is} values were positive in 23 primers, zero in BPPCT014, and negative in the remaining sixteen SSRs, indicating a high level of heterozygosis in the genotypes analyzed. Regarding power of discrimination, the BPPCT015 and CPPCT028 were the best at discriminating between two random cultivars (PD=0.73 and 0.72, respectively), whereas the less informative was BPPCT014 (PD=0.06). Generally, genetic parameters were higher in modern than in local cultivars. The total number of alleles across all 40 SSR loci was higher in local cultivars (172) than in modern cultivars (159) (see supplementary material in annexes 11.2.1).

4.4.3. Population structure

The peach collection, including local cultivars and modern cultivars, was evaluated for population stratification or admixture using STRUCTURE software. Bar plots were obtained with different values of K, the assumed number of subpopulations. The maximum rate of change in the log probability of the data occurred at K=3. In general, there were two populations with subpopulation one comprising modern cultivars and subpopulation two representing local cultivars. However, there was a little

bit of admixture in each subpopulation suggesting allele sharing (Figure 4.1). For comparison, at K=3 (Figure 4.1b), the results were congruent, suggesting a more complex structure that with K=2 (Figure 4.1a). When increasing K, the subpopulations became almost inseparable (Figure not shown).

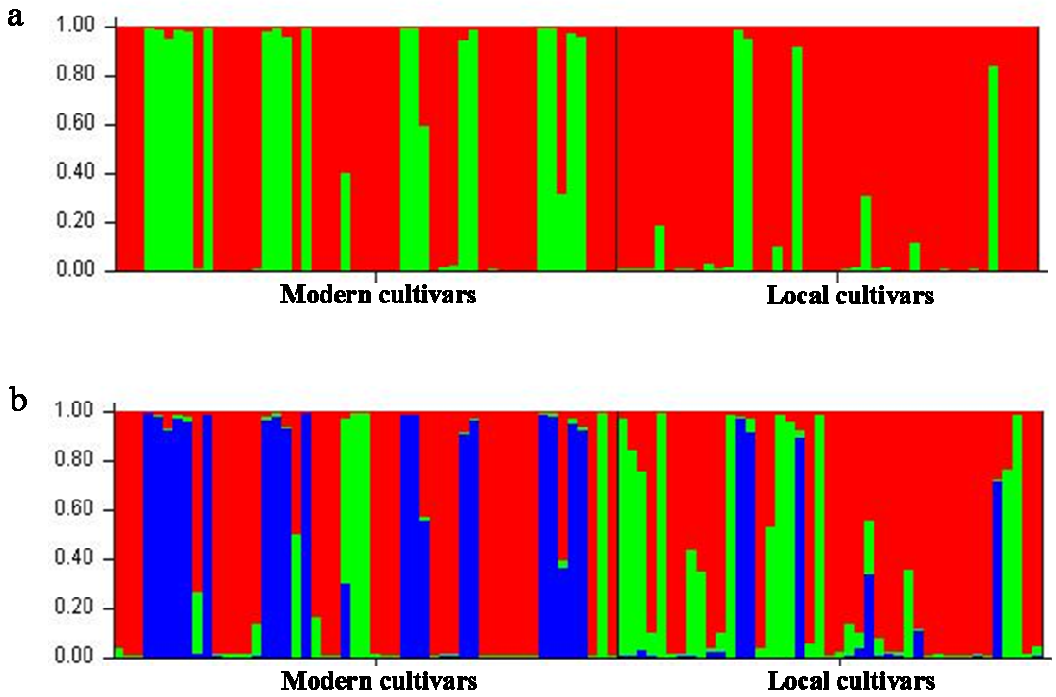

Figure 4.1. STRUCTURE bar plots based on 94 peach/nectarine cultivars at K=2 (a) and K=3 (b). Green and blue represent individuals within the subpopulations. Any blue or green bar that is not completely filled indicates admixture.

Clusters obtained by STRUCTURE for population stratification were compared with the UPGMA analysis. The pattern of diversity in morphological characteristics within the germplasm is shown in Figure 4.2. A tree constructed from the SSR data divided the cluster into sub-clusters characterized by correspondence with fruit characteristics and local or modern cultivars. For example, nectarines, modern cultivars, and melting flesh varieties such as 'Big Top', 'Fantasia', 'Flamekist', 'Flavortop', 'Queen Giant', and 'Venus' are grouped in the same cluster. However, melting peaches 'Benasque', 'Lovell', and 'Redhaven' group according to their origin. 'Lovell' grouped close to 'Halford', 'Gomes', and 'Starn', all USA cultivars, and 'Redhaven' grouped close to 'Babygold 6', 'Babygold 7', and 'Babygold 8', also all from the USA. Furthermore, some of the cultivars are clustered together following the reported parentage (Table 4.1). Thus, 'Andora' and 'Carolyn' are clustered together as they came

from the same cross ('Libee' x 'Lovell'). This was also the case with 'Starn' and 'Shasta', 'Suncling' and 'Babygold 9', 'Andross' and 'Everts' or 'Fantasia' and 'Flamekist', that share a common parent ('Paloro', 'PI35201', 'Dix 5A-1' and 'Gold King', respectively).

In the dendrogram, there is a clear agreement between clusters representing genetic diversity and population structure at K=2, particularly, the differentiation of local cultivars and modern cultivars (Figure 4.2a). Most accessions grouped with either local cultivars (green) or modern cultivars (red).

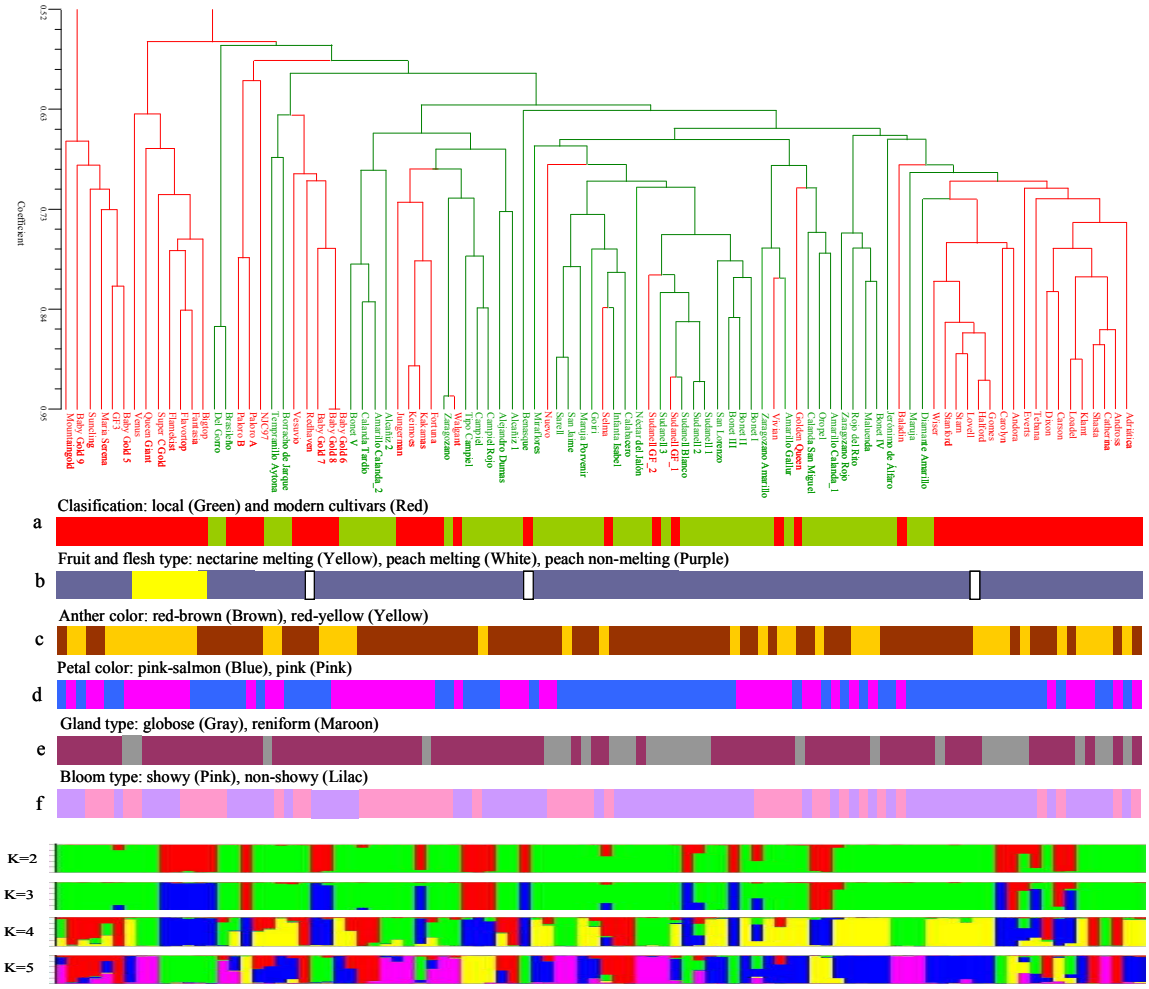

Figure 4.2. Dendrogram of 94 peach/nectarine cultivars based on pair-wise genetic distances with 40 SSRs, and population structure based on different K values (K=2, 3, 4, and 5) separating individuals based on (a) local versus modern cultivars, (b) fruit characteristics, (c, d, f) flower and (e) leaf characteristics.

Also, there was clear separation between peaches and nectarines (Figure 4.2b) and by leaf gland (Figure 4.2e). At K=2, we observed a split between local cultivars and modern cultivars. At K=3, the clusters of local and modern cultivars split into two subpopulations and most cultivars fell into either a group with red-brown anthers (Figure 4.2c) and pink-salmon petals (Figure 4.2d) for local cultivars or a group with red-yellow anthers (Figure 4.2c) and pink petals (Figure 4.2d) for modern cultivars. For nectarines, there is a clear connection between red-yellow anthers, pink petals, and leaf reniform gland. For peaches, the results are mixed. Finally, the separation between showy and non-showy flowers was difficult because the clusters were mixed (Figure 4.2f). With increasing K, the red subpopulation remained almost inseparable (at K=4 and K=5, Figure 4.2), while the green subpopulation became divided into smaller subpopulations.

4.4.4. Linkage disequilibrium

Even though the density of coverage of the genome was low (the average distance between pairs of markers was 10 cM), we detected some trends of LD between pairs of markers (Table 4.5). For the whole set of varieties, overall LD was low, with some indication of higher LD up to 20 cM, and a decay at farther distances, to approximately the same level shown by unlinked markers. The same trend was observed for the local and modern cultivars. For the groups determined with the STRUCTURE analysis, LD relationship with distance was variable. Groups Q1 and Q3 showed higher LD overall, and it extended even to 30 cM at group Q1.

Table 4.5. Linkage disequilibrium scores (r^2), averaged for distance classes and germplasm groups according to the analysis with software STRUCTURE (Q1-Q3) and previous knowledge of the varieties (local vs. modern).

			Structure groups			Breeding history	
Range (cM)	N*	Total n=94	Group Q1 n=20	Group Q2 n=55	Group Q3 n=19	Local n=43	Modern n=51
0-10	20	0.044	0.128	0.027	0.120	0.058	0.068
10-20	24	0.069	0.144	0.029	0.140	0.053	0.100
20-30	21	0.026	0.128	0.045	0.047	0.039	0.048
>30	23	0.023	0.078	0.021	0.106	0.036	0.035
Intrachromosomal	88	0.041	0.120	0.030	0.105	0.046	0.063
Interchromosomal	692	0.028	0.098	0.033	0.105	0.037	0.045

*number of marker pairs included in each class.

For group Q2, LD was no different from background at any distance. Except for groups Q2 and Q3, intrachromosomal LD was slightly higher than interchromosomal LD. Attending to the distribution of LD across linkage groups, the markers of LG5 presented clearly higher scores than interchromosomal LD, or even intrachromosomal LD at the other linkage groups (Figure 4.3 and supplementary material in annexes 11.2.2). LG7 also presented higher values than others at groups Q1 and Q3, but showed low values for the whole sample, or the local and modern cultivars (see supplementary material in annexes 11.2.2).

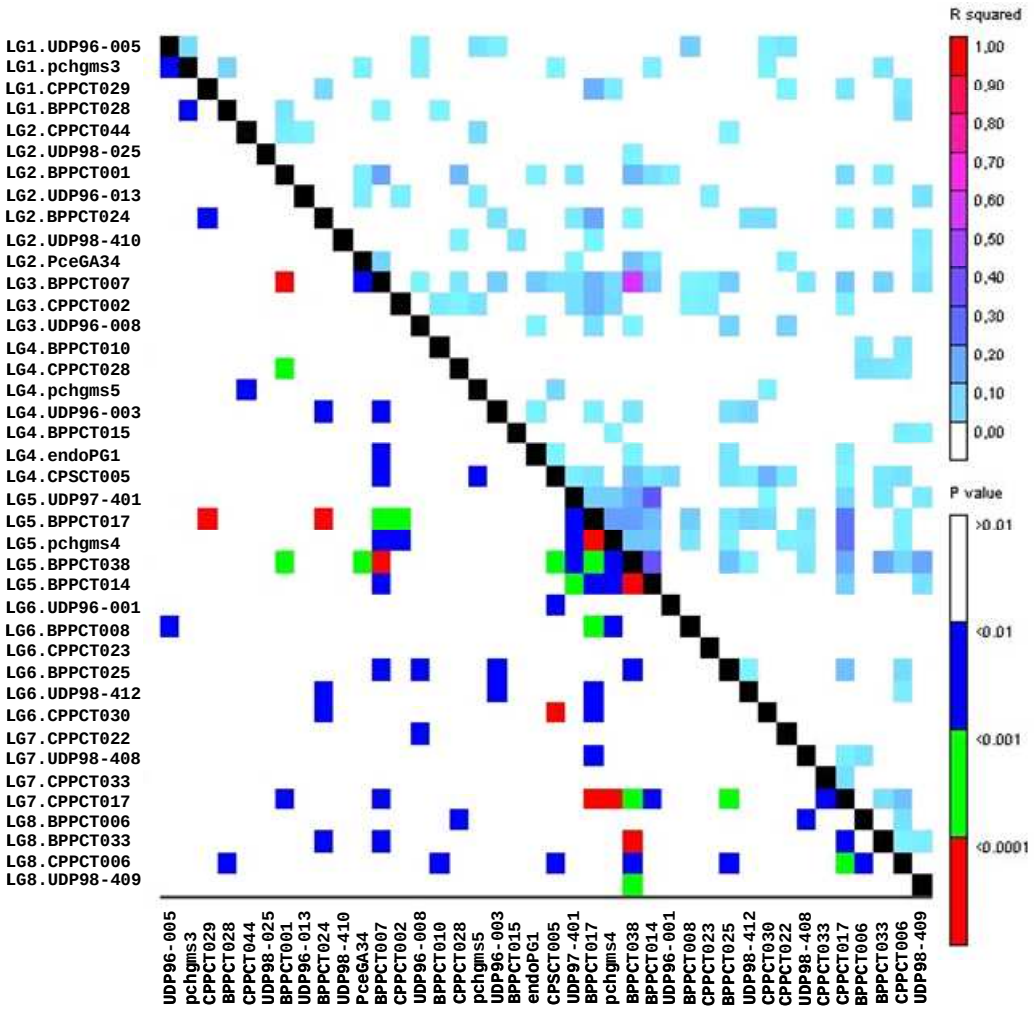

Figure 4.3. Linkage disequilibrium plot based on 40 SSR markers screened in 94 peach/nectarine cultivars. At the right side are represented the r^2 values and at the left side the *p*-values, according the colors of the legend.

4.4.5. Association mapping

Analysis of marker-trait associations using 40 SSR markers with 26 pomological traits was done using TASSEL software. After the Bonferroni procedure the number of

associations was reduced from 296 to 55 using a modelling coefficient of membership (Q) values estimates from STRUCTURE as co-variate and to 61 without co-variate. We will focus on significant associations obtained using Q values since they are more conservative (Table 4.6). Henceforth, our attention will be on associations identified based on endoPG1, marker involved in softening, BPPCT015, and CPPCT028, all located on LG4. The power of discrimination of these markers was higher than others located on the same LG (see supplementary material in annexes 11.2.1, 0.51, 0.73, and 0.72, respectively). BPPCT015 marker was significantly associated with harvest date (p=0.0000072), flavonoids (p=0.000081) and sorbitol contents (p=0.000013) (Table 4.6). CPPCT028 was associated with anther color (p=0.000011), flesh fruit color (p=0.0000001), harvest date (p=0.00037), phenolics (p=0.000019), RAC (p=0.00039) and total sugars (p=0.00016) contents, while endoPG1 was associated with flesh firmness (p=0.000070) and total sugars content (p=0.00061).

Table 4.7 shows the association between the genotype and haplotype with the pomological traits analysed. The 167_167 genotype of BPPCT015 was associated with low concentrations of flavonoids, and sorbitol content which are also linked to medium harvest date. In contrast, the 220_229 genotype was associated with late harvest, and high concentrations of flavonoids and sorbitol. Furthermore, the 136_136 genotype of CPPCT028 was strongly associated with low concentrations of total phenolics, relative antioxidant capacity and total sugars, which are also linked to medium harvest date. The 136_138 genotype of CPPCT028 was associated with late harvest date and high concentrations of total phenolics, RAC and total sugars. The 192_196 genotype of endoPG1 was associated with high firmness, and low to medium concentrations of total sugars, while the 192_228 genotype was associated with high concentrations of total sugars, which are also negatively linked to firmness. Only two haplotypes were associated with one trait. In particular, the 169/136 haplotype from BPPCT015/CPPCT028 was linked to early to medium harvest date while the 209/134 haplotype was strongly associated with late harvest date.

Table 4.6. p-values for pomological traits marker-locus-trait using the TASSEL program. For multiple test of genotypes was applied Bonferroni procedure (Schulze and McMahon 2002).

	AC	FC	Harvest date	FF	RI	Phenolics	Flavonoids	Vitamin C	Anthocyanins	RAC	Sucrose	Sorbitol	TS
BPPCT001		*									**		
BPPCT006						*		*	**				
BPPCT007		*				*							
BPPCT015			**0.0000072**				**0.000081**		Δ			**0.000013**	
BPPCT017					*							***	
BPPCT025		*							*				
BPPCT038					**				*				
CPPCT028	**0.000011**	**0.0000001**	**0.00037**			**0.000019**	Δ			**0.00039**			**0.00016**
CPPCT030		*	*							***			
endoPG1				**0.000070**									**0.00061**
PceGA34		*				***					*		**
pchgms5		**											
UDP96-001			**		***		Δ		*				
UDP96-003	*	*				*		*	*				
UDP96-008									*				
UDP96-013	**	*				*			Δ	Δ	Δ		
UDP98-025	**				**								
UDP98-409	*	*									*		
UDP98-410		**				*	*	**	*				
UDP98-412										***			

The *p*-values for associations are considered when at least one allele is associated with the SSR. *p<0.00001, **p=0.00001-0.0001, ***p=0.0001-0.0012 (considering associations with co-variate), Δ considering associations without co-variate. *AC* anther color, *FC* fruit flesh color, see Table 4.3 for the rest of abbreviations.

Table 4.7. Characteristics and mean values of pomological traits for each genotype and haplotype of BPPCT015, CPPCT028 and endoPG1 markers.

	Genotypes							Haplotypes	
	BPPCT015		CPPCT028			endoPG1		BPPCT015/ CPPCT028	
	167_167	**220_229**	**134_136**	**136_136**	**136_138**	**192_196**	**192_228**	**169/136**	**209/134**
Anther color	-	-	Red-brown	-	-	-	-	-	-
Fruit flesh color	-	-	Yellow	-	-	-	-	-	-
Harvest date	216	244	-	199	251	-	-	209	257
Flesh firmness	-	-	-	-	-	50	27	-	-
Total phenolics	-	-	-	21	56	-	-	-	-
Flavonoids	12	47	-	-	-	-	-	-	-
Relative Antioxidant Capacity	-	-	-	316	997	-	-	-	-
Sorbitol	7	27	-	-	-	-	-	-	-
Total sugars	-	-	-	97	135	90	127	-	-

(-): no associations between traits and genotypes and/or haplotypes. See Table 4.3 for abbreviations, units, maximum, minimum and mean values for the pomological traits evaluated.

4.5. DISCUSSION

4.5.1. Phenotypic evaluation

A broad phenotypic variation was found for all the parameters studied in the 94 peaches and nectarines cultivars except for bloom date. Harvest date varied among cultivars with values in the range of 185-275 Julian days. This trait has been established as characteristic of each cultivar, and quantitatively inherited (Dirlewanger et al., 1999). Moreover, harvest date may change every year depending on the environmental conditions and/or cultivars but harvest season remains constant (Mounzer et al., 2008). All pomological traits evaluated were in the same range than those reported by other authors in other peach cultivars (Cantín et al., 2009a, 2009b; Cevallos-Casals et al., 2006; Gil et al., 2002; Tavarini et al., 2008; Tomás-Barberán et al., 2001).

4.5.2. Allelic variation, fixation index, heterozygosity measures

The 42 SSR markers covering the peach genome used to screen the 94 peach/nectarine cultivars were previously used for cultivar identification and genetic mapping (Testolin et al., 2000) and for phylogenetic studies in peach and other *Prunus* species (Aranzana et al., 2003; Bouhadida et al., 2007, 2009, 2011). The successful amplification of these markers in peach and other *Prunus* species demonstrates the high synteny across this genus (Aranzana et al., 2003). Markers BPPCT001, BPPCT006, BPPCT008, CPPCT006, CPPCT022, CPPCT029, PceGA34, pchgms3, and UDP98-412 were also used to study genetic variation in peach (Bouhadida et al., 2007, 2011), with reported polymorphism similar to ours. The mean value found in this study was of 5.10 alleles per locus, which is slightly lower that the 6.36 observed by the Aranzana et al. (2010) and 6.73 by Bouhadida et al. (2011). The observed heterozygosity averaged (0.48) over the 40 SSR loci was slightly higher than reported values of 0.35 (Aranzana et al., 2003, 2010) and 0.23 (Bouhadida et al., 2011). High F_{is} values in combination with homozygosity (or individuals showing only one band) in these primers suggest the presence of a null allele (Brookfield, 1996). The presence of null alleles affecting heterozygosity could cause such differences. The fixation index and the power of discrimination was slightly lower than others reported (Aranzana et al., 2003; Bouhadida et al., 2011). The differences found in this study could be due either to the different plant material used or to the use of SSRs markers with lower PD. The modern

cultivars in our collection were as genetically diverse as the local cultivars. These results are different to those found in a self-incompatible species such as cherry (Mariette et al., 2010), where local cultivars were more diverse than modern cultivars. This is congruent with current understanding of the evolutionary history of clonally propagated domesticated plants (McKey et al., 2010). It is noteworthy that peach is the less polymorphic species within the *Prunus* because of its condition of self-compatibility.

4.5.3. Population structure

The analysis performed with the STRUCTURE software showed that using K=2 the results suggested that our peach germplasm comprises two main subpopulations with some degree of admixture within both subpopulations (modern and local cultivars). With K=3 and higher, the differentiation was not so apparent. Similar studies in peach reported three unstructured populations including 94 melting peaches, 39 non-melting peaches, and 91 nectarines, indicating a strong subpopulation structure (Aranzana et al., 2010). In our study, nectarines grouped in one cluster similar to what the authors above showed (see Figure 2). Further, according to these authors, some non-melting peaches such as 'Jerónimo', 'Calabacero', 'San Lorenzo', and 'Maruja' grouped according to their Spanish origin while 'Babygold 7', 'Babygold 8', 'Andross', and 'Catherina' grouped according to their foreign origin; a finding similar to our results. The domestication of peach was likely a complex process with several origins resulting from clonal propagation of desirable genotypes and sexual reproduction with local wild peaches. Domestication and breeding generally cause diversity loss, resulting in bottleneck and genetic drift. Diversity after a bottleneck depends on the ratio of wild and cultivated population sizes and the duration of the bottleneck (Haudry et al., 2007). In many fruit species, domestication occurred relatively late, so the bottleneck was relatively recent and its duration short. Although the population genetic parameters obtained suggest that Spanish local cultivars are slightly less diverse than modern cultivars, we interpret these results with caution, since our sampling was limited to the material conserved in our collection. In particular, our local cultivars were selected from populations that have been seed-propagated, possibly over many generations, while the modern cultivars were obtained by crossing two individuals and selecting progeny. Other studies in peach addressing genetic variability of introduced and local Spanish cultivars showed differentiation of accessions according to adaptation to different

environmental conditions (Bouhadida et al., 2011). In particular, Ebro Valley cultivars clustered with the USA releases, suggesting a common gene pool. These results agree, considering the active exchange of germplasm between both countries and the extensive use of Spanish cultivars in American peach breeding programs (Okie, 1998).

4.5.5. Linkage disequilibrium

The overall level of LD detected was rather low, but this depends on the density of marker coverage, which was rather sparse in this study. The average interval was 10 cM, with a maximum of 16 cM at LG1, and a minimum of 8 cM at LG5, but the correlation of intrachromosomal LD with mean interval size across LG was low and non-significant (data not shown). Looking at trends of LD, it decreased with distance, fading away after 20 cM. This value is in the same range as the extent of LD found also in peach by Aranzana et al. (2010). The higher LD observed in LG5 was evident for all groups of varieties, except for Q2 (see supplementary material in annexes 11.2.3-11.2.7). This means that the haplotypes of markers at this LG tend to be more homogeneous within groups than at other LGs. This may have been caused by a selection event of a founder effect affecting specifically genes of this LG, and that did not affect the group of varieties in Q2. One possible cause was the presence of a distinct group of nectarines (7 individuals), which was included within the modern cultivars and the Q1 groups, respectively for the two classifications considered. This group is characterized by the presence of the allele that confers the non-hairy trait, at locus *G* in LG5. We can speculate that the varieties carrying this allele may have experienced linkage drag for the rest of LG5 during breeding, and this may have influenced the level of LD detected for this LG at the groups containing the nectarines. To test this hypothesis, we repeated the analyses of LD for the modern and Q1 groups excluding the nectarines, and the result was the same. Therefore, this higher level of LD at LG5 was not caused by the presence of the nectarine group.

4.5.6. Marker-trait associations and phenotypic correlations

Genome-wide analysis using a GLM procedure in TASSEL identified three loci, BPPCT015, CPPCT028, and endoPG1, which were previously mapped to chromosome 4 and associated with pomological traits in the peach/nectarine germplasm. We analyzed these markers separately because they are on LG4 and showed high polymorphism and power of discrimination.

Different combinations of genotypes/haplotypes associated with important pomological traits were obtained. For example, the 192_196 and 192_228 genotypes of endoPG1 associated significantly with low/high content of total sugars and high/low firmness. Both parameters are indirectly linked because when fruits are ripe, they have low firmness and high total sugars content. Also, the significant negative correlation obtained between them confirmed the associations found. On the contrary, we did not find significant associations between endoPG1 and flesh type and stone type. This lack of association is probably because melting and freestone peaches and nectarines are not well represented in our germplasm. Only 10 cultivars out of 94 cultivars belong to the melting type and 5 cultivars out of 94 belong to freestone. The lack of melting flesh type material in our collection happened because historically, the Spanish peach industry was based on non-melting flesh peaches, primarily derived from native populations, both for fresh market and canning purposes (Badenes et al., 1998; Cambra, 1988; Herrero, 1953). Other important associations were found between the 167_167 and 220_229 genotypes of BPPCT015, the 136_136 and 136_138 genotypes of CPPCT028, with other pomological traits (i.e. different content in antioxidants and sugars). In addition, associations were found between the haplotypes 169/136 and 209/134 of BPPCT015/CPPCT028 with harvest date.

Furthermore, the correlations found in this work among several pomological traits confirm the associations discussed above. For example, high sorbitol was associated to high flavonoids and late harvest, and it exist significant positive correlations among harvest date, SSC, flavonoids, sorbitol and total sugars. Genotypes with high sorbitol are currently of interest for fruit breeders (Ledbetter et al., 2006) since this sugar can be alternatively used as sweetener for diabetics (Cantín et al., 2009a). Moreover, from a practical point of view, the significant positive correlations found between SSC and total sugars, and the fact that those characters were associated, suggest that high SSC can be used as an indirect measure to select genotypes for high total sugars and flavonoids content.

The results found in this study support the potential of the SSR association mapping for agronomical and biochemical important traits in peach. Besides several studies in identifying marker-trait association have been published in other plant species in the Rosaceae family (Cevik et al., 2010; Oraguzie et al., 2010), to our knowledge this is the first study concerning association mapping with pomological traits in peach.

Previously in peach, several QTLs affecting pomological and agronomic traits that have been on the *Prunus* reference map were reported on LG4 for SSC, TA and pH (Cantín et al., 2010a); SSC, glucose fructose, sorbitol, blooming and harvest date (Arús et al. 2012 and references therein). Other QTLs for fructose, sorbitol content and several organic acids were also located on LG4 on a region corresponding to bin 4:27 of T × E (Ogundiwin et al., 2009). In addition, it is remarkable to note that other authors found QTLs for glucose, fructose and sorbitol in peach linked to the BPPCT015 marker (Illa et al., 2011) and for ripening date in almond linked to the CPPCT028 (Sánchez-Pérez et al., 2007). Other QTL explaining maturity date was mapped near the EPPISF032 marker on LG4 (Eduardo et al. 2011) and others controlling antioxidant compounds content (Abidi, personal communication) were located on this linkage group. Besides of these QTLs, several candidate genes linked to a potential role acidity, and phenolic content and fruit growth were mapped on other LGs 3, 5, and 7 (Le Dantec et al., 2010). Regarding bloom date we did not find any correlation or association in our study. However, Fan et al. (2010) found strong QTLs on LG1 during four years in a segregating family. These differences could be probably due to the different plant material used in both studies apart of the environmental effects on bloom date as it was already discussed by these authors. The range of blooming date in the population varied from 16 days (year 2006) to 53 days (year 2007) while our 94 genotypes showed only eight days of variation among genotypes. Likewise, some SSR markers linked to specific monogenic traits have been developed in peach although few practical examples have been described in MAS. The *endoPG* gene has been used in marker assisted selection for distinguishing between melting and non-melting at the seedling stage in peach breeding programs (Peace et al., 2005). Concerning the showy flower type (*Sh*), Fan et al. (2010) located the gene on LG8 1cM from CPPCT006 and Eduardo et al. (2011) described the character cosegregating with ssrCITA15 on the same LG. Another marker, MA014a, apparently was defined controlling flat fruit (*S*) and aborting fruit (*Af*) as single gene (Dirlewanger et al., 2006), however, some discrepancies were described for other authors (Cantín et al., 2010b).

Based on the significant marker-trait association highlighted above, marker-assisted breeding facilitate selection, including prediction of genotype of progeny, leaving only selections with favourable genotypes/alleles for desired pomological traits, and characterising parents used in peach breeding programs. Additionally, this work

provided promising results concerning association mapping with pomological traits that could be applied in other *Prunus* species because of the complete synteny found inside the Rosaceae family.

The present study demonstrates for the first time evidence concerning the utility of association genetics and its potential to generate useful marker-trait associations for application in peach breeding. STRUCTURE analysis identified two main groups, local and modern cultivars, with some admixture within groups. The local cultivars were slightly less diverse than modern cultivars, probably because they were mainly non-melting peach types while the modern cultivars comprised both melting and non-melting peach and nectarine varieties. In addition, our results indicate a subpopulation structure and a relatively high level of linkage disequilibrium conservation. Furthermore, significant associations were observed between genotypes and haplotypes of markers BPPCT015, CPPCT028, and endoPG1 and pomological traits. In particular, two genotypes from BPPCT015 were associated with low and high values of harvest date, flavonoids and sorbitol content. Also, two genotypes from CPPCT028 were associated with low and high values of harvest date, total phenolics, RAC and total sugars. Finally, two genotypes of endoPG1 were linked to flesh firmness and total sugars. As these traits are linked, using a marker to select for one trait would mean indirect selection for other traits, capturing correlated responses. The associations determined in this study would be very useful for deployment for marker-assisted selection (MAS) in peach breeding programs although further research is needed to validate these associations in other populations from a different genetic background. New studies are in progress mapping thousands of SNPs (*RosBREED_Peach* chip from Illumina® Infinium®) to facilitate genome-wide scans and validate marker-locus-trait associations for application in breeding.

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Capítulo 5

Association mapping analysis
for quality traits in peach and
nectarine cultivars using SNP markers

5.1. ABSTRACT

Marker-trait associations based on a set of 94 individuals from a germplasm collection was carried out in this study, including local Spanish and modern cultivars maintained at the Experimental Station of Aula Dei, Spain. Phenotypic evaluation based on agronomical, pomological and fruit quality traits was performed.

In a previous study, we have investigated the genetic structure among 94 peach and nectarine cultivars to reveal useful marker-trait with SSRs. The population structure analysis using STRUCTURE identified two subpopulations, the local and modern cultivars, with admixture within both groups. Significant marker-trait associations were determined by TASSEL with modelling coefficient of membership (Q) values as covariates.

In this chapter, a set of 3,851 out of a total of 8,144 SNPs markers developed by the Illumina Infinium BeadArray technology platform and covering the peach genome were analyzed for genome-wide association studies (GWAS). We used the population structure information obtained in the previous study. A total of 347 significant associations between these markers and eight fruit quality traits including blooming and harvest date, ripening index, anthocyanins, flavonoids, relative antioxidant capacity, sorbitol and total sugars, were found. To our knowledge, this is the first study with significant associations using SNP markers in peach cultivars from a germplasm collection.

Keywords: *Prunus persica*, fruit quality, unstructured population, single nucleotide polymorphism

5.2. INTRODUCTION

Peach is a model plant inside the family Rosaceae due to its small genome size of ~ 230 Mb (http://www.rosaceae.org/ peach/genome) with eight haploid chromosomes (Arús et al., 2012). Also, it is one of the best genetically characterized *Prunus* species, with known genes controlling important traits that display Mendelian inheritance patterns such as flesh colour, flesh adherence to the stone, or acidity (Dirlewanger and Arús, 2004).

Linkage disequilibrium (LD) or association mapping is the non-random association of alleles at distinct loci in a sample population, and it is being now routinely exploited to map disease genes in humans (Hirschhorn and Daly, 2005). In crop plants, the potential of exploiting LD in population-based association mapping, with the objective of estimating the position of a gene conferring a specific trait or phenotype by using LD between alleles of genetically mapped markers, has become a focus of considerable interest. Several factors influence LD in populations. As spurious associations between phenotypes and marker loci may be caused by population structure (Ganopoulos et al., 2011; Mariette et al., 2010), the structure and extent of LD within a sample population must be known before selecting an appropriate association mapping strategy (Lander and Schork, 1994). Whole-genome association studies in crop plants are currently limited by the number of markers available, their format, and cost. The resolution of association mapping generates correlations with the pattern of LD extent. In peach, different studies have been carried out using SSRs markers in cultivars with different genetic origin indicating that linkage disequilibrium (LD) in this crop is quite high (Aranzana et al., 2010; Cao et al., 2012; Font i Forcada et al., 2012).

Peach has a more narrow genetic base (Scorza et al., 1985) in comparison with other species such as grape (Barnaud et al., 2006) or maize (Remington et al., 2001), where the studies suggest that single nucleotide polymorphisms (SNPs) estimate a much lower decay of LD than SSRs. However, the greater frequency of SNPs over SSRs makes the former more useful when the polymorphism within specific genes is desired for targeted investigations. The number and marker type used for investigating population structure has a significant effect on the rate of significant associations.

SNPs are the most abundant form of genetic variation within plant genomes (Zhu et al., 2003). Several species with well known genome sequence such as

arabidopsis (Drenkard et al., 2000), barley (Rostoks et al., 2005) or maize (Batley et al., 2003) were studied previously for SNP analysis. Recently, SNPs have started to be used to study the whole-genome scans for diversity analysis, germplasm management, genetic fingerprinting, parentage verification candidate genes and gene mapping in the Rosaceae family (Ahmad et al., 2011; Cabrera et al., 2012; Chágne et al., 2008; Fernández i Marti et al., 2012; Le Dantec et al., 2010; Martínez-García et al., 2012; Verde et al., 2012; Wu et al., 2008). Multiplex SNP genotyping enables cost effective marker-assisted selection strategies, whole genome fingerprinting and genome-wide association studies (GWAS). Molecular markers are now widely employed in plant breeding for the acceleration of plant selection gains through marker-assisted selection (MAS) on the basis of individual genes or at the whole genome level through the selection of entire chromosomal segments (Collard and Mackill, 2008). The ideal marker system should be highly polymorphic and evenly distributed across the genome, as well as provide codominant, accurate and reproducible data which can be generated in a high-throughput and cost-effective manner. In association mapping, a dense set of SNP markers covering the entire genome is needed for finding a casual mutation or a SNP that is in linkage disequilibrium with the casual mutation (Flint-Garcia et al., 2005). Association mapping studies requires genotyping platforms capable of producing multi-locus genotypes in a large panel of individuals. Several high-throughput platforms have been developed that allow rapid and simultaneous genotyping of hundreds of thousands of SNPs.

The Illumina's Infinium BeadArray Technology is now being used for genetic analysis in several crop species, such as barley (Rostoks et al., 2006), soybean (Hyten et al., 2008) and maize (McMullen et al., 2009). Furthermore, high-throughput genotyping arrays using the GoldenGate® Assay (Illumina, Inc., San Diego, CA) have previously been used for SNP genotyping in soybean (Hyten et al., 2008), wheat (Akhunov et al., 2009), and maize (Yan et al., 2010). Recently, the International Peach SNP Consortium (IPSC) has pursued a genome-scale SNP discovery in peach using next generation sequencing platforms to develop and characterize a high-throughput Illumina InfiniumH SNP genotyping array platform. The IPSC peach 9 K SNP array v1 achieved an average spacing of 26.7 kb between SNPs and distributed over all eight peach chromosomes (Verde et al., 2012).

In this study, we have employed population genetics analyses found in a previous study. The aim of the present work was to find significant associations between markers and agronomical and pomological traits using a medium-size SNP panel covering the peach genome. To our knowledge nothing is known so far in peach. This is the first approach of GWA on peach breeding.

5.3. MATERIAL AND METHODS

5.3.1. Plant material, fruit sampling and evaluation of quality traits

A set of 94 peach and nectarine cultivars (43 native local Spanish cultivars and 51 modern cultivars from U.S., France, Italy, New Zealand, and South Africa) were used in this study, as described by Font i Forcada et al. (2012).

The germplasm was evaluated for the following parameters: blooming and harvest date (Julian's day), fruit weight (g), flesh firmness (N), soluble solids content (SSC) (ºBrix), titratable acidity (TA) (g malic acid/100 g FW), ripening index (RI) (SSC/TA), and concentrations of vitamin C (mg AsA/100 g FW), anthocyanins (mg C3GE/kg FW), total phenolics (mg GAE/100 g FW), flavonoids (mg CE/100 g FW), relative antioxidant capacity (mg TE/g FW), and sucrose, glucose, fructose, sorbitol and total sugars (g/kg FW). Phenotypic evaluations were carried out during three consecutive years. The procedures used in this study including plant material, sampling and evaluations are described in chapter 4 which correspond to the previous study published by Font i Forcada et al. (2012).

5.3.2. DNA isolation and SNP analysis

Young leaves were collected from each cultivar, frozen immediately in liquid nitrogen, and stored at -20ºC. DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Dusseldorf, Germany) following the manufacturer's instructions.

DNA from the 94 cultivars from the germplasm collection was genotyped using a panel of SNP markers spanning the entire peach genome (9K SNP) from Illumina's Infinium BeadArray technology platform and included a set of 8,144 SNPs. They were distributed over all eight peach chromosomes with an average spacing of 26.7 kb between each other (Verde et al., 2012).

The SNP array was developed for use on worldwide breeding germplasm and includes Sanger-based eSNPs from genome sequence of 'Lovell' generously provided by the International Peach Genome Initiative (www.rosaceae.org/peach/genome). They also include SNPs identified from Illumina 80 bp paired-end genome sequencing of 23 important founder peach accessions ('Admiral Dewey', 'Slappey', 'Babcock', 'Elberta', 'Carmen', 'Chinese Cling', 'Mayflower', 'Bolinha', 'Yellow St. John', 'J.H. Hale', 'Rio Oso Gem', 'Diamante', 'Dixon', 'Early Crawford', 'Florida prince', 'Dr. Davis', 'O'Henry', 'Okinawa', 'Nemaguard', 'Lovell', 'Georgia Belle', and 'Oldmixon Free') and the almond 'Nonpareil' (Verde et al., 2012).

The density of the new 9k SNP array v1 developed by The International Peach SNP Consortium (IPSC) (Verde et al., 2012) to cover the peach genome and the number of markers are significant higher (553 SNPs on scaffold 1, 581 on scaffold 2, 443 on scaffold 3, 707 on scaffold 4, 310 on scaffold 5, 500 on scaffold 6, 360 on scaffold 7 and 397 on scaffold 8) compared to other marker systems previously used (40 SSRs) for genome scan in peach (Font i Forcada et al., 2012).

5.3.3. Statistical analysis

The maximum, minimum, means, and Pearson's correlation coefficients for all phenotypic data are included in chapter 4 which correspond to the study previously published by Font i Forcada et al. (2012).

Population structure analysis

The program STRUCTURE (version 2.3) implements a model-based clustering criterion for inferring population structure using genotypic data from unlinked markers (Pritchard et al., 2000). STRUCTURE analysis was performed using 40 SSRs on the whole dataset from the previous study (Font i Forcada et al., 2012). We used the statistic ΔK based on the rate of change in the log probability of the data (Evanno et al., 2005) and it seemed to be the best fit for our data at K=3. For each value of K, STRUCTURE produces a Q-matrix that is the membership coefficient for each accession in each subgroup.

Association mapping analysis

Association analysis was conducted using a general linear model (GLM) analysis in TASSEL (version 3.0) (Yu and Buckler, 2006). Therefore, the population membership estimates (Q1, Q2 and Q3) obtained from STRUCTURE analyses were fitted as a covariate in a GLM as described by Font i Forcada et al. (2012). The model is performed to examine significant associations between the phenotypic traits and SNPs markers. Alleles with frequency lower than 5% were removed (Wilson et al., 2004). A standard correction was performed by applying Bonferroni procedure (Schulze and McMahon, 2002) and significant markers were declared at the $p<0.05$ threshold.

5.4. RESULTS AND DISCUSSION

5.4.1. Population structure

Results obtained in chapter 4 concerning population structure were used for this study as an approach to compare significant associations found in both studies. The maximum rate of change in the log probability of the data occurred at K=3 where the results suggested a more complex structure that with K=2, and when increasing K, the subpopulations became almost inseparable (Font i Forcada et al., 2012).

Similar studies in peach reported a strong subpopulation structure, detecting six major groups when using 244 peach varieties mostly from Spain and USA (Aranzana et al., 2010). A similar study divided into five groups the 104 peach landraces accessions from China, which well agreed with their geographical distribution (Cao et al., 2012). Such structure could be caused by artificial or natural selection, genetic drift or the species-dependent (Flint-García et al., 2005).

5.4.2. SNP filtering

Of the 8,144 candidate SNPs, after discovery and amplification on the Infinium HD BeadChips Illumina, 3,851 high quality SNPs remained for the final analysis of association studies, as summarized on Table 5.1.

The final number of SNPs (Table 5.1) was distributed as follow: 553 SNPs on the scaffold 1; 581 on the scaffold 2; 443 on the scaffold 3; 707 on the scaffold 4; 310 on the scaffold 5; 500 on the scaffold 6; 360 on the scaffold 7; and 397 on the scaffold 8.

Table 5.1. Workflow for SNP detection, filtering and final choice employed for association analysis.

	Initial Number of markers	Remaining markers
Detection and validation Peach 9K array ►	8,144 SNPs	
After removing monomorphic markers ►	1,912 SNPs ►	6,232 polymorphic
After removing markers with gene train score < 0.4 ►	1,052 SNPs ►	5,180 SNPs
After removing markers with similar pattern ►	622 SNPs ►	4,558 SNPs
Final choice (Minor allele frequency, MAF < 5%) ►	707 SNPs ►	**3,851** SNPs

5.4.3. Association mapping

The mean phenotypic data obtained during three years of evaluation (Table 4.3, described in Font i Forcada et al., 2012) was used to test the association analysis with the 3,851 polymorphic SNPs markers. Table 5.2 shows the summarized associations and the range for each marker, scaffold, position and p-value (see supplementary material in annexes 11.3 for the full table). The figures 5.1, 5.2 and 5.3 showed the –log (10) of the p-value against de position expressed on Mbp (million of base pair) of the SNPs based on the 9k SNP array v1.

Eight out the seventeen quality traits used, showed associations with the SNPs (Table 5.2). Figures 5.1, 5.2 and 5.3 represent the associations by trait and by scaffold. A total of 347 associations in different scaffolds were found with blooming date (scaffolds: 1, 3 and 4), harvest date (scaffolds: 1, 2, 3, 4, 5, 6, 7 and 8), RI (scaffold: 7), anthocyanins (scaffolds: 1, 2 and 4), flavonoids (scaffolds: 1 and 6), RAC (scaffolds: 1 and 3), sorbitol (scaffolds: 2, 4, 6 and 8), and total sugars (scaffolds: 4, 6 and 8). Only harvest date showed associations in all scaffolds. The maximum number of associations was found in scaffold 2 (164), followed by scaffold 1 (55) and scaffold 4 (42), and the minimum number of associations (5) were found on scaffold 3.

Also, significant associations were found with the same traits when using 40 SSRs markers (Font i Forcada et al., 2012), covering the peach genome. These authors showed association between SSR markers and harvest date in linkage group (LG) 4 and 6 (LG), RI in LG2, LG5 and LG6, flavonoids in LG2 and LG4, anthocyanins in LG2, LG3, LG4, LG5, LG6 and LG8, RAC LG4 and LG6, sorbitol in LG4 and LG5, and total sugars in LG2 and LG4. In order to compare the associations found in both studies, we tried to establish the positions in the genome of the markers used in both studies

(Table 5.3). The position on the physical map for the UDP98-410, endoPG1 and BPPCT015 SSRs markers, that the authors have found associations, are unknown, but for the remaining markers their position with LGs. On the genome database for Rosaceae, we found that the nearest SNP marker to CPPCT028 it was SNP_IGA_450711; for the UDP96-003 marker it was SNP_IGA_395202; for the CPPCT030 marker it was SNP_IGA_700469; and for the UDP96-001 marker it was SNP_IGA_630302 (Table 5.3).

Table 5.2. List of the SNPs associated with different pomological traits and their p-value.

Trait	Marker	Scaffold	Number of associations	Position	p-value*
Blooming date	SNP_IGA_37843	1	1	12,641.440	0.0000025655
	SNP_IGA_365780	3	1	20,635.992	0.0000009567
	SNP_IGA_430583-441904	4	6	15,574.015-18,522.596	0.0000000116-0.0000012142
Harvest date	SNP_IGA_46754-132155	1	23	14,980.305-44,936.042	0.0000000023-0.0000017881
	SNP_IGA_137253-287700	2	144	461.255-25,228.844	0.0000000001-0.0000024599
	SNP_IGA_303724-363719	3	3	4002.228-19,759.990	0.0000002418-0.0000020747
	SNP_IGA_403353-450711	4	15	8,996.802-20,165.259	0.0000000000-0.0000019947
	SNP_IGA_543247-600691	5	13	276.220-14,995.466	0.0000000497-0.0000017344
	SNP_IGA_619807-700469	6	4	4,759.496-28,045.174	0.0000000040-0.0000020777
	SNP_IGA_746619-792898	7	9	7,470.226-22,673.209	0.0000000005-0.0000015070
	SNP_IGA_797680-879224	8	17	1,271.540-18,309.578	0.0000000002-0.0000020747
RI	SNP_IGA_784373-786935	7	10	18,510.773-19,542.449	0.0000008821-0.0000017596
Anthocyanins	SNP_IGA_53531-96167	1	4	15,750.283-28,550.473	0.0000000002
	SNP_IGA_181444	2	1	3,800.271	0.0000000002
	SNP_IGA_392956-395202	4	4	5,689.470-6,168.570	0.0000000002
Flavonoids	SNP_IGA_82861-112690	1	18	23,722.082-36,758.815	0.0000001371-0.0000025583
	SNP_IGA_628833-638859	6	15	7,901.344-11,016.846	0.0000000213-0.0000018901
RAC	SNP_IGA_48586-112690	1	9	15,234.386-36,758.815	0.0000004932-0.0000017499
	SNP_IGA_303724	3	1	4,002.228	0.0000007245
Sorbitol	SNP_IGA_152976-287700	2	19	1,761.256-25,228.844	0.0000001038-0.0000023958
	SNP_IGA_442063-450711	4	10	18,548.028-20,165.259	0.0000000126-0.0000003412
	SNP_IGA_700469	6	1	28,045.174	0.0000009341
	SNP_IGA_878717-879224	8	5	18,085.149-18,309.578	0.0000000297-0.0000007114
Total sugars	SNP_IGA_442063-449112	4	7	18,548.028-19,905.501	0.0000007096-0.0000017609
	SNP_IGA_636024-637355	6	5	10,460.202-10,606.410	0.0000000365-0.0000002198
	SNP_IGA_870629-879224	8	2	15,787.171-18,309.578	0.0000015839-0.0000023052

* $p \leq 0.01$ (after Bonferroni correction $p \leq 0.0000026$). When more than one association, the min and max p-values were included on a range.

Among them, the common associations found in both studies were obtained with the UDP98-410, BPPCT015, CPPCT028, UDP96-003, CPPCT030, endoPG1 and UDP96-001 markers with harvest date, anthocyanin, sorbitol and total sugars, respectively. Other study in association mapping with SSRs and 104 peach landraces from China showed the CPPCT005 marker in LG4 and the UDP98-407 marker in LG6, both of them associated with blooming date (Cao et al., 2012). Unfortunately, the positions of these markers on the physical map are unknown (www.rosaceae.org). Other study with SSRs (Fan et al., 2010) also found QTLs for blooming date in LG1, but the positions of these markers on the physical map are also unknown. We found associations between SNPs markers and blooming date in scaffold 1, 3 and 4, but the study with SSRs (Font i Forcada et al., 2012) did not show any association with blooming date.

Table 5.3. List of markers (SSR and SNP) associated with pomological traits, their position on the genetical (LG) and physical (scaffolds, Mbp) maps and the nearest marker associated (www.rosaceae.org).

SSR marker	LG	Scaffold	Position on physical map (Mbp)	Nearest marker	Trait
UDP98-410	2	unknown	unknown	unknown	Anthocyanin
BPPCT015	4	unknown	unknown	unknown	Harvest date
BPPCT015	4	unknown	unknown	unknown	Total sugars
BPPCT015	4	unknown	unknown	unknown	Sorbitol
endoPG1	4	unknown	unknown	unknown	Total sugars
CPPCT028	4	4	2,086.534-2,086.8311	SNP_IGA_450711	Harvest date
UDP96-003	4	4	8,757.450-8,757.639	SNP_IGA_395202	Anthocyanin
CPPCT030	6	6	26,851.012-26,851.314	SNP_IGA_700469	Harvest date
UDP96-001	6	6	7,040.757-7,041.024	SNP_IGA_630302	Harvest date

These results could be very useful because many of the associated markers were located in common regions where major genes or QTLs have been previously identified and mapped on the *Prunus* reference map (Arús et al., 2012). Pomological traits associates in scaffold 4 seem consistent with previous studies where QTLs were mapped on LG 4 for blooming date (Fan et al., 2010), harvest date (Arús et al., 2012 and references therein) and sugars content (Arús et al., 2012 and references therein).

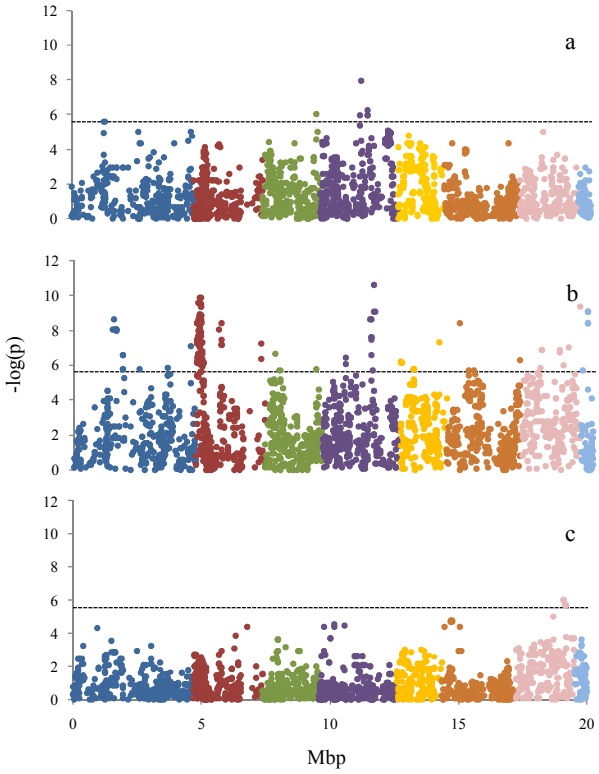

Figure 5.1. Genome scan showing –log (p) value for marker associations with a) blooming date, b) harvest date, and c) ripening index. The different colours represent the different linkage group from 1 to 8.

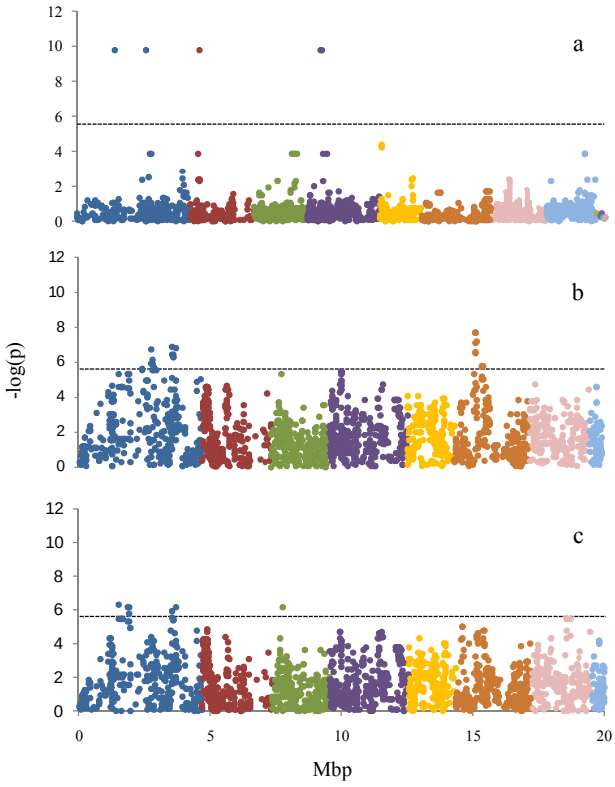

Figure 5.2. Genome scan showing –log (p) value for marker associations with a) anthocyanins, b) flavonoids, and c) relative antioxidant capacity. The different colours represent the different linkage group from 1 to 8.

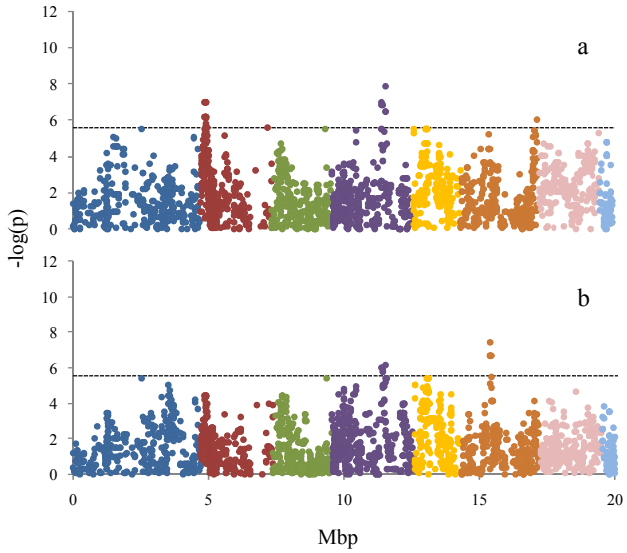

Figure 5.3. Genome scan showing –log (p) value for marker associations with a) sorbitol and b) total sugars. The different colours represent the different linkage group from 1 to 8.

The present assay using the IPSC peach SNParray v1 to find association with pomological traits in a germplasm collection is the first study reported in peach. Due to the density of markers, this work would reinforces the study recently published by Font i Forcada et al. (2012). Further discussion and other statistical analysis should be due in order to compare those results found with different markers type (SSRs and SNPs) to know if there is any impact on the results of GWA mapping in peach. Peach association mapping is an alternative to QTL mapping based on crosses between different accessions because of the multiple advantage compared to bi-parental populations. Additionally, this work provided promising results concerning association mapping with pomological traits that could be applied in other *Prunus* species because of the complete synteny found inside the Rosaceae family, and it would be very useful to make predictions of genetic progress in a breeding program.

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Capítulo 6

Agronomical and fruit quality traits of two peach cultivars on peach-almond hybrid rootstocks growing on Mediterranean conditions

6.1. ABSTRACT

The agronomical and fruit quality trait influence was evaluated for five almond x peach hybrid and one *P. davidiana* x peach hybrid rootstocks. The six rootstocks, Adafuel, Adarcias, Cadaman, Felinem, Garnem and GF 677, were budded with 'Tebana' peach and 'Queen Giant' nectarine cultivars during the summer of 1997, and trees were established in two adjacent plots during the winter of 1998-1999. The trial was located in the Ebro Valley (Zaragoza, Spain), on a heavy and calcareous soil typical of the Mediterranean area.

At the twelfth year after budding, growing conditions generated varying levels of tree mortality, the highest with Felinem and Garnem rootstocks. In contrast, all Adarcias and GF 677 trees survived and the mortality rate was low in Adafuel and Cadaman. The lowest vigour was induced by Adarcias for both cultivars, a 37% and 48% reduction in trunk cross-sectional area (TCSA) for 'Tebana' and 'Queen Giant' respectively compared to vigour on GF 677. For 'Queen Giant', cumulative yield was greater on Felinem, although no significant differences were found with Garnem. Other rootstocks that showed high cumulative yields were Adafuel and GF 677. The highest yield efficiency was recorded on Cadaman rootstock with both varieties, although differences were not significant with Felinem for 'Queen Giant'.

On average, the highest fruit weight was recorded on Adafuel and Cadaman for both cultivars. For 'Queen Giant', the greatest soluble solids content (SSC) was recorded on Adarcias and Cadaman, and the lowest on Garnem and GF 677. The highest titratable acidity was also induced by Cadaman rootstock but it did not differ significantly from Adarcias. Correlations between some agronomical and fruit quality traits were found. The less vigourous rootstocks seem to induce a better fruit quality to the studied cultivars based on fruit sugar content. Our results show the relationship between the characteristics on plant adaptability and development, such as yield, vigour or fruit weight, and the factors of fruit quality value.

Keywords: acidity, firmness, fruit weight, SSC, TCSA, yield

6.2. INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is one of the most important temperate fruits trees grown in the world, after crops such as apples or pears. Peach production comes mainly from China, Mediterranean area (Italy and Spain) and United States (FAOSTAT, 2011).

Stone fruit rootstock development is the aim of several breeding programs around the world (Moreno and Webster, 2004). The hybrids of almond x peach are largely used as rootstocks for peach trees in the Mediterranean countries, because they are tolerant to lime induced Fe chlorosis and they are graft-compatible with peach cultivars (Bernhard and Grasselly, 1981; Moreno et al., 1994). They are vigourous and appropriate for use in poor dry soils (Cambra, 1990). New selections have also been developed with resistance to biotic stresses such as root-knot nematodes (*Meloidogyne spp*.) (Felipe, 2009; Pinochet, 2009) and tolerance to replant conditions (Jiménez et al., 2011). Different studies with *Prunus* sp. have demonstrated that rootstock influences the performance of the grafted scion cultivar. There have been numerous reports of a relationship between rootstocks and water relations, leaf gas exchange, mineral uptake, plant size, blossoming, fruit bud survival, yield efficiency and tree vigour (Albás et al., 2004; Zarrouk et al., 2005). Also, it has been demonstrated that rootstock influences the fruit quality of the scion cultivar. Thus, previous research has shown the rootstock effects on fruit quality parameters like soluble solids content and firmness (Albás et al., 2004; Caruso et al., 1996; Giorgi et al., 2005; Loreti and Massai, 2002; Remorini et al., 2008). Fruit quality was defined by Kramer and Twigg (1996) as the conjunction of physical and chemical characteristics which give good appearance and acceptability to the consumable product. The three more important components in the organoleptic quality of fruit are aroma, sugar content and acidity, which are related to many chemical and physical properties of fruits, and these properties are highly influenced by rootstocks. Different studies in peach (Byrne et al., 1991) have investigated the relationships between some fruit quality traits with agronomical parameters, such as between trunk cross-sectional area (TCSA) and fruit weight and between TCSA and soluble solids content (SSC).

The present work was carried out over twelve years of study, to evaluate the effect of different almond x peach hybrid rootstocks on tree growth and survival, yield

and fruit quality characteristics of 'Queen Giant' and 'Tebana' cultivars on heavy and calcareous soil conditions, typical of the Mediterranean area.

6.3. MATERIALS AND METHODS

6.3.1. Plant material

Five almond x peach hybrid [*Prunus amygdalus* Batsch x *P. persica* (L.) Batsch] and one *P. davidiana* x peach hybrid [*Prunus davidiana* (Carrière) Franch x *P. persica* (L.) Batsch] rootstocks were evaluated in this study. They were budded with 'Tebana' peach and 'Queen Giant' nectarine cultivars during the summer of 1997 (Figure 6.1). The cultivars were of possible interest in the Ebro Valley area, because of their maturity time and good fruit quality. The six rootstocks were compared in a trial established during the winter of 1998-1999 in two adjoining plots, one for each cultivar.

Table 6.1. List of studied rootstocks, description and origin.

Rootstock	Species	Genetic background	Origin[a]	References
Adafuel	*P. amygdalus x P. persica*	'Marcona' seedlings (open-pollinated)	CSIC, Spain	Cambra (1990)
Adarcias	*P. amygdalus x P. persica*	Open-pollinated	CSIC, Spain	Moreno and Cambra (1994)
Cadaman	*P. davidiana x P. persica*	Controlled cross	INRA (France-Hungary)	Edin and Garcin (1994)
Felinem	*P. amygdalus x P. persica*	'Garfi' almond x 'Nemared' peach	CITA, Spain	Felipe (2009)
Garnem	*P. amygdalus x P. persica*	'Garfi' almond x 'Nemared' peach	CITA, Spain	Felipe (2009)
GF 677	*P. amygdalus x P. persica*	Open-pollinated	INRA, France	Bernhard and Grasselly (1981)

[a] CSIC = Consejo Superior de Investigaciones Científicas; INRA = Institut National de la Recherche Agronomique; CITA = Centro de Investigación y Tecnología Agroalimentaria de Aragón.

Rootstocks chosen for this study were Adafuel (Cambra, 1990) and Adarcias (Moreno and Cambra, 1994; Moreno et al., 1994), selections from the Experimental Station of Aula Dei (CSIC); Garnem and Felinem (Felipe, 2009), selections from the Centre of Research and Agro-food Technology of Aragón (CITA); Cadaman (Edin and Garcin, 1994), a French-Hungarian co-obtention; and GF 677 rootstock (Bernhard and

Grasselly, 1981), the most widespread rootstock in the Mediterranean peach-growing area, was the standard (Table 6.1).

6.3.2. Field trial

The experiment was located in the Ebro Valley (North-Eastern of Spain) at the Experimental Station of Aula Dei (CSIC-Zaragoza, Spain), on a heavy and calcareous soil, with 27% total calcium carbonate, 8% active lime, water pH 8.3, and a clay-loam texture (Figure 6.1). Trees were trained to a low density open-vase system (6 × 5 m). Cultural management practices, such as fertilization, winter pruning, and spring thinning, were conducted as in a commercial orchard. Open vase trees were pruned to strengthen existing scaffold branches and eliminate vigorous shoots, inside and outside the vase, that would compete with selected scaffolds or shade fruiting wood. Moderate-sized fruiting wood (0.3-0.6 m long) was selected. Trees were hand-thinned at 45-50 days after full bloom (DAFB) leaving approximately 20 cm between fruits. The plot was level-basin irrigated every 12 days during the summer. Guard rows were used to preclude edge effects. The experiment was established in a randomized block design with five single-tree replications for each scion-stock combination.

6.3.3. Growth, yield determinations and harvest

Trunk girths were measured during the dormant season 20 cm above the graft union, and the trunk cross-sectional area (TCSA) was calculated. At harvest, all fruits from each tree were counted and weighed to determine total yield per tree (Kg/tree) and mean fruit weight. Cumulative yield per tree and yield efficiency (cumulative yield in kilograms per tree per final TCSA) of each scion-stock combination were computed from the harvest data.

6.3.4. Fruit sampling

Over the last 3 years of study, 20 fruits were hand-picked at commercial maturity, to assess optimum maturity for a given scion-rootstock combination. They were considered ripe when they no longer grew and exhibited the ground colour representative for each cultivar. Fruit samples were harvested by a single person to keep consistency of maturity grade. They were used to determine fruit quality parameters

such as soluble solids content (SSC), titratable acidity, firmness and colour during three years (2008-2010).

6.3.5. Evaluation of fruit quality traits

The effects of almond x peach rootstocks on fruit quality parameters were studied for at least three years to estimate seasonal effect on agronomical and fruit quality parameters.

Fruit size (g) was calculated considering the total number of fruits and the total yield per tree. SSC of fruit juice was measured with a digital refractometer (Atago PR-101, Tokyo, Japan) and was expressed as ºBrix. Titratable acidity (TA) of samples was determined using an automatic titrator (Metrohm Ion analysis, 807 Dosing Unit, Switzerland). Ten grams of homogenized samples were diluted with 90 g of distilled water, and microtitrated with 0.1 N NaOH. The results were expressed as g malic acid/100 g FW. Ripening index was calculated based on the SSC/TA ratio. Flesh firmness was measured on two paired sides of each fruit, by removing 1 mm thick disk of skin from each side of the fruit, and using a penetrometer (Model FT-327). The two readings were averaged for each fruit and data were expressed in Newtons (N). Colour determinations were measured on the two opposite sides of the fruits. Values of L* (brightness or lightness), a* (-a* = greenness, +a* = redness), b* (-b* = blueness, +b* = yellowness), C* (chroma) and H (lightness's angle) were measured using a colourimeter (Chroma Meter, CR-400 Konica Minolta, Japan).

6.3.3. Data analysis

Data were analyzed statistically using SPSS 17.0 (SPSS, Inc, Chicago, USA). Data were evaluated by two-way variance (ANOVA) analysis. When the F test was significant, means were separated by Duncan's multiple range ($P \leq 0.05$). Regression analysis was carried out by Pearson's correlation.

6.4. RESULTS

6.4.1. Tree mortality

Mortality rate was high for some of the rootstocks tested, particularly Felinem and Garnem (Figure 6.1). These two rootstocks experienced the highest tree mortality

with 100% of dead trees for the 'Tebana' cultivar. Therefore, these scion-rootstock combinations were not included in the rest of the study.

For 'Tebana' cultivar, Garnem rootstock had lost all replicates at the third year after budding (2001). Felinem experienced more progressive tree mortality with 16.5%, 67% and 16.5% of dead trees in 2000, 2001 and 2006, respectively (Figure 6.1). Lower mortality was found for Adafuel and Cadaman with only a single dead tree (16.5%). In contrast, all trees budded on Adarcias and GF 677 survived well to the end of the experiment.

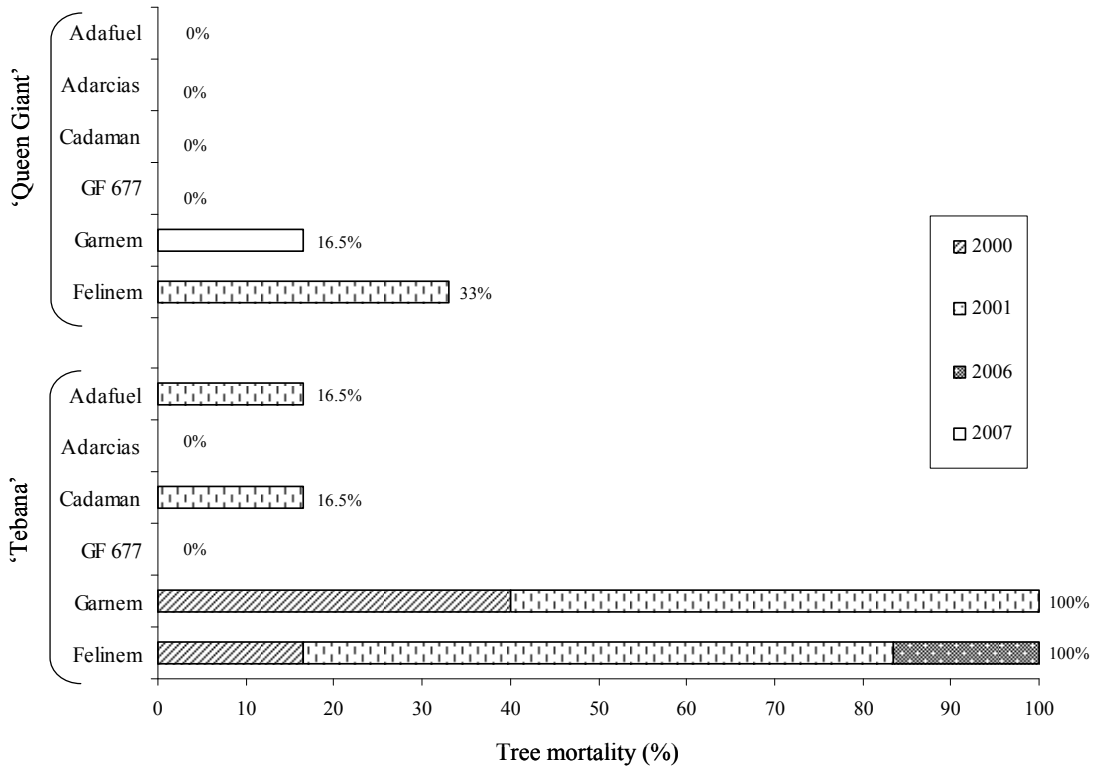

Figure 6.1. Tree mortality rate (%) from the second (2000) to the twelfth (2010) year after budding.

'Queen Giant' showed a 33% mortality rate on Felinem at the third year after budding. Nine years after budding, mortality on Garnem was 16.5%. No dead trees were found for Adafuel, Adarcias, Cadaman and GF 677.

6.4.2. Tree growth, yield, cumulative yield and yield efficiency

Results for 'Tebana' include only Adafuel, Adarcias, Cadaman and GF 677 rootstocks, due to the high mortality of trees on Felinem and Garnem (Figure 6.2). At

the twelfth year after budding (2010), the lowest vigour was induced by Adarcias for both cultivars (Table 6.2).

This rootstock showed 37% and 48% reductions in TCSA for 'Tebana' and 'Queen Giant' respectively, compared to TCSA on GF 677. Contrastingly, the highest TCSA was shown by Garnem and GF 677, although not significant differences were found with Felinem for 'Queen Giant' (Table 6.2). For this cultivar, vigour of Cadaman was intermediate, showing a 31% reduction in TCSA compared to GF 677 at the end of the experiment. A similar trend was found from year 2001 to 2010 (Figure 6.2).

Table 6.2. Effect of rootstock on TCSA (trunk cross-sectional area), cumulative yield and yield efficiency of 'Queen Giant' and 'Tebana', at the twelfth year after budding (2010).

Cultivar	Rootstock	TCSA (cm^2)	Cumulative yield ($kg\ tree^{-1}$)	Yield efficiency ($kg\ cm^{-2}$)
'Queen Giant'	Adafuel	225.0 bc	224.0 b	0.99 a
	Adarcias	155.1 a	164.4 a	1.06 bc
	Cadaman	206.3 b	279.3 b	1.35 d
	Felinem	254.7 cd	306.9 c	1.20 cd
	Garnem	272.4 d	278.5 bc	1.02 ab
	GF 677	297.5 d	244.2 b	0.82 a
'Tebana'	Adafuel	238.0 b	255.6 b	1.07 a
	Adarcias	146.1 a	154.4 a	1.06 a
	Cadaman	209.0 b	306.1 b	1.47 b
	GF 677	231.0 b	289.5 b	1.25 a

For each cultivar, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

In general, throughout the last 6 years of the study, highest fruit yields were induced by Felinem and GF 677 for 'Queen Giant', and Cadaman and GF 677 for 'Tebana' (data not shown). For 'Queen Giant', cumulative yield was greater on Felinem, although not significantly different from Garnem. For 'Tebana', cumulative yield was higher on Cadaman, GF 677 and Adafuel (Table 6.2). The lowest cumulative yield was recorded on the less vigourous rootstock Adarcias. Yield efficiency was greatest on Cadaman for both cultivars, but not significantly different for 'Queen Giant' on Felinem. For this cultivar, the lowest yield efficiency was recorded on Adafuel and GF 677, although they did not differ significantly from Garnem.

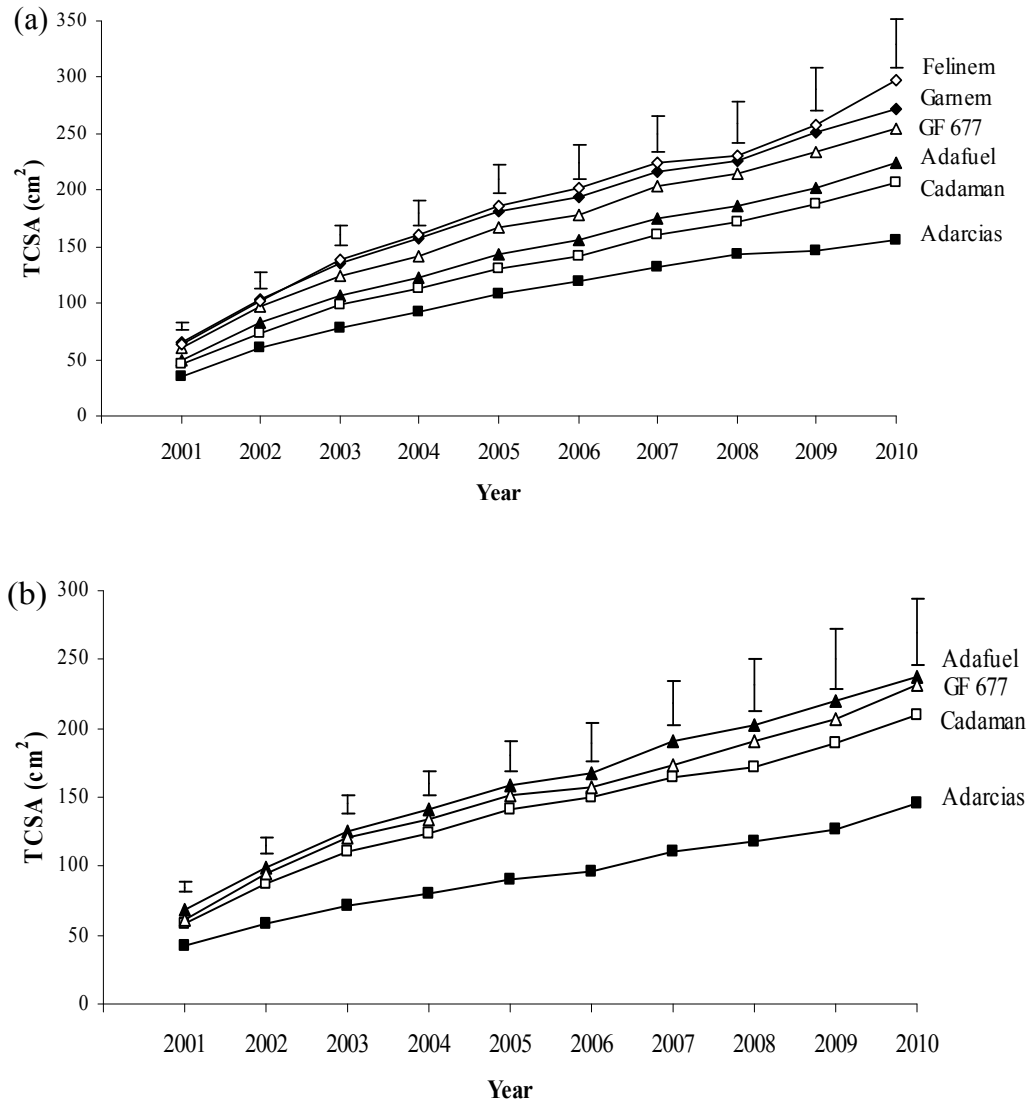

Figure 6.2. Effect of rootstock on TCSA (cm^2) of 'Queen Giant' (a) and 'Tebana' (b) cultivars during 10 years of study. Vertical lines indicate LSD ($P \leq 0.05$).

6.4.3. Fruit quality traits

Table 6.3 shows factors affecting fruit quality parameters in both cultivars. ANOVA results showed no significant interaction between rootstock and year, except for the ripening index of 'Queen Giant' with a significance value of 0.05. The significant effect of year was found for all traits except for SSC in 'Tebana' cultivar.

For 'Queen Giant', the highest mean fruit weight was recorded on Adafuel, Cadaman and GF 677, and the lowest on Adarcias, although not significantly different from Felinem (Table 6.4). For soluble solids content (SSC), the greatest values were recorded on Adarcias and Cadaman (Table 6.4) and the lowest on Garnem and GF 677,

while Adafuel and Felinem did not significantly differ from either of them. Small but consistent differences in titratable acidity (TA) were found among rootstocks throughout the years of study (Table 6.4).

Table 6.3. ANOVA analysis of the effect of rootstock and year on fruit quality traits in 'Queen Giant' and 'Tebana' cultivars for the average of the 3 years of study.

Cultivar	Source of variation[1]	FW	SSC	TA	RI	FF	L*	a*	b*	C*	H
'Queen Giant'	Rootstock (R)	***	*	***	**	*	ns	ns	ns	ns	ns
	Year (Y)	*	***	*	***	***	***	***	***	***	***
	RxY	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
'Tebana'	Rootstock (R)	ns	**	ns	ns	ns	ns	ns	ns	*	ns
	Year (Y)	**	ns	***	***	***	***	***	***	***	***
	RxY	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]Data were evaluated by two-way variance (ANOVA); ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns, not significant. FW, fruit weight; SSC, soluble solids content; TA, titratable acidity; RI, ripening index; FF, flesh firmness; L*, a*, b*, C* and H, chromatic parameters.

On average, the highest TA was induced by Cadaman, although it did not differ from Adarcias. The lowest TA was recorded on Garnem, GF 677, Felinem and Adafuel. The highest average ripening index (RI) values were recorded on Adafuel, Adarcias, Felinem and GF 677 and the lowest on Cadaman, although Garnem did not differ from any of them. No consistent differences were found among rootstocks for fruit firmness during the study, except for the first year of analysis (2008) when Cadaman produced the highest firmness of fruits, although it did not differ from Adafuel (Table 6.4).

Throughout the study, no consistent differences for fruit quality parameters were found among rootstocks for 'Tebana' cultivar, with the exception of fruit weight (Table 6.4) and chromatic parameters in 2010 (data not shown). On average, Adafuel and Cadaman rootstocks resulted in the largest fruit weight of 'Tebana' peaches, whereas Adarcias and GF 677 induced the lowest (Table 6.4).

Table 6.4. Effect of rootstock on fruit weight, soluble solids content, titratable acidity, ripening index and flesh firmness of 'Queen Giant' and 'Tebana' cultivars at the tenth (2008), eleventh (2009) and twelfth (2010) year after budding.

Cultivar	Character	Rootstock	2008	2009	2010	Average
'Queen Giant'	Fruit weight (g)	Adafuel	246 c	223 b	233 ab	234 c
		Adarcias	189 a	185 a	221 ab	198 a
		Cadaman	226 bc	228 b	234 ab	229 c
		Felinem	201 ab	219 b	215 a	212 ab
		Garnem	215 bc	210 ab	231 ab	219 b
		GF 677	229 bc	223 b	248 b	233 c
	SSC (ºBrix)	Adafuel	11.7 ab	10.3 ab	10.1 ab	10.7 ab
		Adarcias	12.6 b	11.0 b	10.8 b	11.5 b
		Cadaman	12.2 b	10.7 ab	10.3 ab	11.1 b
		Felinem	11.7 ab	10.9 ab	10.2 ab	10.9 ab
		Garnem	11.5 ab	9.9 a	9.8 a	10.4 a
		GF 677	10.7 a	10.5 ab	10.5 ab	10.6 a
	Titratable acidity	Adafuel	0.85 ab	0.75 ab	0.82 a	0.81 a
		Adarcias	0.81 a	0.82 bc	0.90 ab	0.88 ab
		Cadaman	0.94 b	0.87 c	0.99 b	0.93 b
		Felinem	0.77 a	0.73 a	0.88 ab	0.79 a
		Garnem	0.77 a	0.75 a	0.83 a	0.78 a
		GF 677	0.74 a	0.77 ab	0.87 a	0.79 a
	Ripening index	Adafuel	13.0 a	15.0 b	14.7 b	14.2 b
		Adarcias	15.0 a	13.5 ab	11.9 a	13.4 b
		Cadaman	12.9 a	12.3 a	10.9 a	12.0 a
		Felinem	14.8 a	14.9 b	12.7 ab	14.1 b
		Garnem	14.8 a	13.3 ab	11.5 a	13.2 ab
		GF 677	14.4 a	13.6 ab	13.3 ab	13.8 b
	Flesh firmness (N)	Adafuel	24.1 ab	32.2 a	35.5 a	30.6 a
		Adarcias	20.2 a	34.6 a	38.2 a	30.8 a
		Cadaman	26.7 b	36.1 a	40.8 a	34.5 a
		Felinem	17.7 a	35.7 a	38.6 a	30.7 a
		Garnem	17.2 a	30.5 a	33.9 a	27.2 a
		GF 677	13.7 a	31.9 a	36.3 a	27.3 a
'Tebana'	Fruit weight (g)	Adafuel	174 a	208 a	214 b	199 b
		Adarcias	164 a	184 a	195 ab	181 a
		Cadaman	176 a	184 a	215 b	192 b
		GF 677	177 a	184 a	184 a	182 a
	SSC (ºBrix)	Adafuel	11.5 a	11.6 a	11.2 a	11.4 a
		Adarcias	12.3 a	12.2 a	11.8 a	12.1 a
		Cadaman	12.0 a	12.2 a	12.1 a	12.1 a
		GF 677	11.6 a	11.4 a	11.0 a	11.3 a
	Titratable acidity	Adafuel	0.33 a	0.40 a	0.55 a	0.43 a
		Adarcias	0.36 a	0.42 a	0.54 a	0.44 a
		Cadaman	0.35 a	0.39 a	0.56 a	0.43 a
		GF 677	0.38 a	0.38 a	0.49 a	0.42 a
	Ripening index	Adafuel	34.0 a	28.9 a	24.5 a	29.2 a
		Adarcias	34.3 a	29.4 a	22.0 a	28.6 a
		Cadaman	34.5 a	31.4 a	24.5 a	30.1 a
		GF 677	31.0 a	29.8 a	23.0 a	27.9 a
	Flesh firmness (N)	Adafuel	21.6 a	30.6 a	34.3 a	28.8 a
		Adarcias	19.5 a	30.1 a	34.6 a	28.1 a
		Cadaman	18.3 a	28.8 a	33.3 a	26.8 a
		GF 677	25.7 a	29.1 a	33.3 a	29.4 a

For each year and character, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test. SSC, soluble solids content; titratable acidity (g malic acid per 100 g FW); ripening index, SSC/TA.

Table 6.5. Rootstock effect on chromatic parameters (L*= lightness; a*= redness and greenness; and b*= yellowness and blueness; C*= chroma; H= lightness's angle) of 'Queen Giant' budded on different rootstocks, at the eleventh and the twelfth year after budding.

Character	Rootstock	2009	2010	Average
L*	Adafuel	40.8 a	49.9 a	45.4 a
	Adarcias	41.5 a	50.4 b	46.0 ab
	Cadaman	42.0 a	51.1 b	46.5 b
	Felinem	41.8 a	45.9 a	43.9 a
	Garnem	41.9 a	45.4 a	43.7 a
	GF 677	41.5 a	45.2 a	43.3 a
a*	Adafuel	41.9 b	35.6 a	38.7 a
	Adarcias	42.2 b	37.7 a	39.9 a
	Cadaman	40.9 ab	35.4 a	38.2 a
	Felinem	41.4 ab	39.2 a	40.3 a
	Garnem	41.6 ab	39.4 a	40.4 a
	GF 677	39.8 a	39.7 a	39.7 a
b*	Adafuel	18.5 a	21.4 b	19.9 b
	Adarcias	18.1 a	21.0 ab	19.6 ab
	Cadaman	18.9 a	19.2 ab	19.0 ab
	Felinem	18.5 a	19.2 a	18.9 a
	Garnem	18.1 a	19.3 a	18.7 a
	GF 677	17.9 a	19.1 a	18.5 a
C*	Adafuel	45.9 b	42.1 a	44.0 a
	Adarcias	46.0 b	43.5 a	44.8 a
	Cadaman	42.2 ab	41.6 a	42.0 a
	Felinem	40.5 ab	43.9 a	44.7 a
	Garnem	45.1 ab	44.1 a	44.6 a
	GF 677	43.7 a	44.2 a	43.9 a
H	Adafuel	23.8 a	32.5 b	28.1 a
	Adarcias	23.2 a	30.1 ab	26.7 a
	Cadaman	24.8 a	31.8 ab	28.3 a
	Felinem	24.1 a	26.5 a	25.3 a
	Garnem	23.1 a	26.3 a	25.0 a
	GF 677	24.1 a	25.8 a	25.1 a

For each year and parameter, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Significant differences were found between rootstocks in L*, a*, b*, C* and H colour parameters for 'Queen Giant' cultivar (Table 6.5). In 2009, Adafuel and Adarcias induced the highest values for a* and C* parameters, and GF 677 induced the lowest value, although not significantly different from the other rootstocks. In 2010, Adarcias and Cadaman induced higher values for L* parameter, compared to the other rootstocks. For b* and H parameters, Adafuel induced the highest values although not significantly different from Adarcias and Cadaman. In the year 2010, Cadaman induced the highest C* value and GF 677 the lowest on 'Tebana' peach (data not shown). No significant differences were found between Adafuel and Adarcias.

6.4.5. Phenotypic correlations

A high significant and positive correlation (Table 6.6) was observed between TCSA and yield for 'Queen Giant' (r = 0.556, $P \leq 0.01$) and 'Tebana' (r = 0.688, $P \leq 0.01$). However, in 'Tebana' cultivar, a significant negative correlation was found between TCSA and fruit weight.

In 'Queen Giant', a significant positive correlation was found between TA and flesh firmness (FF), as well as between RI and SSC. On the contrary, a significant negative correlation was found between TCSA and SSC and between TA and fruit weight. In both cultivars, we found a significant positive correlation between yield and FF, SSC and fruit weight, and FF and SSC. Significant negative correlations were also found between SSC and yield, as well as between FF and RI (Table 6.6).

Table 6.6. Pearson's correlations coefficients between traits observed over three years (2008-2009-2010) in almond x peach hybrid rootstocks budded with 'Queen Giant' and 'Tebana' cultivars for the average of the 3 years of study.

Cultivar	Trait	TCSA	Fruit weight	SSC	TA	FF	RI
'Queen Giant'	Yield	0.556**	ns	-0.505*	ns	0.482**	ns
	Year	ns	0.520**	0.427**	ns	ns	ns
	H (colour)	ns	ns	ns	-0.315**	ns	ns
	Fruit weight	ns	-	0.362**	-0.319*	ns	ns
	SSC	-0.491**	-	-	ns	0.311*	0.582**
	TA	ns	-	-	-	0.408*	ns
	FF	ns	-	-	-	-	-0.431*
'Tebana'	Yield	0.688**	ns	-0.379*	ns	0.300**	ns
	Year	ns	0.630**	0.392**	ns	ns	ns
	H (colour)	ns	ns	ns	-0.315**	ns	ns
	Fruit weight	-0.479**	-	0.392*	-0.437*	ns	ns
	SSC	ns	-	ns	ns	ns	ns
	TA	ns	-	-	-	0.695*	ns
	FF	ns	-	-	-	-	-0.717*

ns, not significant; *P≤0.05; **P≤0.01. Abbreviations: TCSA, trunk cross-sectional area; SSC, soluble solids content; TA, titratable acidity; FF, flesh firmness; RI, ripening index.

For both cultivars, significant correlations were observed between year and fruit weight, as well as between year and SSC. There was no correlation between year and TA. Only hue angle (H) showed a significant negative correlation with TA (r = -0.315, $P \leq 0.01$) in both cultivars, meaning that decreasing the TA will increase the H parameter. No significant relationship was found between colour measurements and FF, SSC or fruit weight (Table 6.6).

6.5. DISCUSSION

Twelve years after budding, growing conditions generated varying levels of tree mortality, the highest with Felinem and Garnem rootstocks. For these two rootstocks, 100% of trees died for 'Tebana' cultivar. The 'Tebana' plot situation was established closer to the irrigation canal than the plot with 'Queen Giant', and likely more prone to flooding. For Adafuel and Cadaman, the mortality rate was low. No dead trees were found on Adarcias and GF 677 at the end of the experiment. In these growing conditions, tree mortality could be attributed to the sensitivity of almond x peach hybrid rootstocks to root asphyxia (Felipe, 2009) or susceptibility to various root rot pathogens such as *Phytophtora* spp (Zarrouk et al., 2005).

The lower vigour of Adarcias has already been mentioned (Moreno et al., 1994). Consequently, Adarcias may be suitable for reducing excessive growth of peach cultivars or to increase planting density and to decrease management costs (Moreno and Cambra, 1994). The higher vigour induced by Felinem, Garnem and GF 677 on 'Queen Giant' and Adafuel, Cadaman and GF 677 on 'Tebana' is comparable to that induced by Adafuel with a similar productivity for 'Catherine' and 'Flavortop' cultivars, as described by Moreno et al. (1994). The greater vigour, on fertile and well-irrigated soils, may become excessive for good orchard practice unless some irrigation and other cultural practices are modified. Vigourous rootstock appears suitable for peach production under replanting conditions or in poor and calcareous soils that might otherwise not be favourable for growing peach (Cambra, 1990; Moreno et al., 1994).

Cadaman rootstock induced higher yield efficiency in both cultivars, because of its intermediate vigour and high yield. On the contrary, the tendency of Garnem and GF 677 to show low yield efficiency, is probably due to their high vigour and the resulting high TCSA, as previously reported (Zarrouk et al., 2005). The highest yield of Felinem and GF 677 with 'Queen Giant' and Cadaman and GF 677 with 'Tebana' was already mentioned by Zarrouk et al. (2005) for the first bearing years. In the study performed by Jiménez et al. (2008), GF 677 and Felinem seem to be better adapted than Cadaman and Garnem, among other rootstocks, to calcareous soils with high lime content. This is probably because of a more chlorosis-tolerant almond parent. Such adaptation probably results in higher vigour and yield for GF 677 and Felinem rootstocks. In Zarrouk et al. (2005) it is interesting to note that the most vigourous rootstocks, such as Felinem and Garnem (in our study), have best efficiency of some mineral nutrition (Ca).

Furthermore, a positive correlation was found between yield efficiency and flower nutrient concentration for Mg, showing that Cadaman has the maximum value of yield efficiency (in our study) and a maximum concentration of Mg in Zarrouk et al. (2005).

The statistical analysis showed the significant effect of year for all quality traits, except for SSC in 'Tebana' cultivar. The year-to-year variation in fruit quality parameters may be explained by the differences in annual temperatures and crop load over the 3 years of study. However, no interaction was found between rootstock and year, except for the ripening index of 'Queen Giant'. This result suggests that ripening index is more influenced by the environmental conditions over the growing season than the other traits, in agreement with Brooks et al. (1993).

Although all rootstocks exhibited acceptable fruit weight, Adafuel and Cadaman produced the largest fruit weight on both cultivars. The tendency of Adafuel to produce higher fruit weight has been previously reported by Albás et al. (2004) and Moreno et al. (1994). Cadaman, with an intermediate level of vigour, tends to show higher fruit weight probably due to its higher productive efficiency. In 'Tebana' cultivar the negative correlation between TCSA and fruit weight is probably due to GF 677 (one of the most vigourous rootstocks) inducing lower fruit weight, in agreement with Tsipouridis and Thomidis (2005).

The tendency of Adarcias and Cadaman to induce higher soluble solids content could be related with their lower vigour, showing a stronger sink competition of fruit compared to vegetative development. The tendency of Adarcias to induce high SSC was already reported by Albás et al. (2004). For 'Queen Giant', Cadaman showed, in general the highest titratable acidity, although it did not differ from Adarcias. Despite higher acidity of fruits on these rootstocks, their SSC was not affected. High sugar contents and, to a lower extent, high acid contents seem to be favourable to fruit quality as evaluated by consumers (Crisosto and Crisosto, 2005).

The negative correlation between yield and SSC for 'Queen Giant' and 'Tebana' cultivars confirms the sink competition among fruits by the assimilate supply (Mounzer et al., 2008). Titratable acidity was negatively correlated with fruit weight for both cultivars, showing that titratable acidity decreases with fruit mass (Cantín et al., 2010). No significant differences for flesh firmness were found among rootstocks, with the exception of the first sampling year for 'Queen Giant'. Firmness was significantly

positive correlated with TA, reflecting the decrease of acidity with fruit softening. Also, the positive correlations between SSC and firmness are in agreement with other studies in peach (Abidi et al., 2011; Cantín et al., 2010) and in sweet cherry (Jiménez et al., 2004), showing that firmer fruits have higher sugar content.

Fruits on Cadaman rootstock showed, in general, the most luminous colour (higher L* parameter), although it did not differ from Adarcias. Conversely, Adafuel and Adarcias seem to induce redder and darker fruit to 'Queen Giant' nectarines (higher a* and b* parameters). However, in the study of Albás et al. (2004) 'Catherina' trees on GF 677 induced a darker red skin than Adafuel and Adarcias (a* and b* parameters). Significant negative relationships were observed between the Hue angle (H parameter) and titratable acidity. In general, high Hue angle values could indicate low acidity, in agreement with Génard et al. (1994) and Ruíz and Egea (2008). Colour measurements in general are good predictors for fruit quality parameters except for the fruit firmness because fruits with the same hue angle may have different firmness (Lewallen and Marini, 2003).

The results of correlations among agronomical and fruit quality parameters show the important relationships between the characteristics of yield, vigour, fruit weight and fruit quality traits. However, for each rootstock type, the most appropriate combination of plant training and cultivation system can help to increase the yield efficiency and fruit size, while retaining their adaptability and fruit quality. These results underline the important relationships between plant adaptability and development and the major factors of fruit quality.

6.6. CONCLUSIONS

The results of this study show the influence of different peach-almond hybrid rootstocks on tree performance. In these growing conditions, Adarcias and GF 677 rootstocks superior adaptation is obviated by the absence of dead trees, twelve years after budding, especially when compared with Garnem and Felinem, likely the most susceptible rootstocks to root asphyxia conditions. Cadaman induced the highest yield efficiency for both cultivars. Cadaman and Adarcias rootstocks seem to induce higher fruit quality, probably because of their lower vigour and stronger sink competition of fruit versus vegetative growth.

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Capítulo 7

Fruit sugar and phytochemical constituents of peach and nectarine cultivars on almond x peach hybrid rootstocks

7.1. ABSTRACT

The influence of five almond x peach hybrid rootstocks (Adafuel, Adarcias, Felinem, Garnem and GF 677) and one *P. davidiana* x peach hybrid (Cadaman) on individual and total sugars in fruit flesh, as well as total phenolics, flavonoids, anthocyanins, vitamin C and antioxidant capacity contents were evaluated. The six rootstocks were budded with 'Tebana' peach and 'Queen Giant' nectarine and grown under field conditions. Some of these rootstocks are the most widely used in the Mediterranean area. Significant differences were found between rootstocks for the different fruit quality traits evaluated. Regarding individual and total sugars for both cultivars, Adarcias was the rootstock inducing the highest sweetness. The highest content of the phenolics, flavonoids, anthocyanins, vitamin C and RAC was found on Adarcias, Garnem and GF 677 rootstock for 'Queen Giant' cultivar and on Cadaman (phenolics, flavonoids and RAC) and GF 677 (anthocyanins) rootstocks for 'Tebana' cultivar. Significant correlations were found among individual sugar contents, as well as between tree vigour and fruit sucrose, glucose and fructose, and between some phytochemical parameters, such as flavonoid and phenolic content. Thus, the less vigorous rootstocks, Adarcias and Cadaman, seem to induce the highest fruit quality, showing higher content of individual and total sugars. Selecting the right combination of the rootstock and cultivar is important for the best chemical characteristics of peach fruit. Also, it indicated the importance of the sugar profile in the global quality of peaches and nectarines.

Keywords: *Prunus persica*, sugars, vitamin C, antioxidant capacity

7.2. INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is the second most important temperate fruit tree grown in the European Union, after apples. Peach production comes mainly from China, Mediterranean area (Italy and Spain) and United States (FAOSTAT, 2012). Breeding programs are active in the release of new rootstocks and scions, improving peach adaptability to soil (Felipe, 2009; Moreno et al., 1994) and fruit quality (Byrne et al., 1991; Monet and Bassi, 2008). Peach fruit quality is mainly determined by the genotype of each cultivar, although other factors such as rootstock, position of the fruit in the canopy, pruning and thinning practices, and yearly climate are known to influence fruit quality. According to different studies (Albás et al., 2004; Giorgi et al., 2005; Massai and Loreti, 2004; Remorini et al., 2008; Orazem et al., 2011a; Scalzo et al., 2005), the rootstock influences tree vigour, yield and fruit quality parameters like soluble solids content, specific sugars, acidity, organic acids, and several antioxidant compounds although this effect is better known for agronomic and basic parameters like soluble solids content and titratable acidity.

In recent years, strong attention to fruit quality has been reported because it presents multiple health benefits (Prior et al., 1998). Sugars are very important parameters for market quality of peaches because they are appreciated by consumers (Kim et al., 2009; Subedi and Walsh, 2009). The most abundant sugar in ripe peaches is sucrose, followed by the reducing sugars (glucose and fructose) and lower contents of sorbitol (Brooks et al., 1993). A ripe peach is characterized by high sucrose, between 50-75% of the total sugar content, contrary to unripe peach where sucrose content is lowest (Byrne et al., 1991; Kakiuchi et al., 1981; Moriguchi et al., 1990). Fructose is one of the most chemically reactive of the natural sugars and sorbitol is known to play a significant role in the translocation of photosynthates (Moriguchi et al., 1990). Fructose has been shown to be sweeter than sucrose by as much as between 1.75-1.8 times (Doty, 1976, Wu et al., 2003), while glucose is reported to be less sweet than sucrose (Yamaguchi et al., 1970). Sucrose and fructose has been shown to have beneficial effects on gastrointestinal health (Muir et al., 2009). In the field of human nutrition, there is also increasing interest in fruits that are rich in sorbitol, since this sugar alcohol is more beneficial than others with regard to diet control, dental health and to avoid gastrointestinal problems. Also, sorbitol can be used as a glucose substitute for diabetics and as an alternative natural sweetener to sucrose (Forni et al., 1992).

The development of some degenerative illnesses is caused by free radicals present in the human organism which cause oxidative damage to lipids or proteins. Therefore, the antioxidant compounds are capable to neutralize these free radicals, and prevent diseases such cancer (García-Alonso et al., 2004). Eating fruits and vegetables, also reduces inflammation and blood pressure (Wang and Lin, 2000). The antioxidant activity of peach fruit is dependent on genotype, rootstock or climatic conditions such as temperature (Cantín et al., 2009b; Gil et al., 1995; Scalzo et al., 2005). Total antioxidant capacity (Prior and Cao, 2000) and phenolic compounds play an important role in food quality, and they can also contribute to aroma or flavour. Anthocyanins give specific coloration to fruits and vegetables and also have health benefits in the prevention of chronic diseases, such as cardiovascular disease and certain types of cancer (Ruíz et al., 2005).

In summary, a key to the commercial expansion of peach production is the promotion and maintenance of the highest possible standards of fruit quality and to understand the role of rootstocks on the sugar profile and other phytochemicals substances. Until recently, this effect has been more studied in cultivars than in rootstocks and little is known of the effect of rootstock on sugar profile and phytochemical composition.

The present work aimed to evaluate the effect of different peach-almond hybrid rootstocks on sugar content and phytochemical fruit quality of peaches and nectarines when grow under typical Mediterranean conditions.

7.3. MATERIALS AND METHODS

7.3.1. Plant material

Five almond x peach hybrid [*Prunus amygdalus* Batsch x *P. persica* (L.) Batsch] and one *P. davidiana* x peach hybrid [*Prunus davidiana* (Carrière) Franch x *P. persica* (L.) Batsch] rootstocks were evaluated in this study. The six rootstocks were budded with 'Tebana' peach and 'Queen Giant' nectarine cultivars during the summer of 1997, and trees were established in a trial during the winter of 1998-1999. Rootstocks chosen for this study were Adafuel (Cambra, 1990) and Adarcias (Moreno and Cambra, 1994; Moreno et al., 1994) as selections from the Experimental Station of Aula Dei (CSIC); Garnem and Felinem (Felipe, 2009) as selections from the Centre of Research and

Agro-food Technology of Aragón (CITA); Cadaman (Edin and Garcin, 1994) as a French-Hungarian release; and GF 677 (Bernhard and Grasselly, 1981) rootstock used as the standard, since it is the most widespread rootstock in the Mediterranean peach-growing area. The effect of almond x peach rootstocks on fruit sugar content and phytochemical constituents of peach cultivars was studied for three years (2008, 2009 and 2010) to estimate seasonal effect on fruit quality.

7.3.2. Field trial

The experiment was located in the Ebro Valley at the Experimental Station of Aula Dei (CSIC-Zaragoza, Northeast, Spain), on a heavy and calcareous soil, with 27% total calcium carbonate, 8% active lime, water pH 8.3, and a clay-loam texture. Trees were trained to a low density open-vase system (6 × 5 m). Cultural management practices, such as fertilization, winter pruning, and spring thinning, were conducted as in a commercial orchard. Open vase trees were pruned to strengthen existing scaffold branches and eliminate vigorous shoots, inside and outside the vase, that would compete with selected scaffolds or shade fruiting wood. Moderate-sized fruiting wood (0.3-0.6 m long) was selected. Trees were hand-thinned at 45-50 days after full bloom (DAFB) leaving approximately 20 cm between fruits. The plot was level-basin irrigated every 12 days during the summer. Guard rows were used to preclude edge effects. The experiment was established in a randomized block design with five single-tree replications for each scion-stock combination.

7.3.3. Growth and yield determinations

Trunk girths were measured during the dormant season 20 cm above the graft union, and the trunk cross-sectional area (TCSA) was calculated. At harvest, all fruits from each tree were counted and weighted to determine total yield per tree (Kg/tree) and mean fruit weight. Cumulative yield per tree and yield efficiency (cumulative yield in kilograms per tree/TCSA) of each scion-stock combination were computed from the harvest data.

7.3.4. Sampling

During harvest, 20 mature fruits of each tree were randomly selected. Fruits were peeled, and a portion of the mesocarp was removed from the middle of each opposite sides and cut into small pieces. A composite sample of 5 g was built by mixing all pieces from all the selected fruits. It was frozen in liquid nitrogen and kept at -20ºC until analysed. Samples for vitamin C determination were kept at -20ºC in metaphosphoric solution (5% HPO_3) until analysis for preservation of oxidation.

For analysis of sugar content, samples were homogenized with 10 mL of extraction solution consisting of 800 mL/L ethanol/Milli-Q water. The mixture was centrifuged at 20,000 g for 20 min at 4ºC. For analysis of antioxidant compounds, samples were homogenized using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington) with 10 mL of extraction solution consisting of 0.5 N HCl in methanol/Mili-Q water (80% v/v). Extracts were centrifuged at 20,000 g for 20 min at 4ºC, and the supernatant was collected and stored at -20ºC. Finally, to determine vitamin C, samples were homogenized with 5% HPO_3 and then centrifuged at 20,000 g for 15 min at 4ºC, and the supernatant stored at -20ºC.

7.3.5. Evaluation of basic fruit quality traits

Fruit weight was calculated from the total number of fruits and the total yield per tree, as previously reported (Font i Forcada et al., 2012). Soluble solids content (SSC) of fruit juice was measured with a digital refractometer (Atago PR-101, Tokyo, Japan). The titratable acidity (TA) of samples was determined using an automatic titrator (Metrohm Ion analysis, 807 Dosing Unit, Switzerland) and ripening index was calculated based on the SSC/acidity ratio. Flesh firmness Flesh firmness measurements were performed by a hand penetrometer with an 8 mm flat probe. Values of L* (brightness or lightness), a* (-a* = greenness, +a* = redness), b* (-b* = blueness, +b* = yellowness), C* (chroma) and H (lightness's angle) were measured using a colorimeter (Chroma Meter, CR-400 Konica Minolta, Japan).

7.3.6. Determination of sugars

For the analysis, 250 µL of the homogenized extract was incubated at 80 °C for 20 min in 200 µL of 800 mL/L ethanol/water, with 5 g/L manitol added as an internal standard. Samples were purified using ion exchange resins (Bio-Rad Barcelona, Spain)

as reported by Moing et al. (1992). Samples were then vacuum concentrated and then resuspended to 1 mL of Milli-Q water, before HPLC analysis. The most important sugars found in fruit flesh (sucrose, glucose, fructose and sorbitol) were analyzed by High Performance Liquid Chromatography (Bio-Rad, Barcelona, Spain), 300x7.8 mm column (Aminex® HPX-87C, CA, USA) with a refractive index detector (Waters 2410), consisted of a pump and manual injection (20 µl injection volume) interfaced to a PC Millenium 3.2 software (Waters) as described by Cantín et al. (2009a). A distilled deionized water solution was used as mobile phase with a flow rate of 0.6 mL/min at 85°C. HPLC peaks were identified using commercial standards of analytical grade (Panreac Quimica SA, Barcelona, Spain). Sugar concentrations were expressed as g per kg of fresh weight (FW).

7.3.7. Antioxidant compounds analysis

The antioxidant compounds were analysed using a spectrophotometer photodiode array detector DU 800 (Beckman Coulter, Inc., Fullerton, CA) as described by Cantín et al. (2009b). Standard calibration curves were daily prepared. The Folin-Ciocalteau reagent at 0.25 N was used to determine the total phenolic content. Absorbance was measured at 725 nm and the results were expressed as mg of gallic acid (3,4,5-Trihydroxy-benzoic acid) equivalents (GAE) per 100 g FW. The flavonoid content absorbance was measured at 510 nm and the results were expressed as mg of catechin equivalents per 100 g of FW. For determining anthocyanin content, spectrophotometric readings at 535 nm were taken subtracting absorbance at 700 nm (due to turbidity). Anthocyanins were expressed as mg of cyanidin-3-glucoside equivalents (C3GE) per kg of FW using a molecular weight of 494 and a molar extinction absorptivity coefficient ε = 25,965/cm M. The relative antioxidant capacity (RAC) was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). Absorbance was measured at 515 nm and the results were expressed as µg of Trolox equivalents per g of FW. For vitamin C determinations, absorbance was determined at 525 nm and the results were expressed as mg of ascorbic acid (AsA) per 100 g of FW.

7.3.8. Data analysis

The means from five replicates were analyzed statistically using SPSS 19.0 (SPSS, Inc, Chicago, USA). When the F test was significant, means were separated by Duncan's multiple range ($P \leq 0.05$). Data were analyzed to determine the significance of

differences between rootstocks. In addition, the analyses of bilateral Pearson correlation were carried out to conclude relationships between parameters. Principal components analysis (PCA) was also used to study correlations among agronomic, fruit quality, sugar content and phytochemical constituents, to interpret relationships between rootstocks and to detect clustering formation and establish relationships between rootstocks and fruit quality traits. A 2D PCA plot was designed using combined data from three years of the study.

7.4. RESULTS AND DISCUSSION

7.4.1. Influence of environmental conditions on sugar and phytochemical compounds

Results for 'Tebana' include only Adafuel, Adarcias, Cadaman and GF 677 rootstocks, due to the high mortality of trees on Felinem and Garnem (Zarrouk et al., 2005). Tree mortality was attributed to sensitivity to root asphyxia, because these rootstocks are better adapted to well drained soils (Felipe, 2009; Font i Forcada et al., 2012).

Table 7.1 shows factors affecting fruit quality parameters in both cultivars. ANOVA results showed that rootstock influenced all traits except sorbitol and RAC for 'Queen Giant', and sucrose and flavonoids for 'Tebana'. It has been shown that levels of individuals sugars in peach fruit differ among rootstocks (Colaric et al., 2005; Orazem et al., 2011b), which agree with our results. The statistically significant effect of year was found for all traits, except for phenolic content in 'Tebana' cultivar. The year-to-year variation in fruit quality parameters may be explained by the differences in harvest time or annual temperatures over the 3 years of study. Thus, some biochemical traits could be more influenced by the environmental conditions over the growing season than other traits, in agreement with different studies (Brooks et al., 1993; Bureau et al., 2009; Serrano et al., 2005; Tomás-Barberán and Espin, 2001).

The phenolic content in 2008 for both cultivars was, in general, higher than in the following two years (2009 and 2010), probably because ripening index was higher in 2008. A positive and significant correlation between ripening index and phenolic content was found in 2008 ($r = 0.58$; $P \leq 0.05$).

Table 7.1. ANOVA analysis of the effect of rootstock and year on fruit quality traits in 'Queen Giant' and 'Tebana' cultivars for the average of the 3 years of study.

Cultivar	Source of variation[1]	SUC	GLU	FRU	SOR	TS	Phen.	Flav.	Anthoc.	Vit. C	RAC
'QG'	Rootstock (R)	**	***	***	ns	**	*	***	**	**	ns
	Year (Y)	***	***	***	**	**	***	***	**	***	***
	RxY	ns	ns	ns	ns	ns	ns	***	ns	*	**
'Tebana'	Rootstock (R)	ns	*	***	***	*	**	ns	***	***	**
	Year (Y)	***	***	***	*	**	ns	***	***	***	*
	RxY	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]Data were evaluated by two-way variance (ANOVA); ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns, not significant. Abbreviations: QG, 'Queen Giant'; SUC, sucrose; GLU, glucose; FRU, fructose; SOR, sorbitol; TS, total sugars; Phen, phenolics; Flav, flavonoids; Anthoc, anthocyanins; Vit C, vitamin C; RAC, relative antioxidant capacity.

This positive correlation was also reported by Proteggente et al. (2002). In contrast, the content of anthocyanins, vitamin C and RAC were generally higher in 2009 than in 2008 and 2010, for both cultivars. The average temperatures recorded in the 'Experimental Station of Aula Dei' from March to July, was higher in 2009 (18.1ºC ± 4.6) than in 2008 (16.8ºC ± 4.8) and 2010 (17.6ºC ± 4.7). Serrano et al. (2005) reported that the location, year or climate had a significant effect on the anthocyanin and flavonoid content in sweet cherry. Similarly, Tomás-Barberán and Espin (2001) showed that temperature had a marked effect on anthocyanin production in apples or plums. No significant interaction between rootstock and year was found, except for the content of flavonoids, vitamin C and RAC of 'Queen Giant'.

Therefore, the chemical composition of sugar and other biochemical compounds of peach and nectarines are significantly affected by rootstocks as well as by others factors, such as climate, harvest conditions and scion genotype. All these parameters may have significant roles in determining fruit quality such as nutraceutical composition and bioactivity of the organic compounds involved.

7.4.2. Content on sugar profile

Rootstock influenced the levels of sucrose, glucose, fructose, sorbitol and total sugars in fruit for both cultivars (Table 7.2). As previously reported, levels of individual and total sugar content in peach fruit differed among rootstocks (Albás et al., 2004; Orazem et al., 2011a, 2011b) and peach cultivars (Abidi et al., 2011; Cantín et al., 2009a; Colaric et al., 2005).

Regarding sucrose levels for the 'Queen Giant' cultivar, in average, the highest and lowest values were induced by Adarcias and Garnem respectively, while no significant differences were found with Adafuel, Cadaman, Felinem, and GF 677. Results obtained for 'Tebana' followed the same tendency as 'Queen Giant' (Table 7.2). In detail, we can observe that, although no significant differences were found in the sucrose content Adarcias and Cadaman induced the highest values in 2008, as well as Adarcias in 2009 and Cadaman in 2010, and GF 677 the lowest in all years. Among rootstocks, values of sucrose ranged from 36.0 (Garnem, in 2009) to 59.9 (Adarcias, in 2008) g kg^{-1} FW for 'Queen Giant' and from 60.8 (GF 677, in 2009) to 79.1 (Adarcias, in 2008) g kg^{-1} FW for 'Tebana' cultivar. For glucose content in 'Queen Giant', Adarcias and Cadaman showed the highest values and Garnem and GF 677 induced the lowest, while no significant differences were found with Adafuel and Felinem rootstocks. In 'Tebana', no significant differences were found among rootstocks. Among rootstocks, values for glucose ranged from 6.9 (Garnem, in 2009) to 14.6 (Adarcias, in 2008) g kg^{-1} FW for 'Queen Giant' and from 5.9 (Adafuel, in 2009) to 13.6 (Adarcias, in 2008) g kg^{-1} FW for 'Tebana' cultivar. For fructose content in 'Queen Giant', Adarcias again had the highest value, although no significant differences were found with Cadaman and Felinem, while GF 677 induced the lowest value with the latter not differing from Adafuel and Garnem. In 'Tebana', Adarcias had the highest content in 2008, 2009 and for the average values although no significant differences were found with Cadaman and GF 677. Among rootstocks, values of fructose ranged from 9.6 (Garnem, in 2009) to 13.4 (Adarcias, in 2008) g kg^{-1} FW for 'Queen Giant' and from 8.0 (GF 677, in 2010) to 13.9 (Adarcias, in 2008) g kg^{-1} FW for 'Tebana' cultivar. For sorbitol content in 'Queen Giant', no significant differences were found among rootstocks, in spite of the tendency of Adarcias to show a higher value. In 'Tebana', Adarcias and Cadaman induced higher values than Adafuel and GF 677, both in 2008 and in the average values for the three years.

Table 7.2. Mean values of individual and total soluble sugars of 'Queen Giant' and 'Tebana' budded on different rootstocks, in the tenth (2008), eleventh (2009) and twelfth (2010) year after grafting.

Cultivar	Character	Rootstock	2008	2009	2010	Average
'Queen Giant'	Sucrose	Adafuel	53.45 ab	43.01 ab	44.72 a	47.06 ab
		Adarcias	59.91 b	46.23 b	49.43 b	51.86 b
		Cadaman	52.35 ab	40.85 ab	43.71 a	45.64 ab
		Felinem	52.94 ab	42.84 ab	45.19 a	46.99 ab
		Garnem	48.78 a	36.02 a	44.76 a	43.19 a
		GF 677	54.54 ab	46.84 b	45.12 a	48.83 ab
	Glucose	Adafuel	12.86 ab	7.84 ab	10.01 ab	10.24 ab
		Adarcias	14.58 c	9.38 c	10.87 ab	11.61 b
		Cadaman	13.48 bc	8.71 b	11.13 b	11.11 b
		Felinem	13.38 bc	7.52 ab	11.22 b	10.71 ab
		Garnem	11.67 ab	6.98 a	10.52 ab	9.72 a
		GF 677	10.68 a	8.73 b	9.80 a	9.74 a
	Fructose	Adafuel	12.50 ab	10.7 ab	10.49 a	11.23 ab
		Adarcias	13.40 b	11.98 b	11.34 a	12.24 c
		Cadaman	13.30 b	10.62 ab	11.28 a	11.73 bc
		Felinem	13.25 b	10.89 ab	11.44 a	11.86 bc
		Garnem	12.03 ab	9.59 a	10.79 a	10.80 ab
		GF 677	10.98 a	9.79 a	10.02 a	10.26 a
	Sorbitol	Adafuel	1.41 a	1.84 a	1.73 a	1.66 a
		Adarcias	2.05 a	1.76 a	2.21 a	2.01 a
		Cadaman	1.74 a	1.51 a	2.13 a	1.79 a
		Felinem	1.34 a	1.66 a	1.90 a	1.63 a
		Garnem	1.61 a	1.33 a	2.27 a	1.74 a
		GF 677	1.41 a	1.79 a	2.14 a	1.78 a
	Total Sugars	Adafuel	80.21 ab	63.41 ab	67.05 a	70.22 ab
		Adarcias	89.95 b	69.36 b	73.86 b	77.72 b
		Cadaman	80.87 ab	62.18 ab	68.25 a	70.43 ab
		Felinem	80.91 ab	62.90 ab	69.76 a	71.19 ab
		Garnem	74.09 a	53.91 a	68.34 a	65.45 a
		GF 677	77.61 a	67.15 b	67.09 a	70.62 ab
'Tebana'	Sucrose	Adafuel	72.82 a	63.78 a	62.19 a	66.26 a
		Adarcias	79.13 b	67.01 b	63.76 a	69.97 a
		Cadaman	79.10 b	64.40 a	66.10 b	69.87 a
		GF 677	71.36 a	60.81 a	61.20 a	64.46 a
	Glucose	Adafuel	9.87 a	5.95 a	6.67 a	7.50 a
		Adarcias	13.61 a	7.64 a	6.82 a	9.36 a
		Cadaman	12.06 a	7.80 a	7.53 a	9.13 a
		GF 677	10.99 a	7.43 a	6.84 a	8.42 a
	Fructose	Adafuel	9.95 a	9.10 a	7.86 a	8.97 a
		Adarcias	13.88 b	10.84 b	8.08 a	10.93 b
		Cadaman	12.41 ab	9.73 a	8.81 a	10.32 ab
		GF 677	11.11 a	8.85 a	8.02 a	9.33 ab
	Sorbitol	Adafuel	1.72 a	1.63 a	2.31 a	1.89 a
		Adarcias	2.96 b	2.58 b	2.61 a	2.72 b
		Cadaman	2.80 b	2.32 ab	3.21 a	2.78 b
		GF 677	1.59 a	1.80 ab	2.57 a	1.99 a
	Total Sugars	Adafuel	94.36 a	80.51 a	79.05 a	84.64 a
		Adarcias	109.58 a	88.10 b	81.27 a	92.98 b
		Cadaman	105.86 a	83.87 a	85.65 a	91.79 b
		GF 677	95.05 a	78.90 a	78.56 a	84.17 a

For each year, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test. All individual sugars and total sugars are expressed as g kg $^{-1}$ FW.

Among rootstocks, values of sorbitol ranged from 1.6 (GF 677, in 2008) to 3.2 (Cadaman, in 2010) g kg^{-1} FW for 'Tebana' cultivar. Finally, for total sugars and for 'Queen Giant', Adarcias induced the highest average value while Garnem showed the lowest, although no significant differences were found with Adafuel, Cadaman, Felinem and GF 677. In 'Tebana', Adarcias and Cadaman also induced higher average values that Adafuel and GF 677. Among rootstocks, values of total sugars ranged from 53.9 (Garnem, in 2009) to 89.9 (Adarcias, in 2008) g kg^{-1} FW for 'Queen Giant' and from 78.6 (GF 677, in 2010) to 109.6 (Adarcias, in 2008) g kg^{-1} FW for 'Tebana' cultivar. As previously reported for different peach and nectarine cultivars, sucrose is the sugar present at the highest concentration, followed by glucose, fructose and sorbitol (Abidi et al., 2011; Cantín et al., 2009a; Génard et al., 2003; Robertson et al., 1990).

Summarizing, Adarcias rootstock induced the highest values of individual and total sugars for both cultivars, while Garnem showed the lowest for 'Queen Giant' and GF 677 for 'Tebana'. Thus, for 'Queen Giant', Adarcias induced a 21%, 28% and 8% increase in total sugars compared to Garnem, in 2008, 2009 and 2010, respectively. Regarding 'Tebana', Adarcias rootstock showed a 15%, 12% and 3% increase in total sugars compared to GF 677 in 2008, 2009 and 2010, respectively.

7.4.3. Content on phytochemical constituents

For 'Queen Giant', Adarcias and Garnem rootstocks showed higher values of phenolic content than Cadaman, Felinem and GF 677, while no significant differences were found among them and Adafuel (Table 7.3). In the case of 'Tebana', Cadaman induced the largest amount of phenolics compared to Adafuel, Adarcias and GF 677. Among rootstocks, values of phenolics ranged from 18.0 (Cadaman, in 2010) to 36.6 (Garnem, in 2008) mg GAE/100 g FW for 'Queen Giant', and from 26.9 (GF 677, in 2008) to 38.2 (Cadaman, in 2008) mg GAE/100 g FW for 'Tebana'.

As for flavonoid content for 'Queen Giant', the average of the three years showed that Adarcias induced the highest content, although no significant differences were found with Adafuel, Felinem and Garnem.

Table 7.3. Effect of rootstock of total phenolics, flavonoids, anthocyanins, vitamin C and antioxidant capacity (RAC) of 'Queen Giant' and 'Tebana' in the tenth (2008), eleventh (2009) and twelfth (2010) year after budding.

Cultivar	Character	Rootstock	2008	2009	2010	Average
'Queen Giant'	Total phenolics	Adafuel	31.4 ab	21.5 a	20.2 a	24.4 ab
		Adarcias	34.4 ab	24.9 b	21.8 a	27.0 b
		Cadaman	25.5 ab	21.8 a	18.0 a	21.8 a
		Felinem	30.3 ab	23.0 ab	18.5 a	23.9 a
		Garnem	36.6 b	23.0 ab	21.0 a	26.9 b
		GF 677	23.4 a	22.8 a	19.2 a	21.8 a
	Flavonoids	Adafuel	7.6 ab	3.4 ab	3.2 a	4.7 bc
		Adarcias	10.3 bc	4.6 b	4.6 b	6.5 c
		Cadaman	4.5 a	3.4 ab	3.8 ab	3.9 ab
		Felinem	6.5 a	2.7 a	3.3 a	4.2 bc
		Garnem	13.2 c	2.9 a	2.9 a	6.3 bc
		GF 677	5.2 a	2.7 a	3.0 a	3.4 a
	Anthocyanins	Adafuel	0.25 c	0.30 b	0.29 ab	0.28 bc
		Adarcias	0.20 bc	0.33 b	0.35 b	0.29 c
		Cadaman	0.10 a	0.26 ab	0.19 a	0.18 ab
		Felinem	0.14 ab	0.21 a	0.19 a	0.18 a
		Garnem	0.20 bc	0.25 ab	0.26 ab	0.24 bc
		GF 677	0.22 bc	0.21 a	0.19 a	0.21 bc
	Vitamin C	Adafuel	2.5 a	4.2 a	4.0 b	3.6 a
		Adarcias	3.1 a	4.7 ab	3.9 b	3.9 a
		Cadaman	2.5 a	3.8 a	4.0 b	3.4 a
		Felinem	3.4 a	4.6 ab	4.1 a	4.0 ab
		Garnem	3.7 a	3.4 a	3.3 ab	3.5 a
		GF 677	3.0 a	6.0 b	5.1 b	4.7 b
	RAC	Adafuel	370.7 ab	407.6 ab	385.6 a	388.0 a
		Adarcias	390.6 ab	394.4 ab	360.8 a	381.9 a
		Cadaman	380.4 a	427.0 bc	389.2 a	399.0 a
		Felinem	306.4 ab	360.9 a	357.0 a	341.4 a
		Garnem	399.0 b	378.0 ab	358.5 a	378.5 a
		GF 677	310.4 a	466.5 c	384.0 a	387.0 a
'Tebana'	Total phenolics	Adafuel	31.3 a	27.7 a	23.5 a	27.5 a
		Adarcias	32.9 a	29.3 ab	27.8 ab	30.0 a
		Cadaman	38.2 a	34.8 b	32.7 b	35.2 b
		GF 677	26.9 a	29.7 ab	27.8 ab	28.1 a
	Flavonoids	Adafuel	9.1 a	3.7 a	2.6 a	5.1 a
		Adarcias	10.1 a	4.2 a	3.7 a	6.0 a
		Cadaman	9.6 a	7.6 b	5.6 b	7.6 b
		GF 677	6.1 a	4.8 a	3.9 a	4.9 a
	Anthocyanins	Adafuel	0.19 a	0.36 a	0.33 a	0.30 a
		Adarcias	0.13 a	0.56 b	0.52 ab	0.40 ab
		Cadaman	0.15 a	0.59 b	0.48 ab	0.41 ab
		GF 677	0.24 b	0.66 b	0.59 b	0.50 b
	Vitamin C	Adafuel	2.7 ab	9.9 ab	10.1 a	7.6 a
		Adarcias	3.5 b	12.1 b	11.3 a	9.0 a
		Cadaman	2.5 ab	8.7 a	10.9 a	7.4 a
		GF 677	2.8 a	10.8 ab	10.3 a	8.0 a
	RAC	Adafuel	334.1 a	389.6 ab	350.5 a	365.1 a
		Adarcias	343.7 a	374.0 a	337.4 a	351.7 a
		Cadaman	400.3 b	405.7 b	403.1 a	403.0 b
		GF 677	299.7 a	392.3 ab	399.4 a	363.8 a

For each year, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test. Total phenolics (mg GAE/100 g FW); flavonoids (mg CE/100g FW); anthocyanins (mg C3GE/kg FW); vitamin C (mg AsA/100 g FW); RAC, relative antioxidant capacity (µg Trolox/g FW).

The lowest value was induced by GF 677 rootstock but no significant differences were shown with Cadaman. In contrast, for 'Tebana', Cadaman induced the higher value for two years as well as for the average of the three years (Table 7.3). Among rootstocks, values of flavonoids ranged from 2.7 (GF 677, in 2009) to 13.2 (Garnem, in 2008) mg CE/100g FW for 'Queen Giant' and from 2.6 (Adafuel, in 2010) to 7.6 (Cadaman, in average) mg CE/100g FW for 'Tebana'.

Fruit anthocyanin content for 'Queen Giant' was highest on Adarcias, but no significant differences were found with Adafuel, Garnem and GF 677 rootstocks. The lowest value was induced by Felinem but it did not differ significantly from Cadaman. For 'Tebana', GF 677 and Adafuel induced the highest and lowest values respectively, although they did not differ from Adarcias and Cadaman. Among rootstocks, values of anthocyanins ranged from 0.10 (Cadaman, in 2008) to 0.35 (Adarcias, in 2010) mg C3GE/kg FW for 'Queen Giant', and from 0.13 (Adarcias, in 2008) to 0.66 (GF 677, 2009) mg C3GE/kg FW for 'Tebana'. Thus, phenolic compounds, flavonoids and anthocyanins could be increased or lowered with the selection of a certain rootstock as reported by (Tomás-Barberán et al. 2001) and with different peach cultivars.

Significant differences were also found among rootstocks in the fruit vitamin C content, although it was more evident in the case of 'Queen Giant'. For this cultivar, GF 677 induced higher value than the rest of rootstocks except for Felinem. As for 'Tebana', the highest vitamin C content was shown on Adarcias for two years, but no significant differences were found among rootstocks when averaged for the three years. Among rootstocks, values of vitamin C ranged from 2.5 (Adafuel and Cadaman, in 2008) to 6.0 (GF 677, in 2009) mg AsA/100 g FW for 'Queen Giant' and from 2.5 (Cadaman, in 2008) to 12.1 (Adarcias, in 2009) mg AsA/100 g FW for 'Tebana'.

Concerning RAC, for 'Queen Giant', Garnem induced the highest value in 2008, although no significant differences were found with Adafuel, Adarcias and Felinem. In 2009, GF 677 induced the highest value but did no differ from Cadaman. No significant differences were found in the average values for the three years. Regarding 'Tebana', Cadaman induced the highest content. Among rootstocks, values of RAC ranged from 306.4 (Felinem, in 2008) to 466.5 (GF 677, in 2009) μg Trolox/g FW for 'Queen Giant' and from 299.7 (GF 677, in 2008) to 405.7 (Cadaman, in 2009) μg Trolox/g FW for 'Tebana'. Similar values of RAC were found in peach by Abidi et al. (2011), Cantín et al. (2009b) and Proteggente et al. (2002).

7.4.5. Phenotypic correlations among traits

A correlation analysis between sugars and phytochemical components in peach and nectarine fruits was carried out to determine the significant effect of different almond x peach hybrid rootstocks on fruit quality. Table 7.4 shows the average of the correlations of the three years of study.

7.4.5.1. Correlations between sugar content and other quality traits

Total sugar content was positively and highly correlated with all individual sugars as previously reported (Cantín et al., 2009a; Dirlewanger et al., 1999; Drogoudi et al., 2008). The higher values were shown between total sugars and sucrose for both cultivars (Wu et al., 2003) probably because sucrose is the major sugar in peach flesh. Similarly, correlation values between total sugars and glucose or fructose were also higher than between total sugars and sorbitol. Also, significant correlation values among sucrose, glucose and fructose were higher than values between these sugars and sorbitol. The correlations found between SSC and individual and total sugars were significant for both cultivars (Table 7.4), as reported in peaches and nectarines (Cantín et al., 2009a; Wu et al., 2003) and in apricots (Drogoudi et al., 2008). A significant positive correlation between SSC and fruit weight (FW) was also found in both cultivars, probably because the rate of fruit growth is determined by the amount of carbohydrates (Morandi, 2008). Glucose and fructose sugars were also slightly correlated with FW in the case of 'Tebana'. In contrast, a significant negative correlation between sugar components and yield was found, especially in the case of 'Queen Giant', and between SSC and tree vigour (TCSA) for this cultivar.

Table 7.4. Pearson's correlations coefficients between traits observed over three years in almond x peach hybrid rootstocks budded with Queen Giant and Tebana cultivars.

Cultivar	Trait	SSC	Sucrose	Glucose	Fructose	Sorbitol	TS[a]	Phenolics	Flavonoids	Vit. C	RAC
QG	Yield	-0.48**	-0.58**	-0.59**	-0.63**	ns	-0.63**	ns	ns	ns	ns
	TCSA	-0.49**	ns	ns	ns	ns	ns	ns	ns	ns	ns
	HD	0.42**	ns	ns	ns	ns	ns	0.28*	ns	ns	ns
	FW	0.39*	ns	ns	ns	ns	ns	ns	ns	0.27*	ns
	SSC		0.46**	0.48**	0.53**	ns	0.52**	0.32*	0.38**	ns	ns
	TA		ns	0.29*	0.36**	ns	ns	ns	ns	-0.31*	ns
	FF		-0.48**	-0.53**	-0.39**	ns	-0.51**	ns	ns	ns	ns
	RI		0.42*	0.39*	0.40*	0.33*	ns	ns	ns	ns	ns
	Sucrose		-	0.73**	0.69**	0.31**	0.97**	ns	ns	ns	ns
	Glucose			-	0.81**	ns	0.86**	ns	ns	ns	ns
	Fructose				-	ns	0.82**	ns	ns	ns	ns
	Sorbitol					-	0.27*	ns	ns	ns	ns
	TS[a]						-	ns	ns	ns	ns
	Phenolics							-	0.78**	ns	0.68**
	Flavonoids								-	ns	0.68**
	Vit. C									-	0.36*
Tebana	Yield	ns	ns	-0.40*	ns	ns	ns	ns	ns	ns	ns
	TCSA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	HD	0.39**	ns	ns	ns	ns	ns	0.37*	ns	ns	ns
	FW	0.56*	ns	0.35**	0.38*	ns	ns	ns	ns	ns	ns
	SSC	ns	0.43*	0.37*	0.35*	0.77*	0.48**	0.44*	0.34*	ns	0.47**
	TA		ns	0.35*	ns	ns	ns	ns	ns	0.46**	ns
	FF		-0.49**	-0.63**	-0.49**	ns	-0.59**	ns	ns	ns	ns
	RI		0.54*	0.42*	0.35*	0.41*	ns	ns	ns	-0.39*	ns
	Sucrose		-	0.58**	0.63**	0.47**	0.95**	ns	ns	ns	ns
	Glucose			-	0.87**	0.44**	0.80**	ns	ns	ns	ns
	Fructose				-	0.49**	0.83**	ns	ns	ns	ns
	Sorbitol					-	0.56**	ns	ns	ns	ns
	TS[a]						-	ns	ns	ns	ns
	Phenolics							-	0.73**	ns	0.67**
	Flavonoids								-	ns	0.60*
	Vit. C									-	0.43*

* and ** represent statistical significance at $P \leq 0.05$ and $P \leq 0.01$ respectively; ns, not significant. Abbreviations: QG, Queen Giant; TCSA, trunk-cross sectional area; HD, harvest date ; FW, fruit weight; SSC, soluble solids content; TA, titratable acidity; FF, flesh firmness; RI, ripening index (SSC/TA); TS [a] , Total sugars are the sum of sucrose, glucose, fructose, and sorbitol for each genotype, analyzed by HPLC; vit. C, vitamin C; RAC, relative antioxidant capacity.

Also, significant and negative correlations were showed between TCSA and fruit content in sucrose (r = -0.58; $P \leq 0.01$), glucose (r = -0.45; $P \leq 0.01$), fructose (r = -0.51; $P \leq 0.01$) and total sugars (r = -0.44; $P \leq 0.01$) in 2008. Similarly, for 'Tebana', a significant and negative correlation was also found between TCSA and fructose (r = -0.62; $P \leq 0.05$) in 2008. These negative correlations between TCSA and SSC or sugar contents can be due to a stronger sink competition of vegetative development in more vigorous rootstocks compared to fruit as shown by Morandi (2008).

On the other hand, sugar components were significantly correlated with TA and RI (SSC/TA). In the case of 'Queen Giant', significant positive correlations were found between glucose or fructose with TA, and for 'Tebana' between glucose and TA. All individual sugars showed a significant positive correlation with RI with highest values for sucrose (Cantín et al., 2009a; Wu et al., 2003). On the contrary, significant negative correlations were found between all individual sugars and fruit firmness, with the exception of sorbitol.

However, no significant correlations were found between sugar content and biochemical compounds. In contrast, Abidi et al. (2011) reported a positive and significant correlation between total sugars and total phenolics, vitamin C and RAC. Pirie and Mullins (1977) also found a positive correlation between sugar content in berries and levels of phenolic contents, probably due to the role of sugars in the regulation of phenolic biosynthesis.

The negative correlations between vigour and SSC and the positive correlation between SSC and sugars, confirm that less vigorous rootstocks like Adarcias and Cadaman have the possibility to induce sweeter fruits to the cultivar. Peach fruits from the less vigorous trees also had the highest SSC and sugar contents in the study of Giorgi et al. (2005) probably because dwarfing rootstocks are generally able to send more nutrients to the fruit because there is less competition from the vegetative parts (Chalmers et al., 1981).

7.4.5.2. Correlations between phytochemical components and other quality traits

A significant relationship was observed between RAC and total phenolics, flavonoids and vitamin C for both cultivars (Table 7.4). Similar results were also found in peaches and nectarines by Gil et al. (2002) and in other fruit species such as apple (Lata, 2007), cherries (Serrano et al., 2005) and plums (Gil et al., 2002). Gardner et al.

(2000) also showed the contribution of vitamin C to the antioxidant capacity of different fruit juices, such as orange, apple and pineapple. These results showed that phenolic acids, flavonoid compounds and vitamin C are the main source of the antioxidant capacity in fruits (Cevallos-Casals et al., 2006; Wang et al. 1996; Gil et al., 2002). However, no significant correlation was found between RAC and anthocyanins, according to Cantín et al. (2009a). Positive correlations between FW and vitamin C, between SSC and phenolic content or flavonoids for 'Queen Giant', and between SSC and phenolic content, flavonoids and RAC in the case of 'Tebana' were found to be in agreement with other studies in peach (Cantín et al., 2009b), apricot (Bureau et al., 2009) and sweet cherry (Serrano et al., 2005). The positive correlation between vitamin C and TA for 'Tebana' may be due to the contribution of ascorbic acid to the fruit acidity according to Cantín et al. (2009b). These correlations showed a tendency of bigger and sweeter fruits to have higher levels of these bioactive compounds. The relationship of fruit weight with bioactive compounds could be explained by the well-known influence of the sink size (i.e., fruit weight) on the ability to attract photosynthates from the plant sources, because a sufficient accumulation of sugars in or near the fruit is essential for phenolic compounds synthesis during fruit growth (DeJong, 1999). Thus, rootstocks inducing bigger and sweeter fruits could be also producing fruits with higher content of antioxidant compounds, like Adarcias, Cadaman and GF 677.

7.4.6. Principal components analysis

The principal components analysis (PCA) was used to analyze the data for the 19 agronomical and fruit quality traits obtained from the different rootstocks budded with 'Tebana' peach and 'Queen Giant' nectarine cultivars. A four component model accounted for more than 74% of total variance, with the first two components, PC1 and PC2, explaining 39.1% and 15.3% of total variance, respectively. The distribution of individuals based on the PC1, PC2 and PC3 (Table 7.5) shows their phenotypic variation and how widely dispersed they are along axes. The PC1 mainly contributes to fruit weight, SSC, TA, RI, sucrose, total sugars, phenolics, flavonoids, anthocyanins and vitamin C. The PC2 explains yield, TCSA, cumulative yield and fructose. Finally, PC3 mainly represents yield efficiency, firmness, glucose and sorbitol. Individual trees on the negative side of PC1 such as 6, 7, 8, 9 and 10 (Adarcias rootstock); 11, 12 and 13 (Cadaman rootstock); or 22, 26 and 27 (GF 677 rootstock) were all budded to 'Queen

Giant' and had higher values for glucose and fructose and other fruit quality traits (TA, firmness and fruit weight). Individuals on the positive side of PC1 such as 32, 33, 34, 35 and 36 (Adarcias rootstock) were budded to 'Tebana' and had higher values for sorbitol, SSC and total sugars. Also, the individuals 28, 38, 39 and 40 (Cadaman rootstock); and 42, 43 and 45 (GF 677 rootstock), all with 'Tebana' as the scion cultivar, had higher values on anthocyanins, phenolic content and RAC.

Table 7.5. Eigenvectors of the three principal component (PC) axes of the 19 agronomic and fruit quality traits evaluated on different rootstocks budded with 'Tebana' peach and 'Queen Giant' nectarine cultivars.

	Component loading		
Traits	PC1 (39.11%)	PC2 (15.33%)	PC3 (8.5%)
Trunk cross-sectional area	-0.438	**0.689**	0.009
Yield	-0.173	**0.811**	0.291
Cumulative yield	-0.269	**0.790**	0.485
Yield efficiency	0.081	0.418	**0.697**
Fruit weight	**-0.577**	0.054	0.054
Soluble solid content	**0.749**	-0.228	0.172
Flesh firmness	-0.469	-0.090	**0.585**
Titratable acidity	**-0.884**	-0.288	0.195
Ripening index	**0.893**	0.214	-0.061
Sucrose	**0.880**	-0.016	0.244
Glucose	-0.532	-0.479	**0.554**
Fructose	-0.423	**-0.620**	0.330
Sorbitol	0.455	-0.192	**0.585**
Total Sugars	**0.770**	-0.203	0.430
Phenolic content	**0.814**	0.043	-0.003
Flavonoids	**0.546**	-0.253	0.302
Antocyanins	**0.778**	0.027	0.050
Vitamin C	**0.898**	0.177	-0.055
Relative antioxidant capacity	0.166	-0.003	0.011

The rootstocks for 'Queen Giant' are on the left side of the PCA and the rootstocks for 'Tebana' are on the right side of the PCA (Figure 7.2). This is probably due to the average values for glucose and fructose (left side of the PCA Figure 7.1), higher on 'Queen Giant', and the average values for sucrose, sorbitol, total sugars, phenolics, flavonoids, anthocyanins and vitamin C (left right of the PCA Figure 7.1) are higher on 'Tebana'.

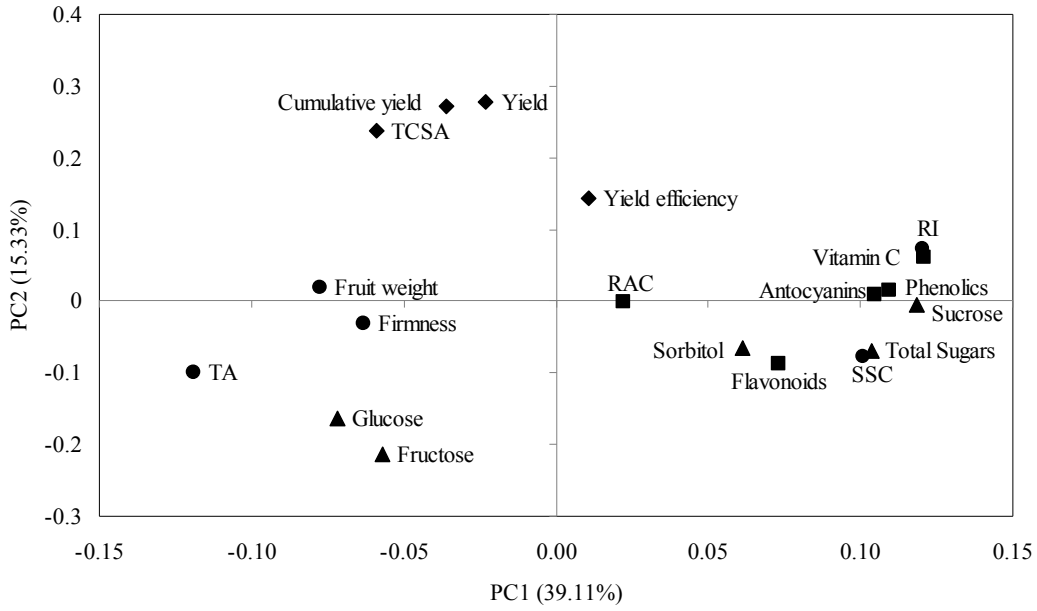

Figure. 7.1. Principal components analysis axes of the 19 agronomic and fruit quality traits evaluated on different rootstocks budded with 'Queen Giant' nectarine and 'Tebana' peach cultivars. Symbols: (♦) agronomical traits, (●) basic fruit quality traits, (▲) sugars and (■) phytochemical compounds.

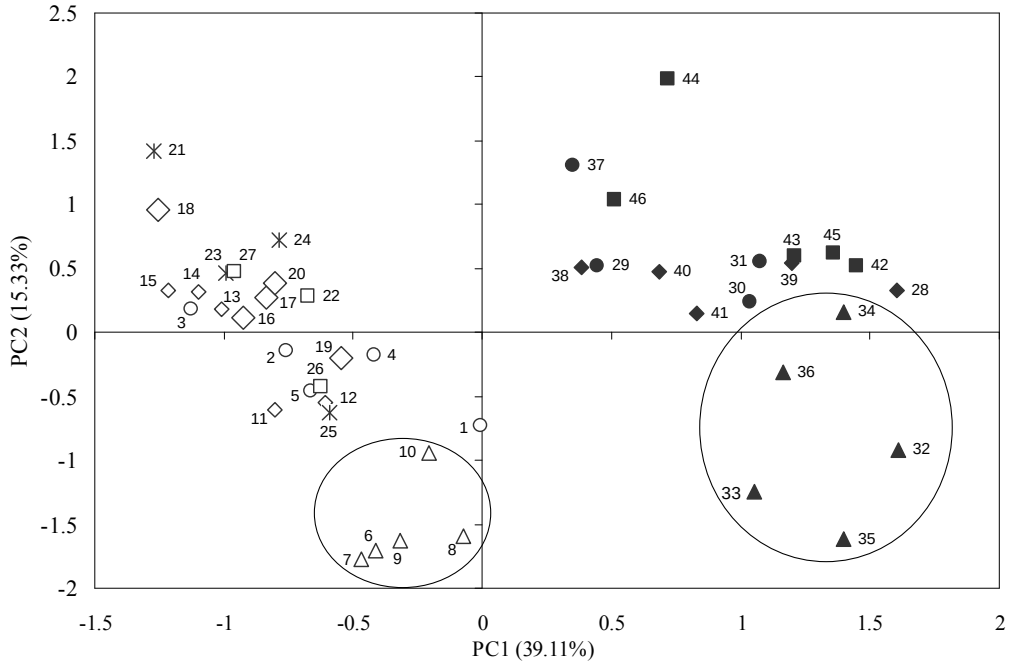

Figure. 7.2. Principal components analysis axes of the 46 individuals evaluated on different rootstocks budded with 'Queen Giant' nectarine (left side) with the symbols: (○) Adafuel, (Δ) Adarcias, (◊) Cadaman, (◊) Felinem, (ж) Garnem and (□) GF 677 rootstocks and 'Tebana'

peach (right side) with the symbols (●) Adafuel, (▲) Adarcias, (♦) Cadaman and (■) GF 677 rootstocks.

7.5. CONCLUSION

Significant differences were found between rootstocks for the different fruit quality traits evaluated. In conclusion, Adarcias and Cadaman rootstocks seem to induce, in general, the higher fruit sweetness based on individuals and total sugars, for both cultivars. For the other biochemical compounds, Adarcias also induced higher values on phenolics, flavonoids and anthocyanins for 'Queen Giant' and on vitamin C for 'Tebana'. Cadaman induced higher values on phenolics, flavonoids and RAC for 'Tebana', and GF 677 induced higher values on vitamin C and RAC for 'Queen Giant' and on anthocyanins for 'Tebana'. This would have a crucial impact on the quality of peach fruit.

We can conclude that selecting the right combination of the rootstock and cultivar, the chemical characteristics of peach fruit could be greatly affected and it should become a more important parameter to be considered in new plantings.

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Capítulo 8

Agronomical parameters,
sugar profile and antioxidant
compounds of Catherine peach cultivar
influenced by different plum rootstocks

8.1. ABSTRACT

The influence of seven plum rootstocks (Adesoto, Monpol, Montizo, Puebla de Soto 67 AD, PM 105 AD, St. Julien GF 655/2 and Constantí 1) on individual and total sugars, as well as on antioxidants content in fruit flesh of 'Catherine' peaches, was evaluated for three years. Agronomical and basic fruit quality parameters were determined for twelve years.

At twelve years after budding, significant differences were found between rootstocks for the different agronomical and fruit quality traits evaluated. Positive and significant correlations were showed between SSC and individual and total sugars. Significant correlations were also found among sugars, phytochemical parameters, yield and vigour.

A clear tendency was showed with the 'Pollizo' plum rootstock Adesoto and PM 105 AD inducing, in general, higher contents on individual (sucrose, fructose and sorbitol), and total sugars, as well as phenolics, flavonoids, vitamin C and RAC. The results obtained with the principal components analysis confirmed the highest content for trees on these two rootstocks for fruit quality traits. ANOVA results showed the absence of interaction between rootstock and year for all traits evaluated indicating that rootstocks had consistent effects on the cultivar. The results of this study show the importance of rootstock on the sugar profile and phytochemical characteristics of peaches.

Keywords: *Prunus persica*, fruit quality, sugars, phytochemical compounds

8.2. INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is one of the most important fruit crop in the world after apples and pears. This crop is economically important with a production around 20.2 million tons in 2010 and with a cultivated area approximately of 1.6 million ha (FAOSTAT, 2012). China, the Mediterranean area (Italy and Spain) and Unites States are the four top producers in the world. In Spain, the peach production (around 1.4 million tons in 2011) contributes to the major production among stone and pome fruits and its production represents 4.5% of total Spanish horticultural products (MAGRAMA, 2012).

The selection of an appropriate rootstock is important because some factors such as yield, vigour or fruit quality depend on the selection of the right scion-rootstock combination. The rootstock effect on tree growth, survival, yield or fruit weight is well known for the most commonly used rootstocks in the peach industry (Font i Forcada et al., 2012; Remorini et al., 2008; Zarrouk et al., 2005). However, most vigorous and high-yielding peach-based rootstocks have been shown to induce lower fruit quality to the budded cultivars, probably due to the higher strength of vegetative growth versus fruit quality (Font i Forcada et al., 2012). Thus, different genetic background of rootstocks can affect fruit quality, indicating that vigour and yield are not the only parameters affected by rootstocks (Giorgi et al., 2005; Orazem et al., 2011a).

In the fruit industry, there is an increasing interest in the nutritional value of fruit quality traits (Byrne et al., 2012; Wolfe et al., 2008) because they represent multiple health benefit effects to human health. Sugar profile, vitamin C, antioxidant capacity and phenolic compounds are the most important phytochemical compounds that could play an important role in gastrointestinal health (Muir et al., 2009) and prevent oxidative stress, diseases such as cancer or cardio-vascular disease (García-Alonso et al., 2004). The most abundant sugar in ripe peaches and nectarines is sucrose, followed by glucose, fructose, and finally sorbitol. Sucrose is important as a sweetener and energy source (Brooks et al., 1993). Fructose is sweetness than sucrose and glucose, and sorbitol is more beneficial than others with regard to diet control (Muir et al., 2009).

Fruit quality depends mainly on the genotype (Tomás-Barberán et al., 2001), but also may be influenced by the rootstock (Scalzo et al., 2005; Tavarini et al., 2011) and climatic conditions, especially yearly climate. The effect of different cultivars on sugar

profile, phenolics content and antioxidant capacity of different type of peaches has been reported (Abidi et al., 2011; Cantín et al., 2009a, 2009b; Drogoudi and Tsipouridis, 2007). The effect of different peach-almond hybrid rootstocks on individual sugars of a peach and a nectarine cultivar was first reported by Albás et al. (2004). Also, Remorini et al. (2008) showed the effect of different rootstocks on some phytochemical compounds, including the total antioxidant capacity of 'Flavorcrest' peaches.

A key to the commercial expansion of peach production is the promotion and maintenance of the highest possible standards of fruit quality. Since rootstock strongly affects agronomic parameters of the budded cultivars, it is also advisable to know the role of rootstocks on the fruit quality and the relationship between agronomical and quality traits. Until present, fruit quality effects have been much more studied in cultivars (Byrne et al., 2012) than in rootstocks and little is known about the influence of rootstocks with different genetic origin on sugar profile and phytochemical composition of the fruit.

At the Experimental Station of Aula Dei (CSIC), a breeding program of *Prunus* rootstocks adapted to growing Mediterranean conditions is carried out. Within this program (Moreno, 2004), local Spanish plums named 'Pollizo' (*P. insititia*) were included for clonal selection as multi-purpose rootstocks for different stone fruit species (Moreno et al., 1995), but especially for peach trees grown in heavy and calcareous soil conditions. In addition, it is commonly assumed that 'Pollizo' plums induce higher fruit quality to peaches than the most frequently used peach x almond hybrids or peach seedlings. Thus, the present study aims to evaluate the effect of seven plum rootstocks of different genetic origins, among them five 'Pollizo' plums, on agronomical and fruit quality traits of peaches.

8.3. MATERIALS AND METHODS

8.3.1. Plant material and field trial

Seven plum rootstocks (Table 8.1), including five 'Pollizo' plums (*P. insititia*): Adesoto, Monpol, Montizo, P. Soto 67 AD and PM 105 AD, a St. Julien GF 655/2 plum (*P. insititia*) and a common plum (*P. domestica*): Constantí 1, were evaluated for three consecutive years (2009-2011).

Table 8.1. List of studied rootstocks, description and origin.

Rootstock	Species	Genetic background	Origin[a]	References
Adesoto[b]	*P. insititia*	op[d] 'Pollizo', clonal selection	CSIC, Spain	Moreno (1990); Moreno et al. (1995)
Monpol	*P. insititia*	op[d] 'Pollizo', clonal selection	CITA, Spain	Felipe (1989)
Montizo	*P. insititia*	op[d] 'Pollizo', clonal selection	CITA, Spain	Felipe (1989)
P. Soto 67 AD[c]	*P. insititia*	op[d] 'Pollizo'	CSIC, Spain	Cambra (1970)
PM 105 AD[c]	*P. insititia*	op[d] 'Pollizo', clonal selection	CSIC, Spain	Moreno (1990)
GF 655/2	*P. insititia*	'St. Julien' clonal selection	INRA, France	Bernhard and Grasselly (1959)
Constantí 1[c]	*P. domestica*	op[d], common plum	CSIC, Spain	Moreno (2004); Cantín et al. (2006)

[a] CSIC = Consejo Superior de Investigaciones Científicas; CITA = Centro de Investigación y Tecnología Agroalimentaria de Aragón; INRA = Institut National de la Recherche Agronomique. [b] Protected grant by Community Plant Variety Office (CPVO). [c] non-released clones from the Aula Dei breeding program. [d] op: open-pollinated.

Trees were established in a field trial during the winter of 1999-2000. Adesoto (formerly Adesoto 101) and PM 105 AD (Moreno, 1990; Moreno et al., 1995) were selected as polyvalent clonal rootstocks for different stone fruit trees, but especially for peaches to avoid water-logging and iron chlorosis in heavy and calcareous soils. Constantí 1 is a local autochthonous plum that has shown a good performance as peach rootstock in field trials at the Experimental Station of Aula Dei (Moreno, 2004; Cantín et al., 2006). Montizo and Monpol are also two 'Pollizo' clonal selections from the 'Centro de Investigación y Tecnología Agroalimentaria de Aragón' (CITA, Spain) (Felipe, 1989). St. Julien GF 655/2 was a rootstock selection developed at the 'Institut National de la Recherche Agronomique' (INRA, France) (Bernhard and Grasselly, 1959).

The experiment was located at the Experimental Station of Aula Dei (CSIC-Zaragoza, Northeastern, Spain), on a heavy and calcareous soil, with 30.5% total calcium carbonate, 8.8% active lime, water pH 7.7, and a clay-loam texture. Trees were trained to a low density open-vase system (5 × 4 m). Cultural management practices, such as fertilization, winter pruning, and spring thinning, were conducted as in a commercial orchard. Open vase trees were pruned to strengthen existing scaffold branches and eliminate vigorous shoots, inside and outside the vase, that would compete with selected scaffolds or shade fruiting wood. Moderate-sized fruiting wood (0.3-0.6 m

long) was selected. Trees were hand-thinned at 45-50 days after full bloom (DAFB) leaving approximately 20 cm between fruits. The plot was level-basin irrigated every 12 days during the summer. Guard rows were used to preclude edge effects. The experiment was established in a randomized block design with six replications for each scion-stock combination except for Adesoto with five replications. All trees budded on Adesoto and PM 105 AD survived well to the end of the experiment. In contrast, a rate of 33% of mortality was found on Montizo, Monpol and P. Soto 67 AD. Lower mortality was found for GF 655/2 and Constantí 1 with only a single dead tree (16.6%).

8.3.2. Fruit sampling and evaluation of agronomic, sugar and phytochemical traits

Twenty mature fruits of each tree were randomly selected at harvest. The mean fruit weight was calculated considering the total number of fruits and the total yield per tree. The trunk cross-sectional area (TCSA), yield and yield efficiency were also calculated for each scion-stock combination as previously reported (Font i Forcada et al., 2012). In the entire fruits, values of L* (brightness or lightness), a* (-a* = greenness, +a* = redness), b* (-b* = blueness, +b* = yellowness), C* (chroma) and H (lightness's angle) were measured using a colorimeter (Chroma Meter, CR-400 Konica Minolta, Japan). Flesh firmness (N) was measured on two paired sides of each fruit, by removing 1 mm thick disk of skin from each side of the fruit, and using a penetrometer (Model FT-327). After skin colour and flesh firmness determinations, the fruits of the sample were peeled, and a portion of the mesocarp was removed from each opposite face and cut into small pieces. A composite sample was built by mixing all pieces from all the selected fruits and soluble solids content (SSC) of fruit juice was measured with a digital refractometer (Atago PR-101, Tokyo, Japan), and was expressed as ºBrix. The titratable acidity (TA) of samples was determined using an automatic titrator (Metrohm Ion analysis, 807 Dosing Unit, Switzerland). Ten grams of homogenized samples were diluted with 90 g of distilled water, and microtitrated with 0.1 N NaOH (Metrohm Ion analysis, 807 Dosing Unit, Switzerland), and was expressed as g malic acid/100 g FW. Ripening index was calculated based on the SSC/acidity ratio.

For sugars analysis, a composite sample of 5 g was frozen in liquid nitrogen and kept at -20ºC until analyzed. Samples were homogenized with 10 mL of extraction solution consisting of 800 mL/L ethanol/Milli-Q water. The mixture was centrifuged at 20,000 g for 20 min at 4ºC. For the analysis, 250 µL of the homogenized extract was

incubated at 80°C for 20 min in 200 µL of 800 mL/L ethanol/water, with 5 g/L manitol added as an internal standard. Samples were purified using ion exchange resins (Bio-Rad Barcelona, Spain) as reported by Moing et al. (1992). Samples were then vacuum concentrated and then resuspended to 1 mL of Milli-Q water, before High Performance Liquid Chromatography (HPLC) analysis. The most important sugars found in fruit flesh (sucrose, glucose, fructose and sorbitol) were analyzed by HPLC (Aminex HPX-87C column, 300 mm x 7.8 mm; Bio-Rad, Barcelona, Spain) with a refractive index detector (Waters 2410) as described by Cantín et al. (2009a). PC Millenium 3.2 software (Waters) was used to perform sugar quantification. A distilled deionized water solution was used as mobile phase with a flow rate of 0.6 mL/min at 85°C. HPLC peaks were identified using commercial standards of analytical grade (Panreac Quimica SA, Barcelona, Spain) and standard calibration curves were used to quantify each different sugar. Sugar concentrations were expressed as g per kg of fresh weight (FW).

For phytochemical analysis, a composite sample of 5 g was frozen in liquid nitrogen and kept at -20ºC until analyzed. Samples were homogenized using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington) with 10 mL of extraction solution consisting of 0.5 N HCl in methanol/Mili-Q water (80% v/v). Extracts were centrifuged at 20,000 g for 20 min at 4ºC, and the supernatant was collected and stored at -20ºC. The antioxidant compounds were analyzed using a spectrophotometer photodiode array detector DU 800 (Beckman Coulter, Inc., Fullerton, CA) as described by Cantín et al. (2009b). Standard calibration curves were daily prepared. The Folin-Ciocalteau reagent at 0.25 N was used to determine the total phenolics content. Absorbance was measured at 725 nm and the results were expressed as mg of Gallic acid (3,4,5-Trihydroxy-benzoic acid) equivalents (GAE) per 100 g FW. The flavonoid content absorbance was measured at 510 nm and the results were expressed as mg of catechin equivalents per 100 g of FW. For determining anthocyanin content, spectrophotometric readings at 535 nm were taken subtracting absorbance at 700 nm (due to turbidity). Anthocyanins were expressed as mg of cyaniding 3-glucoside equivalents (C3GE) per kg of FW using a molecular weight of 494 and a molar extinction absorptivity coefficient ε = 25,965/cm M. The relative antioxidant capacity (RAC) was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). Absorbance was measured at 515 nm and the results were expressed as µg of Trolox equivalents per g of FW. Samples for vitamin C determination were kept at -20ºC in metaphosphoric solution (5% HPO_3) until analysis

for preservation of oxidation. Samples were homogenized with 5% HPO_3 and then centrifuged at 20,000 g for 15 min at 4ºC, and the supernatant stored at -20ºC. Absorbance for vitamin C was determined at 525 nm and the results were expressed as mg of ascorbic acid (AsA) per 100 g of FW.

8.3.3. Data analysis

The data of the means from six replicates were analyzed statistically using SPSS 19.0 (SPSS, Inc, Chicago, USA) and evaluated by ANOVA analysis. When the F test was significant, means were separated by Duncan's multiple range ($P \leq 0.05$). Data were analyzed to determine the significance of differences between rootstocks. The analyses of bilateral Pearson correlation and principal components analysis (PCA) were carried out to study correlations among agronomical, fruit quality, sugars content and phytochemical constituents. A 2D PCA plot was designed using combined data from three years of the fruit quality evaluation and twelve years of the tree agronomical performance.

8.4. RESULTS AND DISCUSSION

8.4.1. Fruit quality traits evaluation

Table 8.2 shows factors affecting agronomical and fruit quality parameters in 'Catherine' peach cultivar. Rootstock influenced the levels of TCSA, yield, cumulative yield, yield efficiency, FW, SSC, TA, RI, sucrose, fructose, sorbitol, total sugars, phenolics, flavonoids, vitamin C and RAC. Similarly, the significant effect of year was found for TCSA, yield, cumulative yield, yield efficiency, FW, SSC, FF, TA, RI, sugars (glucose, fructose and sorbitol), phenolics and anthocyanins. The year-to-year variation in fruit quality parameters may be explained by the differences in annual temperatures and crop load over the 3 years of the study. ANOVA results showed the absence of interaction between rootstock and year for all traits evaluated. This could indicate that rootstocks had consistent effects on the cultivar.

Table 8.2. ANOVA analysis of the effect of rootstock and year on agronomic and fruit quality traits in 'Catherine' cultivar for the average of the 3 years of study.

Source of variation[1]	Rootstock	Year	Rootstock xYear
Trunk cross-sectional area (TCSA)	***	***	ns
Yield	***	***	ns
Cumulative yield	**	**	ns
Yield efficiency	*	**	ns
Fruit weight (FW)	***	***	ns
Soluble solid content (SSC)	***	***	ns
Flesh firmness (FF)	ns	***	ns
Titratable acidity (TA)	***	***	ns
Ripening index (RI)	***	***	ns
Sucrose	**	ns	ns
Glucose	ns	***	ns
Fructose	*	***	ns
Sorbitol	***	***	ns
Total Sugars	***	ns	ns
Phenolics	***	**	ns
Flavonoids	***	ns	ns
Anthocyanins	ns	***	ns
Vitamin C	***	ns	ns
Relative antioxidant capacity (RAC)	***	ns	ns

[1]Data were evaluated by two-way variance (ANOVA); ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns, not significant.

Regarding basic fruit quality parameters (Table 8.3), the average of the three years of study shows that Constantí 1 induced the highest value for fruit weight (FW), although no significant differences were found with the 'Pollizo' Adesoto. In contrast, the lowest FW values were induced by P. Soto 67 AD, PM 105 AD and GF 655/2, but differences were not significant from Monpol and Montizo. Regarding soluble solids content (SSC), the 'Pollizo' PM 105 AD showed the highest value, although no significant differences were found with Adesoto and Monpol. The lowest values were induced by Montizo and GF 655/2, but they did not significantly differ from Constantí 1. Orazem et al. (2011a) reported that Adesoto rootstock induced higher values on FW and SSC when compared to other five different plums and five peach-based rootstocks. Regarding firmness, Montizo showed the higher values in 2009 and 2010, but no significant differences were finally found among rootstocks in the average value for the three years. For TA, no significant differences were observed among rootstocks. For RI, Adesoto and Monpol induced the highest values, while Montizo, GF 655/2 and Constantí 1 showed the lowest, although no significant differences were found with the

rest of rootstocks. In the absence of differences for firmness and TA, higher RI is due to the ability of specific rootstock to induce higher SSC.

Table 8.3. Influence of different plum rootstocks on fruit weight, soluble solids content, flesh firmness and ripening index of 'Catherine' peach fruits in the tenth (2009), eleventh (2010) and twelfth (2011) year after budding.

Character	Rootstock	2009	2010	2011	Average
Fruit weight (g)	Adesoto	164.4 ab	176.2 ab	179.3 bc	173.3 bc
	Monpol	154.4 a	169.2 a	171.2 ab	165.0 ab
	Montizo	162.2 ab	170.1 ab	162.9 ab	165.1 ab
	P. Soto 67 AD	156.2 a	167.8 a	167.6 ab	163.8 a
	PM 105 AD	154.3 a	164.3 a	171.7 ab	163.4 a
	GF 655/2	163.8 ab	172.2 ab	157.5 a	164.1 a
	Constantí 1	169.5 b	180.7 b	181.5 c	177.2 c
SSC (ºBrix)	Adesoto	14.1 ab	13.6 a	13.0 bc	13.5 bc
	Monpol	14.2 ab	13.6 a	12.7 ab	13.5 bc
	Montizo	13.4 ab	12.3 a	11.8 a	12.5 a
	P. Soto 67 AD	14.1 ab	13.4 a	12.1 ab	13.3 b
	PM 105 AD	14.6 b	13.7 a	13.3 c	13.9 c
	GF 655/2	12.8 a	12.8 a	12.2 ab	12.6 a
	Constantí 1	13.4 ab	12.2 a	12.5 ab	12.7 ab
Flesh firmness (N)	Adesoto	32.0 ab	31.0 a	26.2 a	29.7 a
	Monpol	32.0 ab	33.9 ab	26.7 a	30.9 a
	Montizo	35.7 b	37.5 b	20.0 a	31.1 a
	P. Soto 67 AD	33.7 ab	35.1 ab	30.8 a	33.2 a
	PM 105 AD	30.9 ab	31.6 a	25.0 a	29.1 a
	GF 655/2	29.8 a	32.5 a	28.7 a	30.3 a
	Constantí 1	32.9 ab	35.2 ab	26.6 a	31.6 a
RI	Adesoto	31.6 b	23.1 a	21.1 a	25.3 b
	Monpol	29.0 ab	22.8 a	20.3 a	26.5 b
	Montizo	24.5 a	20.6 a	18.5 a	23.7 a
	P. Soto 67 AD	29.0 ab	22.4 a	19.6 a	24.0 ab
	PM 105 AD	29.2 ab	22.5 a	20.5 a	24.1 ab
	GF 655/2	23.7 a	19.4 a	17.5 a	20.2 a
	Constantí 1	26.5 ab	20.5 a	19.6 a	22.2 a

For each year and character, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test. SSC, soluble solids content; RI, ripening index.

The sucrose, glucose, fructose and sorbitol contents of 'Catherine' peaches were analyzed separately by HPLC because they play an important role in peach flavour quality (Esti et al., 1997; Robertson et al., 1988). Sucrose was the sugar present at the highest concentration, as previously reported in peaches and nectarines (Abidi et al., 2011; Cantín et al., 2009a) followed by fructose, glucose and sorbitol. Their levels differed significantly among rootstocks (Table 8.4), as showed by Albás et al. (2004)

comparing three peach-almond hybrids, and Orazem et al. (2011a, 2011b) studying different rootstocks.

Table 8.4. Influence of different plum rootstocks on the individual and total sugars of 'Catherine' peach fruits in the tenth (2009), eleventh (2010) and twelfth (2011) year after budding.

Character	Rootstock	2009	2010	2011	Average
Sucrose	Adesoto	69.9 a	72.1 b	70.2 b	70.7 b
	Monpol	66.9 a	65.0 a	66.5 ab	66.1 a
	Montizo	61.0 a	62.3 ab	67.2 ab	63.5 a
	P. Soto 67 AD	69.0 a	66.9 ab	61.2 a	65.7 a
	PM 105 AD	68.5 a	67.6 ab	67.6 ab	67.9 ab
	GF 655/2	63.0 a	67.0 ab	61.9 ab	64.0 a
	Constantí 1	62.5 a	63.6 ab	66.8 ab	64.3 a
Glucose	Adesoto	7.9 a	8.5 a	7.2 a	7.9 a
	Monpol	9.0 a	9.0 a	8.3 a	8.8 a
	Montizo	9.2 a	9.2 a	8.1 a	8.8 a
	P. Soto 67 AD	8.9 a	8.5 a	7.3 a	8.2 a
	PM 105 AD	8.7 a	8.2 a	7.6 a	8.2 a
	GF 655/2	8.4 a	8.6 a	7.3 a	8.1 a
	Constantí 1	8.5 a	8.8 a	7.7 a	8.3 a
Fructose	Adesoto	10.2 b	9.8 a	9.3 b	9.8 b
	Monpol	9.4 ab	9.1 a	8.5 ab	9.0 ab
	Montizo	9.8 ab	10.1 a	9.0 ab	9.4 ab
	P. Soto 67 AD	9.8 ab	9.7 a	8.5 ab	9.3 ab
	PM 105 AD	8.8 a	9.0 a	8.2 a	8.7 a
	GF 655/2	9.4 ab	10.1 a	8.6 ab	9.4 ab
	Constantí 1	9.6 ab	9.8 a	8.7 ab	9.4 ab
Sorbitol	Adesoto	6.2 b	5.8 b	4.8 b	5.6 b
	Monpol	4.4 a	4.5 a	4.0 ab	4.3 ab
	Montizo	3.8 a	3.9 a	3.1 a	3.6 a
	P. Soto 67 AD	4.7 a	4.7 ab	3.5 ab	4.3 ab
	PM 105 AD	5.1 a	4.8 ab	4.2 ab	4.7 ab
	GF 655/2	3.7 a	4.1 a	3.0 a	3.6 a
	Constantí 1	3.6 a	3.6 a	3.7 ab	3.6 a
Total sugars	Adesoto	94.2 b	96.2 b	91.5 b	94.0 b
	Monpol	89.7 ab	87.6 ab	87.3 ab	88.2 ab
	Montizo	83.8 a	85.5 a	87.4 ab	85.5 a
	P. Soto 67 AD	92.4 ab	89.8 ab	80.5 a	87.5 a
	PM 105 AD	91.1 ab	89.6 ab	87.6 ab	89.5 ab
	GF 655/2	84.5 a	89.8 ab	80.8 a	85.1 a
	Constantí 1	84.2 a	85.8 a	86.9 ab	85.6 a

For each year, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test. All individual sugars and total sugars (g kg $^{-1}$ FW).

For sucrose, the 'Pollizo' Adesoto induced the highest average content, although no significant differences were found with PM 105 AD. Differences were not significant among the later and the rest of the other rootstocks. Values of sucrose ranged among rootstocks and years from 61.0 to 72.1 g kg^{-1} FW. For glucose, no significant

differences were found among rootstocks. Fructose content was higher on Adesoto and lower on PM 105 AD but not significantly different from the other rootstocks. The fructose has been shown to be sweeter than sucrose by as much as between 1.75-1.8 times (Doty, 1976), and both of them have been shown to have beneficial effects on gastrointestinal health (Muir et al., 2009). Consequently, Adesoto could have an additional value to be considered in the future. For sorbitol content, Adesoto again showed the highest content and Montizo, GF 655/2 and Constantí 1 the lowest, but they did not differ significantly from the other rootstocks. The sorbitol content varied greatly among rootstocks ranging from 3.0 to 6.2 g kg^{-1} FW. Sorbitol has been reported as the attribute most related to peach aroma and taste by Colaric et al. (2005). In addition, it is more beneficial than other sugars with regard to diet control, dental health and to avoid gastrointestinal problems, and it can be used as a glucose substitute (Forni et al., 1992). Similar results were found on total sugars content, calculated as the sum of sucrose, glucose, fructose and sorbitol contents. Adesoto induced the highest value while Montizo, P. Soto 67 AD, GF 655/2 and Constantí 1 showed the lowest, but not significantly different from the other two rootstocks (Monpol and PM 105 AD). Total sugar content ranged from 80.8 to 96.2 g kg^{-1} FW. Results for Adesoto agree with the work of Orazem et al. (2011a, 2011b) showing that Adesoto rootstock induced higher values on individual and total sugars compared with the others rootstocks. Again, this is an interesting result as these sugars strongly affect peach flavour quality (Robertson et al., 1988).

Other phytochemical traits seem to follow the same tendency that individual and total sugars content. In the average of the three years of study, the highest value for total phenolics was induced by Adesoto rootstock, although no significant differences were found with P. Soto 67 AD and PM 105 AD. The lowest value was found on Constantí 1, but it did not differ significantly from Monpol (Table 8.5). The phenolics content varied greatly among rootstocks and years ranging from 22.8 to 34.3 mg GAE/100 g FW. Regarding flavonoids content, Adesoto also induced the highest value, but no significant differences were found with Montizo and PM 105 AD. In contrast, GF 655/2 and Constantí 1 induced the lowest values, although they did not significantly differ from Monpol and P. Soto 67 AD. Values for flavonoids ranged from 6.6 to 11.7 mg CE/100 g FW. Concerning anthocyanins content, no significant differences among rootstocks were found. For vitamin C, Adesoto and PM 105 AD induced the highest

values, although they did not differ significantly from Montizo, whereas the lowest values were induced by GF 655/2 and Constantí 1. Values ranged from 5.4 to 9.6 mg ASA/100 g FW.

Table 8.5. Influence of different plum rootstocks on the antioxidant compounds of 'Catherine' peach fruits in the tenth (2009), eleventh (2010) and twelfth (2011) year after budding.

Character	Rootstock	2009	2010	2011	Average
Total phenolics	Adesoto	33.9 b	31.8 c	34.3 c	33.4 c
	Monpol	25.0 a	24.5 ab	28.7 ab	26.1 ab
	Montizo	28.2 a	26.7 bc	28.7 ab	28.0 b
	P. Soto 67 AD	28.5 a	28.0 bc	30.7 bc	28.9 bc
	PM 105 AD	28.8 a	29.7 bc	29.1 ab	29.2 bc
	GF 655/2	26.2 a	25.9 ab	29.9 bc	27.4 b
	Constantí 1	24.2 a	22.8 a	26.0 a	24.3 a
Flavonoids	Adesoto	11.7 c	10.2 bc	10.2 c	10.7 c
	Monpol	7.7 ab	8.1 ab	8.2 ab	8.0 ab
	Montizo	9.9 bc	9.7 bc	8.8 bc	9.5 bc
	P. Soto 67 AD	9.9 bc	9.0 bc	8.2 bc	9.1 ab
	PM 105 AD	10.9 c	11.1 c	9.6 bc	10.6 bc
	GF 655/2	7.7 ab	7.7 ab	7.9 ab	7.8 a
	Constantí 1	7.4 a	6.6 a	7.0 a	7.0 a
Anthocyanins	Adesoto	0.46 a	0.47 a	0.59 a	0.50 a
	Monpol	0.49 a	0.53 a	0.65 a	0.56 a
	Montizo	0.39 a	0.44 a	0.63 a	0.49 a
	P. Soto 67 AD	0.40 a	0.41 a	0.62 a	0.47 a
	PM 105 AD	0.46 a	0.47 a	0.66 a	0.53 a
	GF 655/2	0.47 a	0.46 a	0.64 a	0.52 a
	Constantí 1	0.45 a	0.50 a	0.62 a	0.52 a
Vitamin C	Adesoto	8.9 c	9.1 b	8.6 b	8.8 c
	Monpol	7.4 b	6.9 a	7.6 a	7.3 b
	Montizo	8.0 bc	8.1 ab	8.0 ab	8.0 bc
	P. Soto 67 AD	7.3 bc	7.8 ab	7.4 a	7.5 b
	PM 105 AD	9.0 c	9.6 b	8.8 b	9.1 c
	GF 655/2	5.9 ab	6.2 a	5.6 a	5.9 a
	Constantí 1	5.4 a	6.1 a	5.8 a	5.7 a
RAC	Adesoto	502.2 c	466.7 b	430.0 b	466.3 c
	Monpol	434.5 bc	416.2 ab	414.4 ab	421.7 bc
	Montizo	410.3 ab	398.1 ab	382.9 ab	397.1 ab
	P. Soto 67 AD	436.8 bc	415.5 ab	383.0 ab	413.8 ab
	PM 105 AD	429.0 bc	440.4 b	406.0 ab	425.2 bc
	GF 655/2	413.1 ab	411.9 ab	401.3 ab	408.8 ab
	Constantí 1	345.6 a	350.3 a	358.1 a	351.3 a

For each year, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test. Total phenolics (mg GAE/100 g FW); flavonoids (mg CE/100 g FW); anthocyanins (mg C3GE/kg FW); vitamin C (mg AsA/100 g FW); RAC, relative antioxidant capacity (µg Trolox/g FW).

Finally, in a similar way to phenolics and flavonoids, the highest content of RAC was also induced by Adesoto, although no significant differences were found with PM 105 AD and Monpol. The lowest value was found on Constantí 1, but it did not differ from Montizo, P. Soto 67 AD and GF 655/2. The RAC content varied greatly among rootstocks ranging from 345.6 to 502.2 μg Trolox/g FW.

Sugars content and antioxidant compounds have been influenced by rootstocks, as previously reported (Albás et al., 2004; Orazem et al., 2011b, Scalzo et al., 2005; Tomás-Barberán et al., 2001). The interaction between rootstocks and cultivars influences the levels of sugar profile and phytochemical traits, and this could have a crucial impact on the health promoting properties of peach fruit (Tomás-Barberán et al., 2001). Thus, some cultivars that contain high levels of beneficial traits could be heightened or lowered depending of the rootstock.

In this study, two 'Pollizo' selections (Adesoto and PM 105 AD) induced the highest fruit quality, regarding to SSC, sugars contents and phytochemical compounds, when compared to other 'Pollizo' plums, another *P. insititia* (GF 655/2) or *P. domestica* (Constantí 1) plums as rootstocks for 'Catherine' peach cultivar. In addition, Adesoto induced an intermediate level of vigour, being one of the most high-yielding rootstocks in this trial (data not shown), in good agreement with Orazem et al. (2011a, 2011b), who reported that Adesoto resulted in the best fruit quality (SSC, individual and total sugars levels and phenolic compounds). The present work confirms the good performance of this rootstock, and jointly with PM 105 AD emphasize the interest of some 'Pollizo' rootstocks to reach higher fruit quality peaches.

8.4.2. Correlations between agronomical parameters and fruit sugars content and phytochemical traits

Significant correlations were found among agronomical parameters, sugars profile and phytochemical traits related to fruit quality (Table 8.6). Yield was positively correlated with TCSA, yield efficiency, fruit weight and anthocyanins, but negatively correlated with SSC, sucrose, flavonoids, vitamin C and RAC. Negative correlations between yield and some fruit components, such as SSC or sucrose, can be due to the sink competition of more fruits in development compared to fruit quality (Morandi, 2008). Fruit weight was significantly and positively correlated with SSC, sucrose, glucose, total sugars, phenolics and flavonoids as it was also reported by Cantín et al.

(2010) in different peach and nectarine progenies. That correlation is probably due to the fact that the rate of fruit growth is determined by the amount of available carbohydrates (Morandi, 2008).

All individual sugars were positively and highly correlated with total sugars content. Correlation values between total sugars and glucose or fructose were also higher than between total sugars and sorbitol. Also, significant correlation values among sucrose, glucose and fructose were higher than values between these sugars and sorbitol. Previous studies on fruit sugar content in peaches and nectarines reported similar results (Cantín et al., 2009a; Dirlewanger et al., 1999). On the other hand, total sugars were positive and significantly correlated with phenolics, flavonoids and RAC. Pirie and Mullins (1977) reported a good correlation in grapes between sugar content in berries and levels of phenolic substances, probably due to the role of sugars in the regulation of phenolic biosynthesis. Similarly, Abidi et al. (2011) reported a positive correlation between total sugars and total phenolics, vitamin C and RAC in nectarines. Total sugars, phenolics and flavonoids contents showed a slight significant positive correlation with fruit weight and SSC, showing a tendency of bigger and sweeter fruits to have higher levels of these bioactive compounds. The relationship of fruit weight and SSC with bioactive compounds could be explained by the well-known influence of the sink size on the ability to attract photosynthates from the plant sources, because a sufficient accumulation of sugars near the fruit is essential for phenolic compounds synthesis during fruit growth (DeJong, 1999). Thus, rootstocks inducing bigger and sweeter fruits could be also producing fruits with higher content on antioxidant compounds, as the 'Pollizo' Adesoto rootstock. The correlations between SSC and individual and total sugars were also significant, as previously reported in peaches and nectarines (Cantín et al., 2009a; Wu et al., 2003) or in apricots (Drogoudi et al., 2008). A positive correlation between SSC and phenolics content, flavonoids and RAC was also found in other studies with peaches and nectarines (Abidi et al., 2011; Cantín et al., 2009b), apricots (Bureau et al., 2009) and sweet cherries (Serrano et al., 2005).

Table 8.6. Correlations coefficients between some agronomical and fruit quality traits on different plum rootstocks budded with Catherine cultivar.

Trait	TCSA	YE	FW	SSC	Sucrose	Glucose	Fructose	Sorbitol	TS[a]	Phenolics	Flavonoids	Anthocyanins	Vitamin C	RAC
TCSA	-	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Yield	0.63**	0.36*	0.36**	-0.39**	-0.52**	ns	ns	ns	ns	ns	-0.35**	0.40**	-0.28**	-0.35*
YE		-	ns	ns	ns	-0.36*	ns	ns	ns	ns	ns	ns	ns	ns
FW			-	0.36**	0.42**	0.54**	ns	ns	0.37**	0.26**	0.30**	ns	ns	ns
SSC				-	0.41**	0.52**	0.39*	0.43**	0.43**	0.37**	0.32**	ns	ns	0.29**
Sucrose					-	0.65*	0.63**	0.52**	0.85*	ns	ns	ns	ns	ns
Glucose						-	0.56**	0.64**	0.98*	ns	ns	ns	ns	ns
Fructose							-	0.45*	0.42**	ns	ns	ns	ns	ns
Sorbitol								-	0.41*	ns	ns	ns	ns	ns
TS[a]									-	0.53**	0.46**	ns	ns	0.37**
Phenolics										-	0.71**	ns	0.33**	0.52**
Flavonoids											-	ns	0.36**	0.65**
Anthocyanins												-	ns	ns
Vitamin C													-	0.25**
RAC														-

* and ** represent statistical significance at $P \leq 0.05$ and $P \leq 0.01$ respectively; ns, not significant. Abbreviations: FW, fruit weight; SSC, soluble solids content; TS [a], Total sugars, the sum of sucrose, glucose, fructose, and sorbitol for each genotype, analyzed by HPLC; TCSA, trunk cross-sectional area; RAC, relative antioxidant capacity.

Moreover, we found significant positive correlations between relative antioxidant capacity and total phenolics, flavonoids, and vitamin C, and between vitamin C and phenolics and flavonoids. Thus, flavonoids and total phenolics contribute significantly to the antioxidant capacity of fruits as reported in peaches (Abidi et al., 2011; Cantín et al. 2009b; Gil et al. 2002), in apples (Lata, 2007) or in cherries (Serrano et al., 2005). Gardner et al. (2000) showed the contribution of vitamin C to the antioxidant capacity of fruit juices. These results showed that phenolic acids and flavonoids compounds are the main source of antioxidants in fruits (Cevallos-Casals et al., 2006; Gil et al., 2002). However, no significant correlation was obtained between anthocyanins and RAC in our study, as reported by Cantín et al. (2009b) in peaches and nectarines, probably due to their lower content compared with strawberries, raspberries and plums (Gardner et al., 2000). The high positive correlation found between total phenolics and flavonoids content, indicates that flavonoids are an important group of phenolic compounds in peaches and nectarines with high antioxidant activity.

8.4.3. Principal Component Analysis (PCA) for agronomical parameters, fruit sugar content and phytochemical traits

A principal component analysis (PCA) was performed to understand how 19 agronomical and fruit quality traits contribute to variability among the different rootstocks budded with 'Catherine' peach cultivar (Figure 8.1a, b). The first two PCs (PC1 and PC2) accounted for 58.2% of the total variance. PC1 represented the 35.0% of the variance and PC2 showed the 23.2% of the variance (Table 8.7).

The distribution of individuals based on the PC1, PC2 and PC3 shows the phenotypic variation and how widely dispersed they are along axes. The PC1 represents mainly SSC, TA, RI, phenolic content, flavonoids, sucrose, sorbitol and total sugars. The PC2 explains mainly yield, TCSA, cumulative yield, fruit weight, firmness, RAC and vitamin C. Finally, PC3 mainly contributes to yield efficiency, anthocyanins, glucose and fructose. The results of the analysis of PCA show that the individual trees on the negative side of PC1 such as 55, 66 or 77, corresponding to GF 655/2 rootstock, induced lower TCSA and higher yield efficiency. Individual trees on the positive side of PC1 such as 47, 65 or 78, corresponding to Adesoto rootstock, showed higher values of fruit quality traits, such as SSC, glucose, fructose, sorbitol and total sugars.

Table 8.7. Eigenvectors of the three principal component (PC) axes of the 19 agronomical and fruit quality traits evaluated on different plum rootstocks budded with 'Catherine' cultivar.

	Component loading		
Traits	PC1 (35.0%)	PC2 (23.2%)	PC3 (19.0%)
Trunk cross-sectional area	0.191	**0.894**	0.199
Yield	0.083	**0.693**	-0.491
Cumulative yield	0.015	**0.701**	-0.397
Yield efficiency	-0.296	-0.307	**-0.610**
Fruit weight	0.329	**0.497**	-0.321
Soluble solid content	**0.626**	-0.027	0.173
Flesh firmness	-0.185	**0.348**	0.206
Titratable acidity	**-0.609**	-0.236	-0.262
Ripening index	**0.787**	0.174	-0.133
Antocyanins	-0.133	-0.016	**0.355**
Phenolic content	**0.261**	-0.029	-0.084
Flavonoids	**0.559**	0.132	0.068
Relative antioxidant capacity	-0.197	**-0.430**	-0.015
Vitamin C	-0.118	**-0.449**	-0.244
Sucrose	**0.675**	0.196	0.116
Glucose	0.170	0.367	**0.620**
Fructose	0.076	0.174	**0.595**
Sorbitol	**0.832**	0.015	0.126
Total Sugars	**0.764**	0.098	0.237

Also, individuals 23, 39, 48, 57, 64 and 72, corresponding to PM 105 AD rootstock, had higher values on several phytochemical compounds, such as RAC, phenolics content or vitamin C. The rest of the individual trees corresponding to Constantí 1, Monpol and P. Soto 67 AD had lower or medium values on agronomical parameters and phytochemical compounds.

The results obtained with the PCA confirm that the 'Pollizo' rootstocks Adesoto and PM 105 AD induced the higher values on sugar profile (individuals and total sugars) and phytochemical compounds (phenolics, flavonoids, vitamin C and RAC) of the 'Catherine' peach cultivar. These results agree with Orazem et al. (2011a, 2011b) for the Adesoto rootstock confirming its interest for the peach industry.

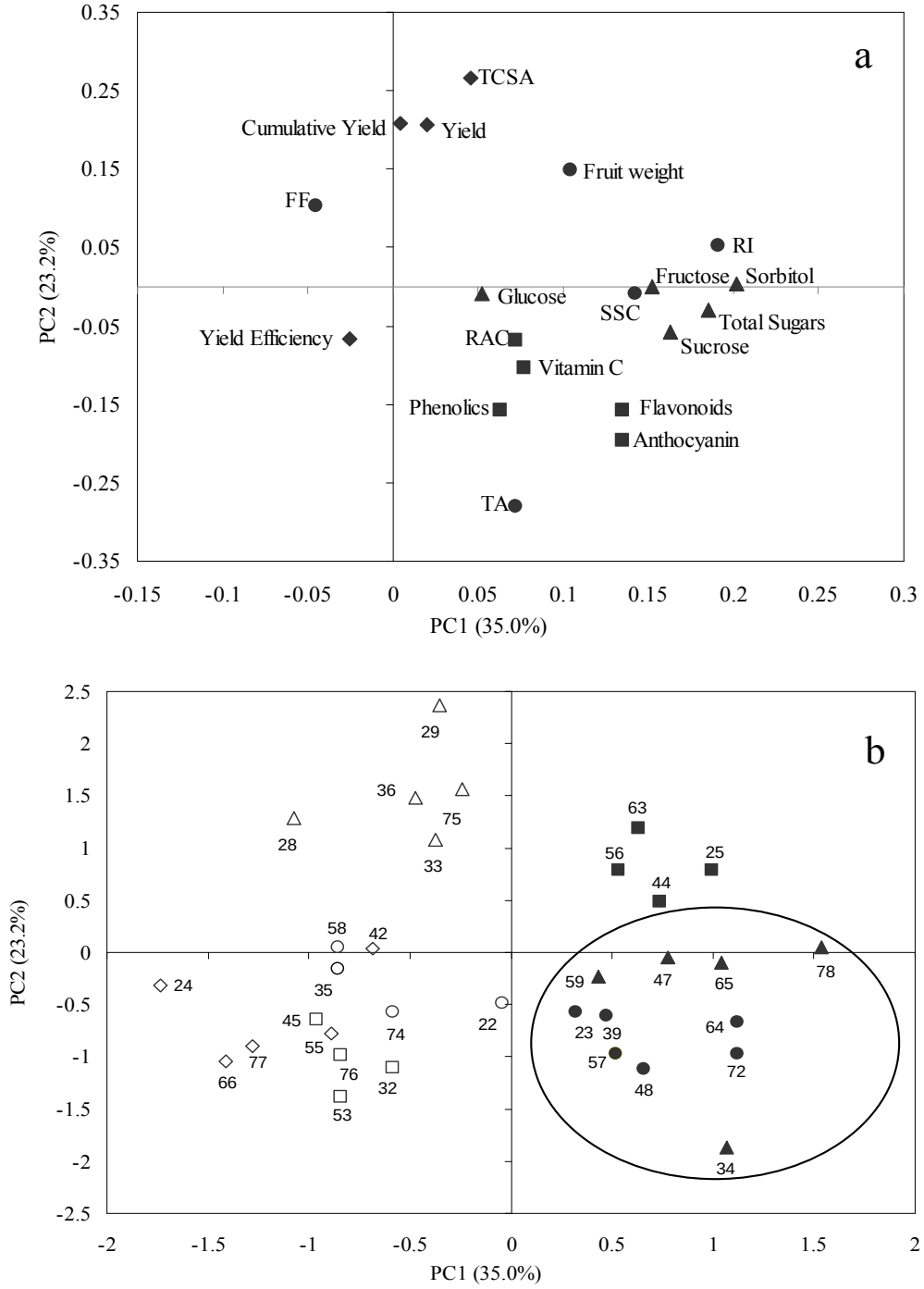

Figure 8.1. Principal components analysis axes of the 19 agronomic, basic fruit quality and phytochemical traits evaluated on different plum rootstocks budded with 'Catherine' peach cultivar. Analysis was performed using mean data of the three years of study (2009-2011). PC1/PC2 loadings plot (a) generated from PCA analysis. Symbols: (♦) agronomical traits, (●) basic quality fruit traits, (▲) sugars and (■) antioxidants compounds. PC1/PC2 scores plot (b) of the 33 individuals evaluated of different rootstocks budded with 'Catherine' peach cultivar. Symbols: (▲) Adesoto, (□) Montizo, (■) Monpol, (O) P. Soto 67 AD, (●) PM 105 AD, (◊) GF 655/2 and (Δ) Constantí 1.

8.5. CONCLUSIONS

The results of this study show the great influence of different plum rootstocks on peach fruit quality. The 'Pollizo' plum rootstocks Adesoto and PM 105 AD, seem to induce higher fruit sweetness, based on sugar profile and SSC, and higher content on antioxidant compounds. Studying relationships among agronomical and phytochemical traits in evaluating the fruit quality could be of great interest for fruit quality. The results of this study show the importance of the sugar profile, because specific sugars play an important role in peach flavour quality, and the phytochemical characteristics to be considered in choosing the best scion-rootstocks graft-combinations in new plantings looking for high quality peaches.

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Capítulo 9

Discusión general

En los frutales caducifolios de clima templado, la calidad del fruto viene determinada principalmente por el genotipo de la variedad cultivada. No obstante, dicha calidad se ve también muy influida por el patrón sobre el que se encuentra injertada, así como por las condiciones ambientales y las técnicas de cultivo empleadas.

En esta tesis se han estudiado 94 variedades de melocotonero y nectarina. De ellas, 43 son variedades autóctonas españolas y 51 son variedades extranjeras. Todas las variedades están incluidas en la colección de germoplasma de la Estación Experimental de Aula Dei (EEAD). Además, se ha evaluado la influencia sobre algunas variedades de melocotonero de 13 patrones *Prunus* con diferente base genética. Se ha determinado su influencia sobre caracteres pomológicos y de calidad del fruto como vigor del árbol, producción, peso del fruto, color, firmeza, sólidos solubles, acidez valorable, índice de maduración, azúcares y compuestos antioxidantes.

Por otra parte, se ha estudiado la colección de melocotonero por sus características pomológicas y moleculares, relacionadas con su estructura poblacional, y se ha calculado el desequilibrio de ligamiento. También se han llevado a cabo diversos estudios de asociación entre marcadores SSRs y SNPs con los caracteres fenotípicos del árbol y del fruto. Las diferentes técnicas empleadas aportarán información y herramientas de trabajo de gran utilidad en la mejora genética del melocotonero.

9.1. Evaluación del comportamiento agronómico, análisis de parámetros de calidad básicos y bioquímicos del fruto en 94 variedades de la colección de melocotonero de la Estación Experimental de Aula Dei

Los resultados obtenidos tras la evaluación de 94 variedades incluidas en la colección de melocotonero de la EEAD han mostrado una gran variabilidad fenotípica para los diferentes caracteres agronómicos, parámetros básicos de calidad y bioquímicos del fruto. La fecha de cosecha varió desde Junio hasta Octubre, siendo las variedades extranjeras ‘Maria Serena’ y ‘Super Crimson Gold’ las más tempranas, y las autóctonas ‘Alcañíz 1’ y ‘Calanda Tardío’ las más tardías. La variabilidad encontrada para las fechas de floración, de cosecha y de producción resulta de gran interés ya que permite disponer de variedades con un amplio calendario para cubrir la demanda del mercado. En cuanto a los caracteres básicos de calidad del fruto como tamaño, color, firmeza, sólidos solubles (SS), acidez valorable e índice de madurez, también se observó una

amplia variabilidad. Cabe destacar las variedades autóctonas 'Borracho de Jarque', 'Amarillo Calanda (131 AD)', 'Bonet III', 'Calanda Tardío' y 'Sudanell 1', así como las variedades comerciales 'Keimoes', 'Lovell' y 'Vivian' por presentar los valores más elevados en firmeza. Para los SS cabe destacar las variedades autóctonas 'Bonet I', 'Bonet III', 'Borracho de Jarque', 'Rojo del Rito' y 'Sudanell 1', y las comerciales 'Golden Queen', 'Halford', 'Nuevo', 'Paloro A', 'Oropel' y 'Vivian' por presentar los niveles más altos. Aunque la variabilidad viene explicada tanto por el componente genético varietal como por la influencia de factores ambientales (Abidi et al., 2011; Cantín et al., 2009a, 2009b; Cevallos-Casals et al., 2006; Gil et al., 2002; Milatović et al., 2010; Tavarini et al., 2008; Tomás-Barberán et al., 2001), permite su utilización en los programas de mejora, destacando los mejores genotipos tras el estudio aquí realizado durante al menos tres años.

El contenido en azúcares es un criterio de calidad del fruto muy importante en los programas de mejora, ya que está muy relacionado con el aroma y el sabor de los frutos (Colaric et al., 2005). Las variedades estudiadas en el presente trabajo presentaron una gran variabilidad, tanto para el contenido en azúcares totales como en los mayoritarios presentes en el fruto del melocotón y nectarina. Se confirmó que el azúcar más abundante es la sacarosa, que osciló entre 35-97 g/kg de peso fresco (PF) en las variedades estudiadas. Siguieron en importancia los contenidos en fructosa (4-15 g/kg PF), glucosa (2-14 g/kg PF) y sorbitol (2-35 g/kg PF). Estos valores están en el rango descrito en otros trabajos (Abidi et al., 2011; Cantín et al., 2009a). Las variedades autóctonas 'Bonet III', 'Calabacero' y 'Calanda San Miguel' mostraron el mayor contenido en azúcares totales. Según los azúcares individuales, entre las variedades que mostraron el mayor contenido para la sacarosa estuvieron: 'Calabacero', 'Diamante Amarillo' y 'Jungerman'; para la glucosa: 'Babygold 9', 'Bonet IV', 'Fantasia' y 'Calabacero'; para la fructosa: 'Amarillo Calanda' (2400 AD), 'Babygold 9', 'Bonet IV', 'Calabacero', 'Fantasia', 'Infanta Isabel' y 'Venus'; y para sorbitol: 'Bonet III', 'Miraflores', 'Rojo del Rito', 'Vivian' y 'Zaragozano Amarillo'. El contenido en compuestos antioxidantes también mostró una gran variabilidad entre variedades. Fue interesante destacar que algunas variedades como 'Alcañíz 2', 'Amarillo de Gallur', 'Goiri' y 'Shasta', presentaron un elevado contenido en vitamina C; 'Alcañiz 1', 'Amarillo Calanda (131 AD)', 'Nuevo' y 'Vivian' en fenoles totales; 'Alcañiz 2', 'Amarillo Calanda (131 AD)' y 'Nuevo' en flavonoides; 'Amarillo de Gallur',

'Borracho de Jarque' y 'Vivian' en antocianinas; y 'Alcañiz 2', 'Amarillo Calanda (131 AD)', 'Zaragozano Amarillo', 'Nuevo y 'Vivian' en RAC, especialmente cuando se compararon con otras variedades de interés económico como son 'Big Top' o 'Venus'.

En los programas de mejora para la obtención de nuevas variedades es muy importante conocer las correlaciones entre los distintos parámetros agronómicos y de calidad básica y bioquímica del fruto. En el presente trabajo, se observaron correlaciones positivas entre el contenido en SS y la firmeza del fruto, como ya se ha mencionado en otros estudios de melocotonero (Abidi et al., 2011; Cantín et al., 2010a, 2010b). Estos dos parámetros son de gran importancia desde el punto de vista comercial ya que un sabor dulce y una firmeza aceptable disminuyen los daños provocados por el transporte, lo que va a garantizar una alta calidad del producto final y su aceptabilidad por parte del consumidor (Colaric et al., 2005). Esto explicaría, por ejemplo, la mayor difusión comercial de algunas variedades autóctonas como 'Miraflores' y 'Sudanell 1' (Badenes, 2000).

Por otra parte, se observaron correlaciones positivas entre fenoles totales, flavonoides y capacidad antioxidante, mostrando la importancia de los compuestos fenólicos como fuente principal de antioxidantes en el fruto (Abidi et al., 2011; Cantín et al., 2009a; Tavarini et al., 2008). Asimismo, las correlaciones positivas observadas entre los azúcares totales y los compuestos antioxidantes, apuntan la posibilidad de seleccionar individuos con una mayor calidad organoléptica y nutricional. Las agrupaciones realizadas según los diferentes caracteres pomológicos del fruto han permitido observar que, en general, el grupo de las variedades modernas presentan un mayor contenido en SS, sorbitol y azúcares totales, diferente a lo obtenido cuando se estudian las variedades individualmente. Esto es debido a que el grupo de las variedades locales tiene un rango más amplio de variabilidad, presentando algunas variedades con contenidos muy altos y otras con contenidos muy bajos, mientras que las modernas tienen un rango más estrecho de variabilidad, y esto hace que el contenido total en un compuesto sea más alto para su totalidad. También podría explicarse por el mayor grado de selección realizado sobre dichas variedades. Para los compuestos antioxidantes, como vitamina C y flavonoides, el grupo de las variedades modernas también presentan un mayor contenido. El análisis de componentes principales mostró el agrupamiento de variedades según sus caracteres pomológicos y de calidad del fruto. Las variedades autóctonas 'Alcañíz 2' y 'Rojo del Rito' y la comercial 'Vivian', que se agruparon juntas, mostraron al mismo tiempo un alto contenido en fenoles, flavonoides,

antocianinas y RAC, aunque menor producción. Además, la variedad 'Alcañíz 2' presentó un alto contenido en vitamina C y RAC. Otras variedades como 'Kakamas' y 'Calabacero' que se agruparon juntas en el eje positivo, presentaron elevados contenidos en azúcares.

9.2. Variabilidad genética, estructura poblacional, desequilibrio de ligamiento y genética de asociación mediante marcadores moleculares microsatélites (SSRs)

Los 42 marcadores SSRs utilizados para la caracterización de las 94 variedades de melocotón y nectarina de la EEAD mostraron que las variedades modernas fueron, en general, genéticamente más diversas que las locales, siendo estos resultados diferentes a los obtenidos para otras especies del género *Prunus*, como es el caso del cerezo (Mariette et al., 2010). Este resultado podría explicarse teniendo en cuenta la historia evolutiva de las diferentes especies en su proceso de domesticación y propagadas clonalmente (McKey et al., 2010), pero también debido a que el melocotonero es la especie menos polimórfica entre las del género *Prunus* por su condición de autogamia. En esta colección, las variedades modernas tienen orígenes muy diversos (EE.UU., Francia, Italia, etc.), mientras que las locales proceden en su mayoría del Valle del Ebro, en España.

Al realizar los análisis con el programa *Sructure* para determinar el nivel poblacional en las 94 variedades estudiadas, se observó que se agrupaban principalmente en dos grupos, el de las modernas en general de origen extranjero, y el de las locales, de origen español. Al comparar la estructura poblacional con el dendograma (UPGMA) y el resultado del programa *Structure*, la concordancia parece confirmar que las variedades que pertenecen a una misma subpoblación, presentan un fondo genético muy similar. Por ello, la selección de individuos de una subpoblación implicaría la drástica reducción de la variabilidad genética de la muestra. La intensa domesticación de esta especie y la mejora genética, que ha utilizado un escaso número de genotipos, han ido causando a lo largo de los años una pérdida muy acusada de la diversidad disponible en melocotonero (Aranzana et al., 2003).

Los análisis de la estructura poblacional se complementaron con el estudio del desequilibrio de ligamiento (DL), a fin de estudiar sus niveles y ver la posibilidad de la aplicación de la genética de asociación en melocotonero. En general, la extensión del DL es dependiente de la población estudiada, ya que se ve muy influido por

características como el tamaño de la población, sistema de reproducción de la especie, estructura de la población, recombinación o selección (Pritchard et al., 2000). Debido al carácter autógamo del melocotonero y al hecho de que se haya utilizado un número reducido de variedades comerciales, como progenitores para diferentes caracteres de interés agronómico o comercial, hace pensar que en el melocotonero existe una elevada conservación del DL. Los resultados mostraron que este DL alcanza su máximo nivel a los 20cM, y después decae con la distancia genética, en concordancia con el estudio de Aranzana et al. (2010).

Tradicionalmente, la identificación y localización de genes se ha estudiado a través de la construcción de mapas genéticos y análisis de QTL en poblaciones procedentes de cruzamientos controlados o descendencias (Aranzana et al., 2003). Sin embargo, en árboles frutales trabajar con un número elevado de generaciones supone una gran limitación, debido al tamaño y al periodo improductivo del árbol frutal. A esto se añade el hecho de que cada población hereda de los progenitores un número limitado de caracteres morfológicos o fenotipos (Aranzana et al., 2003).

Una alternativa para identificar genes de interés se encuentra en los estudios de la genética de asociación, basada en el ligamiento entre marcadores moleculares y caracteres de interés agronómico de individuos “no relacionados” entre sí y que no proceden de un cruzamiento dirigido. En este caso no se requieren plantas pertenecientes a una misma población o cruzamiento, sino variedades autóctonas o comerciales ubicadas en colecciones varietales o bancos de germoplasma.

Las especies más estudiadas en la genética de asociación han sido las de gran importancia económica como el maíz (Remington et al., 2001; Tenaillon et al., 2001), la cebada (Kraaman et al., 2004), la vid (Barnaud et al., 2006) y la caña de azúcar (Janoo et al., 1999), aunque también *Arabidopsis thaliana* (Hagenblad y Nordborg, 2002) por su importancia como planta modelo. Hasta la fecha, son pocos los trabajos publicados en genética de asociación en plantas y más concretamente en frutales (Font i Forcada et al., 2012b). Algunos de ellos estudian el DL o la estructura poblacional, como es el caso del trabajo realizado por Aranzana et al. (2010) en melocotonero o por Mariette et al. (2010) y Ganopoulos et al. (2011) en cerezo. Con el estudio de asociación del genoma completo se ha observado como numerosos marcadores están asociados a caracteres pomológicos de interés. En este trabajo, se seleccionaron los marcadores BPPCT015, CPPCT028 y endoPG1, posicionados en el grupo de ligamiento 4 (GL4), ya que están

relacionados con caracteres muy importantes de la calidad del fruto, como el contenido en azúcares totales, sorbitol o firmeza del fruto. Así, diferentes combinaciones genotipo/haplotipo fueron asociadas a caracteres pomológicos como, por ejemplo, los genotipos 192_196 y 192_228 del marcador endoPG1, que estuvieron asociados significativamente con bajos/altos contenidos en azúcares totales y con menor/mayor firmeza, o el genotipo 167_167 y 220_229 del marcador BPPCT015, y los genotipos 136_136 y 136_138 del marcador CPPCT028, asociados con otros caracteres pomológicos del fruto como contenidos en antioxidantes o azúcares. Además, los haplotipos 169/136 y 209/134 del marcador BPPCT015/CPPCT028 fueron asociados con la fecha de cosecha. A pesar de algunos estudios de identificación de asociaciones entre el fenotipo y genotipo publicados en la familia de las Rosaceas (Cevik et al., 2010; Oraguzie et al., 2010), este sería el primer estudio de asociación de caracteres pomológicos en melocotonero (Font i Forcada et al., 2012b). Previamente, se han cartografiado en melocotonero QTLs ligados a caracteres pomológicos y agronómicos en el GL4 del mapa de referencia de *Prunus* (T × E). Algunos de ellos están relacionados con SS, TA y pH (Cantín et al., 2010a); con SS, glucosa, fructosa, sorbitol, fecha de floración y de cosecha (Arús et al., 2012); con fructosa, sorbitol y algunos ácidos orgánicos (Ogundiwin et al., 2009); con fecha de cosecha (Eduardo et al., 2011); y con el contenido de antioxidantes (Abidi, comunicación personal). Además, hay que remarcar que otros autores encontraron QTLs para el contenido en glucosa, fructosa y sorbitol en melocotonero y ligados al marcador BPPCT015 (Illa et al., 2011), y para el índice de madurez en almendro ligados al marcador CPPCT028 (Sánchez-Pérez et al., 2007). En cuanto a la fecha de floración, no se han encontrado asociaciones con ningún marcador en este trabajo. Sin embargo, Fan et al. (2010) encontraron QTLs en el GL1 para este carácter. Probablemente estas discrepancias se deban a las diferencias en el material utilizado o a los efectos ambientales. Sin embargo, a pesar de la existencia de SSRs ligados a los caracteres monogénicos en melocotonero, son pocos los ejemplos prácticos que se están usando en la actualidad para la selección asistida por marcadores (SAM).

9.3. Marcadores del tipo SNP asociados a los caracteres agronómicos y de calidad del fruto en melocotonero

Además del estudio con microsatélites, en las 94 variedades de melocotón y nectarina mencionadas en el capítulo anterior, se realizó el estudio de genética de

asociación con marcadores del tipo SNPs. La plataforma de Illumina ha comercializado un chip desarrollado por el Consorcio Internacional del melocotón (The International Peach SNP Consortium, IPSC) (Verde et al., 2012), el cual se ha utilizado en este estudio.

La elevada densidad de los marcadores del tipo SNP en todo el genoma del melocotonero permitirá una mayor eficiencia en la búsqueda de asociaciones de interés. Para este análisis se asumió la misma estructura poblacional del capítulo anterior. Del total de 8.144 SNPs analizados, solo 3.851 fueron incluidos en este trabajo. Se obtuvieron un total de 347 asociaciones entre los marcadores SNPs y la fecha de floración y de cosecha, índice de madurez, contenido de antocianinas, flavonoides, capacidad antioxidante relativa, sorbitol y azúcares totales. En el estudio precedente también se encontraron asociaciones entre los marcadores SSRs con la fecha de cosecha, índice de madurez, antocianinas, flavonoides, capacidad antioxidante relativa, sorbitol y azúcares totales (Font i Forcada et al., 2012b). En el caso de los SNPs, la posición de los marcadores viene determinada según el mapa físico mientras que en los SSRs la posición viene determinada según el mapa genético. Se buscó la posición de los SSRs estudiados en el mapa físico (www.rosaceae.org), coincidiendo en algunos casos el grupo de ligamiento (GL) con el scaffold. Así, entre las asociaciones encontradas en ambos estudios para todo el genoma del melocotonero y con la posición coincidente de los marcadores, estarían la fecha de cosecha en los GL o scaffolds 4 y 6 y el contenido en antocianinas en el GL o scaffold 4. En otro estudio de asociación y hasta ahora el único publicado en melocotonero (Cao et al., 2012), se encontraron asociaciones con la fecha de floración pero con distintos SSRs a los aquí empleados.

Fan et al. (2010) encontraron, en su estudio con SSRs, QTLs para la fecha de floración en melocotonero en el GL1. En el presente trabajo con SNPs se han encontrado asociaciones para este mismo carácter en el scaffold 1, a diferencia del estudio precedente con SSRs (Font i Forcada et al., 2012b). Estas discrepancias podrían deberse a la diferente densidad de marcadores utilizada en ambos estudios, así como a los efectos ambientales (Fan et al., 2010).

Este estudio resulta novedoso ya que es la primera aproximación que se realiza asociando los SNPs de la plataforma de 'Illumina Infinium® BeadArray Technology' con los caracteres de calidad del fruto en melocotonero. Otros estudios con SNPs ya han sido publicados para daños por frío en post cosecha en melocotonero (Martínez-García

et al., 2012). Debido a la elevada densidad entre los marcadores SNPs, este trabajo refuerza los resultados obtenidos anteriormente mediante SSRs (Font i Forcada et al., 2012b). Así, el mapeo por asociación en el germoplasma de melocotonero es una alternativa sólida frente al análisis de QTLs en poblaciones de mejora. Además, este trabajo muestra resultados prometedores que podrían aplicarse a otras especies *Prunus* debido a la sintenia existente dentro de la familia de las Rosáceas. Sin embargo, como ya se ha mencionado para el caso de los SSRs, aunque existen muchos QTLs posicionados en el mapa de referencia de *Prunus*, son pocos los ejemplos de selección asistida por marcadores que se están empleando en la práctica en los programas de mejora genética del melocotonero.

9.4. Influencia del patrón sobre el comportamiento agronómico y la calidad del fruto en un ensayo de patrones híbridos almendro x melocotonero para melocotonero

Como ya se ha mencionado, la calidad del fruto en melocotonero viene determinada principalmente por el genotipo de la variedad cultivada. Sin embargo, es también conocido el efecto del patrón sobre las características agronómicas y la calidad del fruto de la variedad injertada (Albás et al., 2004; Cantín et al., 2010b; Jiménez et al., 2007, 2011; Moreno et al., 1994; Tsipouridis y Thomidis, 2005; Zarrouk et al., 2005). Las distintas especies y genotipos de los posibles patrones empleados para el cultivo del melocotonero determinarán diferencias agronómicas de adaptación al suelo, productivas y de calidad del fruto.

En el presente estudio, se evaluaron cinco patrones híbridos de almendro x melocotonero (Adafuel, Adarcias, Felinem, Garnem y GF 677) y un híbrido de melocotonero x *P. davidiana* (Cadaman), con distintos origenes y base genética, y establecidos en un suelo pesado y calizo, típico del área mediterránea. Se observó una influencia significativa del patrón sobre las características agronómicas y de calidad del fruto de las variedades injertadas, el melocotonero ‘Tebana’ y la nectarina ‘Queen Giant’. También se observaron diferencias significativas en la supervivencia de los árboles para las condiciones de cultivo consideradas. Así, se determinó un nivel de mortalidad elevado sobre algunos patrones (Felinem y Garnem), posiblemente debido a su mayor sensibilidad a la asfixia de raíces en suelos pesados (Felipe, 2009) o a su susceptibilidad a la podredumbre de la raíz, debida a la presencia de posibles patógenos

en el suelo (Zarrouk et al., 2005). También se observó una influencia significativa del patrón sobre el vigor del árbol, al igual que se menciona en otros estudios de patrones para melocotonero (Albás et al., 2004; Moreno et al., 1994; Zarrouk et al., 2005). Entre los patrones evaluados, el híbrido Adarcias presentó un menor vigor y una buena productividad, por lo que puede ser muy interesante para reducir el crecimiento excesivo del árbol, aumentar la densidad de plantación y disminuir los costes de mantenimiento en las plantaciones de melocotonero (Moreno y Cambra, 1994). Por el contrario, el gran vigor de Adafuel, Felinem, Garnem y GF 677 los hace más convenientes para el cultivo del melocotonero en condiciones de replantación o en suelos pobres y calizos (Cambra, 1990; Moreno et al., 1994) donde un mayor nivel de vigor puede ser necesario. Sin embargo, la tendencia de Garnem y GF 677 a presentar una menor productividad de las variedades injertadas, probablemente debido a su excesivo vigor (Jiménez et al., 2011), los hace menos aconsejables en condiciones normales del cultivo.

Además de los aspectos agronómicos, también se encontraron diferencias significativas entre los distintos patrones sobre las características básicas de calidad del fruto (SS, firmeza y acidez valorable) de la variedad injertada, tal y como se ha mencionado en otros trabajos (Albás et al., 2004; Moreno et al., 1994; Tsipouridis y Thomidis, 2005). La tendencia de los patrones Adarcias y Cadaman a inducir una mayor calidad del fruto, podría deberse a su menor vigor, probablemente por una menor competencia del desarrollo vegetativo frente al fruto (Albás et al., 2004). Además, Cadaman, con un nivel intermedio de vigor, tiende a inducir una mayor eficiencia productiva. Las correlaciones encontradas entre los parámetros agronómicos y de calidad del fruto confirman la tendencia de los patrones híbridos interespecíficos menos vigorosos a inducir una mayor calidad del fruto, y por ello los hace más interesantes para su uso en las plantaciones comerciales de melocotonero.

9.5. Influencia de patrones híbridos almendro x melocotonero en la composición nutricional del fruto de melocotonero y nectarina

Los trabajos realizados con patrones híbridos almendro x melocotonero (*P. persica* x *P. amygdalus*) son de gran importancia al ser los patrones más utilizados para el cultivo del melocotonero en suelos calizos, ya que son tolerantes a la clorosis férrica (Socias i Company et al., 1995) y algunos de ellos también resistentes a nematodos

(Felipe, 2009; Felipe et al., 1997). Como se ha mostrado en el apartado anterior, el patrón utilizado tiene una influencia significativa sobre la supervivencia del árbol, el vigor y la productividad de las variedades injertadas, así como sobre los parámetros básicos de calidad del fruto (Font i Forcada et al., 2012a). Aunque es mucho más conocido el efecto sobre la calidad del fruto atribuido a las distintas variedades (Abidi et al., 2011; Cantín et al., 2009a, 2009b), también hay que tener en cuenta el efecto del patrón (Albás et al., 2004; Colaric et al., 2005; Orazem et al., 2011b) cuando se requieren estudiar aspectos nutricionales del fruto. La posible implicación de los factores ambientales en los compuestos bioactivos del fruto (Cantín et al., 2009a) plantea la necesidad de realizar ensayos de patrones y variedades en las condiciones reales de cultivo durante varios años.

Los patrones híbridos almendro x melocotonero antes mencionados (*P. persica* x *P. amygdalus; P. persica* x *P. davidiana*), injertados con las variedades 'Queen Giant' y 'Tebana', también mostraron una influencia significativa sobre las características nutricionales del fruto, tanto sobre los azúcares individuales (sacarosa, glucosa, fructosa y sorbitol) y totales, como sobre el contenido en compuestos antioxidantes (fenoles, flavonoides, antocianinas, capacidad antioxidante y vitamina C), presentes en los frutos de melocotón y nectarina.

Así, el híbrido almendro x melocotonero Adarcias (Moreno y Cambra, 1994) y el híbrido de melocotonero x *P. davidiana* Cadaman (Edin y Garcin, 1994), parecen inducir, en general, un mayor contenido del fruto en azúcares individuales y totales, y en compuestos fenólicos para las dos variedades estudiadas. El análisis de componentes principales permitió confirmar que los árboles de los patrones Adarcias y Cadaman presentaban un mayor contenido en azúcares y en compuestos antioxidantes del fruto. Además, se han observado correlaciones positivas entre los SS y los azúcares individuales, como ya ha sido mencionado en otros trabajos en melocotonero (Cantín et al., 2009a; Wu et al., 2003) y en albaricoquero (Drogoudi et al., 2008). También se observaron correlaciones positivas entre los SS y el peso del fruto, probablemente porque la tasa de crecimiento del fruto está determinada por la cantidad de carbohidratos disponibles (Morandi, 2008). Otras correlaciones positivas y significativas fueron las encontradas entre fenoles, capacidad antioxidante, flavonoides y vitamina C, mostrando que los ácidos fenólicos, los flavonoides y la vitamina C son las fuentes principales de la capacidad antioxidante en los frutos (Cevallos-Casals et al.,

2006; Gil et al., 2002; Wang et al. 1996). La correlación positiva encontrada entre la vitamina C y la acidez valorable puede ser debida a la contribución del ácido ascórbico en la acidez de la muestra (Cantín et al., 2009b). Otras correlaciones significativas y positivas fueron las encontradas entre peso del fruto y vitamina C o entre los SS y fenoles y flavonoides, mostrando que los frutos más grandes y más dulces tuvieron un mayor contenido en estos compuestos bioactivos.

Por el contrario, se han observado correlaciones negativas entre el vigor del árbol y los SS y el contenido en azúcares del fruto, lo que confirma el efecto negativo del mayor vigor del árbol sobre la calidad del fruto. Estas correlaciones negativas pueden ser debidas a una mayor competencia del desarrollo vegetativo frente al fruto en los patrones más vigorosos (Morandi, 2008).

Las diferencias encontradas entre años para los parámetros nutricionales estudiados confirman que estos parámetros nutricionales se ven también influenciados por las condiciones ambientales, especialmente la temperatura (Brooks et al., 1993; Bureau et al., 2009; Serrano et al., 2005; Tomás-Barberán and Espin, 2001), por lo que se aconseja realizar este tipo de estudios durante varios años.

9.6. Influencia del patrón sobre el comportamiento agronómico y la calidad del fruto de melocotonero en un ensayo de patrones ciruelo (*P. insititia* y *P. domestica*)

De forma complementaria a los híbridos almendro x melocotonero, los patrones ciruelo (*P. insititia*, *P. domestica*) también son de gran importancia para el cultivo del melocotonero, ya que estos últimos son tolerantes a la asfixia de raíces provocada por los suelos pesados, compactos y con problemas de drenaje (Felipe et al., 1997; Moreno et al., 1995).

En el presente estudio, se evaluaron siete ciruelos 'Pollizo' de Murcia (*P. insititia*): Adesoto, Monpol, Montizo, P. Soto 67 AD y PM 105 AD, un ciruelo San Julián (*P. insititia*): GF 655/2 y un ciruelo común (*P. domestica*): Constantí, con distintos origenes y base genética, y establecidos en un suelo pesado y calizo, típico del área mediterránea.

En el estudio de diferentes patrones ciruelo sobre el comportamiento agronómico, los parámetros básicos y compuestos bioquímicos de calidad del fruto se observó como algunos de los patrones 'Pollizo de Murcia' seleccionados en la EEAD,

presentan gran interés como patrones para melocotonero. Así, se observó que el 'Pollizo de Murcia' Adesoto inducía un mayor contenido del fruto de la variedad injertada en los azúcares solubles (sacarosa, glucosa y sorbitol), así como en los compuestos antioxidantes evaluados (fenoles, flavonoides, vitamina C y capacidad antioxidante). Además, el análisis de componentes principales confirmó que los árboles del patrón Adesoto presentaban un mayor contenido del fruto en los azúcares solubles y en los compuestos antioxidantes analizados. El mayor contenido en azúcares del fruto inducido por el patrón Adesoto también fue mencionado por Orazem et al. (2011a, 2011b) cuando lo compararon con 11 patrones *Prunus*. Asimismo, en el presente trabajo, Adesoto indujo un nivel intermedio de vigor y fue uno de los patrones con mayor productividad, como también se refiere en la bibliografía (Jiménez et al., 2011; Moreno et al., 1995). Igualmente, hay que destacar otro ciruelo 'Pollizo de Murcia', el patrón PM 105 AD, que también indujo niveles elevados en los azúcares solubles (sacarosa, sorbitol y azúcares totales) y en los compuestos antioxidantes evaluados (fenoles, flavonoides, vitamina C y RAC), lo que plantea su interés como patrón para melocotonero.

Estos resultados confirman la influencia significativa de los distintos patrones sobre los niveles de azúcares y compuestos antioxidantes, los cuales determinarán en gran medida la calidad del fruto (Robertson et al., 1988). La interacción entre el patrón y la variedad puede influir los niveles de estos compuestos con gran impacto en la nutrición y la salud humana (Tomás-Barberán et al., 2001). En resumen, se puede mencionar que la correcta combinación de patrón-variedad puede potenciar las características de calidad básicas y nutricionales del fruto.

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Capítulo 10

Conclusiones

1. El estudio de 94 variedades de melocotonero y nectarina de la colección de germoplasma de la Estación Experimental de Aula Dei mostró una gran variabilidad fenotípica para los diferentes caracteres pomológicos del fruto, así como para los parámetros básicos de calidad y compuestos nutricionales del fruto. Esta variabilidad puede ser de gran interés en los programas de mejora del melocotonero.
2. El estudio de los parámetros de calidad del fruto reveló el interés de algunas variedades autóctonas españolas, como 'Alcañíz 1', 'Borracho de Jarque', 'Calabacero', 'Miraflores' y 'Sudanell 1' por la mayor firmeza de los frutos y/o por su mayor contenido en sólidos solubles, azúcares y compuestos antioxidantes. La mayor calidad organoléptica y nutricional de dichas variedades muestran su interés para el consumidor y como fuente de variabilidad para la obtención de nuevas variedades.
3. La caracterización molecular con microsatélites (SSRs) indicó un elevado nivel del desequilibrio de ligamiento en las 94 variedades estudiadas. Se alcanzó su máximo a los 20cM, aunque los niveles observados variaron según la población considerada. El estudio de la estructura poblacional mostró que las variedades se dividían en dos grupos, el de las locales de origen español y el de las modernas, en general de origen extranjero.
4. Los estudios de genética de asociación tanto con los marcadores SSRs como con los SNPs mostraron asociaciones significativas a caracteres pomológicos de interés. Entre las asociaciones confirmadas con ambos marcadores estarían algunos caracteres pomólogicos como la fecha de cosecha y de calidad del fruto, relacionada con el contenido en flavonoides, antocianinas y capacidad antioxidante, situadas en los mismos grupos de ligamiento o 'scaffolds'.
5. En el trabajo con microsatélites se seleccionaron los marcadores BPPCT015, CPPCT028 y endoPG1, en el grupo de ligamiento 4 (GL4), y algunos genotipos y haplotipos relacionados con caracteres muy importantes de la calidad del fruto, como el contenido en azúcares totales, sorbitol o firmeza del fruto. Estos resultados muestran el interés de su utilización en la selección asistida por marcadores.
6. En los ensayos de patrones híbridos con base genética de melocotonero (*P. amygdalus* x *P. persica* y *P. davidiana* x *P. persica*) se observó la tendencia de los patrones Adarcias y Cadaman a inducir una mayor productividad y calidad

del fruto en las variedades injertadas, sobre todo considerando los parámetros de firmeza, sólidos solubles, acidez valorable y azúcares solubles. El menor vigor de dichos patrones, probablemente favorece una menor competencia del desarrollo vegetativo del árbol frente a la calidad del fruto, lo que potencia su interés a nivel agronómico.

7. El estudio de distintos patrones ciruelo (*P. insititia, P. domestica*) mostró su influencia en los parámetros agronómicos y de calidad del fruto de melocotonero. Entre los patrones evaluados, los 'Pollizos de Murcia' Adesoto y PM 105 AD indujeron, en general, una buena productividad y el mayor contenido en sólidos solubles, azúcares y compuestos antioxidantes en el fruto de la variedad injertada, lo que demuestra su interés comercial como patrones para melocotonero.

8. El buen comportamiento agronómico y de calidad del fruto (mayor contenido en azúcares y antioxidantes) de algunas variedades autóctonas españolas y varios patrones *Prunus* seleccionados en la Estación Experimental de Aula Dei, potenciará su difusión al sector, teniendo en cuenta que la calidad del fruto juega un papel importante en la aceptación por el consumidor, tanto por su influencia sobre el sabor como sobre las características nutricionales del fruto.

Capítulo 11

Anexos

11.1. Material suplementario correspondiente al capítulo 3

11.1.1. Supplementary file 1. Full bloom, trunk-cross sectional area (TCSA), yield, annual yield efficiency (AYE), fruit weight (FW), soluble solids content (SSC), flesh firmness (FF), titratable acidity (TA) and ripening index (RI) of the 94 cultivars studied.

	Full Bloom	TCSA	Yield	AYE	FW	SSC	FF	TA	RI
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Adriática	85 ± 1.2	105.2 ± 11.5	19.8 ± 2.5	0.38 ± 0.1	187.0 ± 13.1	13.8 ± 0.9	23.3 ± 1.2	0.5 ± 0.02	28.1 ± 1.1
Alcañiz 1	83 ± 0.5	95.2 ± 8.5	17.9 ± 2.1	0.37 ± 0.03	221.3 ± 14.2	16.1 ± 0.4	37.7 ± 2.5	0.7 ± 0.06	21.9 ± 2.1
Alcañiz 2	85 ± 0.4	74.5 ± 6.3	1.6 ± 0.03	0.02 ± 0.001	187.8 ± 15.1	17.7 ± 0.7	37.4 ± 2.3	0.5 ± 0.01	32.5 ± 2.1
Alejandro Dumas	81 ± 0.5	98.2 ± 11.5	20.6 ± 1.1	0.44 ± 0.1	315.0 ± 15.4	15.1 ± 0.4	51.1 ± 5.3	0.6 ± 0.01	24.7 ± 1.5
Amarillo Calanda (131)	87 ± 1.2	65.6 ± 6.5	9.9 ± 0.8	0.01 ± 0.02	197.5 ± 10.0	15.0 ± 0.4	42.1 ± 4.2	0.6 ± 0.02	23.2 ± 1.1
Amarillo Calanda (2400)	83 ± 0.7	59.1 ± 8.5	9.9 ± 2.2	0.33 ± 0.04	148.6 ± 13.1	16.6 ± 0.1	58.3 ± 0.5	0.8 ± 0.05	21.8 ± 1.5
Amarillo de Gallur	81 ± 1.2	58.1 ± 11.5	9.8 ± 1.6	0.34 ± 0.1	168.0 ± 8.5	16.1 ± 0.9	38.6 ± 2.3	0.6 ± 0.06	25.5 ± 2.3
Andora	84 ± 0.7	73.0 ± 6.5	10.4 ± 1.3	0.28 ± 0.02	169.8 ± 10.0	14.8 ± 0.7	52.0 ± 2.2	0.9 ± 0.02	15.0 ± 0.5
Andross	81 ± 0.5	96.3 ± 6.3	26.6 ± 2.5	0.27 ± 0.04	190.1 ± 15.1	15.7 ± 0.7	34.3 ± 2.1	0.5 ± 0.02	31.2 ± 2.3
Baby Gold 5	81 ± 1.2	109.5 ± 13.5	28.2 ± 2.5	0.51 ± 0.1	172.9 ± 12.5	13.5 ± 0.1	36.9 ± 11	0.6 ± 0.04	24.0 ± 1.0
Baby Gold 6	81 ± 0.4	77.4 ± 8.5	18.3 ± 2.5	0.47 ± 0.02	312.2 ± 18.5	14.3 ± 0.5	34.0 ± 3.6	0.5 ± 0.02	29.6 ± 1.1
Baby Gold 7	81 ± 1.2	51.2 ± 11.5	14.4 ± 3.6	0.56 ± 0.12	200.3 ± 14.2	14.3 ± 0.5	37.8 ± 2.3	0.6 ± 0.03	25.2 ± 1.5
Baby Gold 8	81 ± 0.7	71.3 ± 6.3	10.3 ± 0.8	0.28 ± 0.04	194.3 ± 13.1	16.1 ± 0.7	37.5 ± 4.5	0.6 ± 0.01	28.0 ± 0.5
Baby Gold 9	80 ± 0.7	59.5 ± 6.5	12.5 ± 2.4	0.21 ± 0.02	158.6 ± 12.6	15.8 ± 0.9	40.3 ± 5.6	0.5 ± 0.02	29.4 ± 1.0
Baladin	83 ± 0.5	86.6 ± 11.5	22.7 ± 1.1	0.52 ± 0.04	168.6 ± 11.3	13.6 ± 0.1	28.7 ± 2.5	0.7 ± 0.01	21.1 ± 1.1
Benasque	85 ± 0.8	80.1 ± 8.5	11.8 ± 2.2	0.29 ± 0.12	64.0 ± 15.2	15.7 ± 0.2	17.1 ± 1.5	0.8 ± 0.02	19.4 ± 2.1
Big Top	80 ± 0.5	184.8 ± 6.3	12.3 ± 2.1	0.13 ± 0.02	182.3 ± 12.5	15.4 ± 0.5	38.0 ± 2.3	0.5 ± 0.06	30.6 ± 2.1
Bonet I	81 ± 0.7	108.1 ± 11.5	16.9 ± 3.4	0.31 ± 0.1	170.9 ± 8.5	18.2 ± 0.7	41.9 ± 2.1	0.6 ± 0.01	31.1 ± 0.5
Bonet II	81 ± 1.2	105.2 ± 13.6	18.7 ± 2.6	0.35 ± 0.12	172.1 ± 10.0	15.9 ± 0.4	44.0 ± 1.3	0.6 ± 0.06	26.3 ± 2.1
Bonet III	84 ± 0.7	280.0 ± 15	4.3 ± 0.05	0.03 ± 0.03	187.8 ± 9.6	18.0 ± 0.7	56.4 ± 3.2	0.8 ± 0.02	22.1 ± 1.1
Bonet IV	83 ± 0.4	76.1 ± 2.5	4.2 ± 0.05	0.11 ± 0.02	185.3 ± 13.1	16.7 ± 0.3	49.2 ± 1.6	0.8 ± 0.03	22.5 ± 0.5
Bonet V	83 ± 1.2	126.6 ± 11.5	12.1 ± 2.2	0.19 ± 0.04	208.2 ± 13.1	16.0 ± 0.1	48.9 ± 1.3	0.7 ± 0.01	24.4 ± 1.5
Borracho de Jarque	83 ± 0.5	116.9 ± 10.2	14.2 ± 2.3	0.24 ± 0.1	204.0 ± 15.1	17.7 ± 0.4	61.0 ± 1.5	0.5 ± 0.01	67.0 ± 2.3
Brasileño	81 ± 0.4	108.1 ± 9.8	15.7 ± 2.2	0.29 ± 0.03	165.2 ± 16.3	13.3 ± 0.6	28.6 ± 2.6	0.5 ± 0.01	27.4 ± 1.0
Calabacero	82 ± 0.8	96.3 ± 11.5	12.0 ± 2.1	0.25 ± 0.04	148.8 ± 14.6	15.8 ± 0.1	28.5 ± 1.0	0.5 ± 0.06	30.6 ± 1.1
Calanda San Miguel	85 ± 1.2	138.7 ± 6.3	6.1 ± 0.8	0.08 ± 0.02	165.0 ± 12.4	15.0 ± 0.6	39.7 ± 1.0	0.5 ± 0.01	29.8 ± 1.2
Calanda Tardío	84 ± 0.8	81.9 ± 6.5	2.7 ± 0.01	0.06 ± 0.01	147.5 ± 11.6	17.1 ± 0.1	55.2 ± 2.5	0.9 ± 0.03	19.4 ± 1.1
Campiel Rojo	83 ± 0.7	90.1 ± 8.5	13.6 ± 2.5	0.30 ± 0.1	218.1 ± 15.1	15.5 ± 0.3	44.6 ± 1.3	0.6 ± 0.07	26.1 ± 0.5
Carolyn	84 ± 0.8	78.4 ± 6.3	13.3 ± 2.3	0.33 ± 0.02	179.1 ± 12.5	15.6 ± 0.2	47.3 ± 1.3	0.8 ± 0.01	20.5 ± 2.1
Carson	83 ± 1.2	55.3 ± 8.6	16.8 ± 4.3	0.60 ± 0.12	192.8 ± 12.5	14.5 ± 0.6	33.7 ± 1.6	0.5 ± 0.02	27.7 ± 2.1
Catherina	82 ± 0.7	87.5 ± 11.3	22.8 ± 1.1	0.52 ± 0.03	194.0 ± 8.5	14.9 ± 0.6	27.8 ± 2.5	0.6 ± 0.07	27.8 ± 0.5
Del Gorro	82 ± 0.5	117.5 ± 11.5	12.0 ± 2.2	0.20 ± 0.03	174.6 ± 12.7	14.1 ± 0.7	28.0 ± 2.6	0.6 ± 0.07	25.8 ± 1.5
Diamante Amarillo	84 ± 0.8	58.2 ± 5.6	1.0 ± 0.01	0.05 ± 0.004	101.6 ± 12.0	17.0 ± 0.3	43.9 ± 2.3	0.6 ± 0.06	28.3 ± 1.2
Dixon	84 ± 0.5	105.7 ± 13.6	18.4 ± 1.0	0.34 ± 0.04	197.3 ± 13.1	15.3 ± 0.7	32.1 ± 2.5	0.5 ± 0.02	31.9 ± 0.5
Everst	81 ± 0.9	80.1 ± 6.3	22.3 ± 2.5	0.55 ± 0.02	166.2 ± 10.0	15.3 ± 0.3	40.7 ± 2.4	0.6 ± 0.06	25.1 ± 1.1
Fantasia	80 ± 0.5	101.0 ± 14.7	6.7 ± 0.8	0.13 ± 0.1	189.6 ± 9.6	14.6 ± 0.9	9.1 ± 1.2	0.7 ± 0.02	20.5 ± 1.2
Flamekist	80 ± 1.1	87.7 ± 8.5	6.5 ± 0.8	0.15 ± 0.04	214.7 ± 15.1	16.0 ± 0.3	29.6 ± 1.3	0.8 ± 0.01	20.6 ± 1.1
Flavortop	80 ± 0.7	101.6 ± 6.5	7.1 ± 1.0	0.14 ± 0.03	181.1 ± 15.6	15.8 ± 0.1	28.0 ± 1.1	0.8 ± 0.06	20.0 ± 1.0
Fortuna	81 ± 0.5	48.5 ± 9.2	11.3 ± 1.1	0.46 ± 0.1	173.0 ± 14.3	13.8 ± 0.9	41.3 ± 1.6	0.6 ± 0.06	24.2 ± 2.1
Fraga	83 ± 0.9	107.3 ± 10.2	22.6 ± 2.5	0.42 ± 0.02	203.9 ± 13.1	15.2 ± 0.6	40.8 ± 1.0	0.5 ± 0.01	27.7 ± 1.1
GF3	81 ± 1.2	107.5 ± 10.0	31.6 ± 3.5	0.58 ± 0.04	202.3 ± 15.1	14.3 ± 0.8	28.6 ± 2.5	0.6 ± 0.06	24.2 ± 1.5
Goiri	81 ± 0.7	88.2 ± 11.5	2.1 ± 0.05	0.04 ± 0.01	152.8 ± 12.5	15.3 ± 0.3	31.8 ± 1.3	0.5 ± 0.01	30.4 ± 1.2
Golden Queen	81 ± 0.5	56.8 ± 10.0	10.4 ± 1.0	0.36 ± 0.12	162.2 ± 12.5	17.4 ± 0.8	38.5 ± 2.6	0.6 ± 0.02	27.3 ± 1.2
Gomes	81 ± 0.3	63.5 ± 10.0	6.0 ± 0.6	0.18 ± 0.02	201.3 ± 14.2	16.0 ± 0.2	46.3 ± 4.5	0.8 ± 0.06	19.6 ± 0.5
Halford	80 ± 1.2	85.6 ± 11.5	8.6 ±1.2	0.20 ± 0.12	175.0 ± 8.5	16.7 ± 0.7	46.7 ± 1.2	0.7 ± 0.06	24.3 ± 1.2
Infanta Isabel	81 ± 0.6	65.8 ± 4.5	7.2 ± 2.2	0.21 ± 0.03	157.1 ± 12.3	15.9 ± 0.1	30.2 ± 2.3	0.6 ± 0.02	25.2 ± 2.1
Jerónimo de Alfaro	81 ± 0.4	73.0 ± 6.3	13.2 ± 1.0	0.36 ± 0.04	167.0 ± 11.1	14.9 ± 0.3	38.9 ± 2.3	0.5 ± 0.02	30.6 ± 0.5
Jungerman	81 ± 0.7	111.3 ± 8.9	22.7 ± 2.5	0.40 ± 0.02	204.2 ± 14.2	15.1 ± 0.9	31.2 ± 1.3	0.5 ± 0.02	30.1 ± 1.1
Kakamas	83 ± 0.5	51.8 ± 8.6	7.5 ± 0.6	0.29 ± 0.1	197.0 ± 10.0	15.5 ± 0.5	50.1 ± 1.6	0.7 ± 0.01	23.0 ± 2.0
Keimoes	84 ± 0.5	51.3 ± 8.5	12.3 ± 1.2	0.48 ± 0.03	161.9 ± 10.0	15.9 ± 0.7	53.9 ± 1.2	0.6 ± 0.04	25.1 ± 2.0
Klamt	81 ± 0.4	113.1 ± 10.3	24.7 ± 1.7	0.43 ± 0.04	233.3 ± 15.1	15.6 ± 0.5	37.6 ± 1.3	0.6 ± 0.04	25.2 ± 2.1
Loadel	81 ± 0.5	133.7 ± 11.5	29.6 ± 1.1	0.44 ± 0.12	192 ± 11.1	13.0 ± 0.1	38.2 ± 2.5	0.6 ± 0.06	21.4 ± 1.5
Lovell	82 ± 0.7	125.6 ± 11.5	46.5 ± 3.2	1.31 ± 0.08	223.3 ± 15.1	15.9 ± 1.0	51.6 ± 1.3	0.7 ± 0.02	23.5 ± 1.0
Maluenda	80 ± 1.2	65.5 ± 6.5	5.9 ± 0.6	0.18 ± 0.1	152.7 ± 8.5	14.8 ± 0.9	30.4 ± 1.5	0.6 ± 0.06	23.1 ± 1.2
Maria Serena	82 ± 0.4	73.0 ± 2.3	11.7 ± 3.1	0.32 ± 0.12	160.4 ± 13.1	13.2 ± 0.1	24.6 ± 1.4	0.4 ± 0.05	35.5 ± 1.5
Maruja	83 ± 1.1	87.3 ± 6.3	5.5 ± 0.1	0.12 ± 0.03	145.6 ± 12.5	14.5 ± 0.1	25.8 ± 2.6	0.6 ± 0.06	24.8 ± 1.2
Maruja Porvenir	83 ± 1.2	110.9 ± 11.5	2.8 ± 0.05	0.05 ± 0.001	162.5 ± 8.5	14.4 ± 0.2	33.5 ± 1.2	0.6 ± 0.04	24.4 ± 2.0
Miraflores	84 ± 1.3	77.7 ± 5.6	12.8 ± 1.1	0.32 ± 0.03	167.5 ± 10.2	15.7 ± 0.8	39.9 ± 2.6	0.6 ± 0.04	25.3 ± 0.5
Mountaingold	81 ± 1.2	86.9 ± 4.6	24.8 ± 2.5	0.57 ± 0.04	182.7 ± 11.3	14.1 ± 0.9	40.7 ± 1.0	0.6 ± 0.02	24.4 ± 1.1
Nectar del Jalón	81 ± 0.5	108.5 ± 12.8	8.3 ± 1.2	0.15 ± 0.02	113.9 ± 9.9	15.6 ± 0.6	27.2 ± 2.5	0.6 ± 0.01	25.2 ± 1.2
NJC 97	80 ± 1.2	70.8 ± 8.5	12.5 ± 1.1	0.35 ± 0.12	191.7 ± 14.2	13.5 ± 0.3	20.5 ± 1.3	0.6 ± 0.02	23.4 ± 1.6

(continue)

	Full Bloom	TCSA	Yield	AYE	FW	SSC	FF	TA	RI
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Nuevo	83 ± 0.7	56.0 ± 2.5	2.4 ± 1.2	0.08 ± 0.01	174.0 ± 13.1	17.5 ± 0.7	37.7 ± 1.3	0.5 ± 0.09	33.2 ± 1.2
Oropel	87 ± 0.9	91.7 ± 3.6	19.5 ± 1.1	0.42 ± 0.03	185.5 ± 16.3	17.1 ± 0.1	42.5 ± 4.3	0.6 ± 0.06	26.9 ± 2.1
Paloro A	85 ± 1.3	81.8 ± 4.8	12.0 ± 2.3	0.29 ± 0.12	189.9 ± 9.9	16.5 ± 0.3	40.3 ± 5.6	0.7 ± 0.09	23.2 ± 1.5
Paloro B	85 ± 0.4	280 ± 12	7.0 ± 30.5	0.02 ± 0.03	174.0 ± 13.1	15.5 ± 0.3	37.4 ± 1.5	0.9 ± 0.02	18.3 ± 2.0
Queen Giant	81 ± 0.4	88.2 ± 6.5	7.6 ± 0.6	0.17 ± 0.1	214.5 ± 10.0	12.0 ± 1.2	28.3 ± 2.5	0.7 ± 0.03	17.0 ± 0.8
Redhaven	81 ± 1.5	79.9 ± 8.5	11.1 ± 0.8	0.27 ± 0.04	160.1 ± 12.3	12.1 ± 0.3	28.9 ± 1.6	0.6 ± 0.01	19.0 ± 2.0
Rojo del Rito	83 ± 1.2	66.9 ± 6.3	2.0 ± 0.01	0.06 ± 0.02	187.6 ± 13.1	18.2 ± 0.3	49.7 ± 2.6	0.6 ± 0.01	31.1 ± 1.1
San Jaime	81 ± 1.6	124.3 ± 12.5	12.1 ± 0.9	0.19 ± 0.03	156.5 ± 13.1	15.0 ± 0.7	38.8 ± 2.3	0.6 ± 0.04	27.1 ± 1.5
San Lorenzo	81 ± 0.4	69.4 ± 8.6	10.5 ± 1.1	0.30 ± 0.1	149.4 ± 16.3	15.7 ± 0.1	31.1 ± 1.6	0.6 ± 0.02	27.6 ± 0.5
Sarell	80 ± 0.7	66.1 ± 2.6	10.2 ± 0.8	0.31 ± 0.02	183.8 ± 15.1	14.5 ± 0.4	34.1 ± 1.3	0.6 ± 0.08	25.4 ± 1.0
Selma	81 ± 1.8	104.0 ± 14.6	11.5 ± 0.9	0.22 ± 0.12	155.6 ± 13.1	15.9 ± 0.6	28.2 ± 2.3	0.6 ± 0.02	26.2 ± 1.2
Shasta	79 ± 0.8	43.8 ± 12	8.8 ± 0.6	0.40 ± 0.02	187.7 ± 8.5	15.1 ± 0.3	43.9 ± 4.3	0.6 ± 0.09	25.5 ± 1.7
Stanford	79 ± 0.6	166.9 ± 12.5	27.7 ± 5.6	0.33 ± 0.03	199.0 ± 14.2	14.5 ± 0.1	41.9 ± 1.0	0.5 ± 0.09	27.4 ± 1.5
Starn	80 ± 0.4	79.4 ± 8.5	11.0 ± 2.3	0.27 ± 0.12	192.7 ± 13.2	15.1 ± 0.9	46.6 ± 2.3	0.6 ± 0.01	24.8 ± 1.5
Sudanell 1	82 ± 1.9	69.7 ± 5.9	13.5 ± 2.2	0.38 ± 0.04	148.7 ± 10.3	18.1 ± 0.9	52.2 ± 4.3	0.6 ± 0.02	31.1 ± 2.0
Sudanell 2	84 ± 1.2	72.5 ± 3.7	15.5 ± 1.2	0.42 ± 0.03	155.3 ± 13.2	16.4 ± 0.4	45.3 ± 1.3	0.7 ± 0.02	23.7 ± 1.1
Sudanell 3	84 ± 0.7	101.7 ± 11.5	16.2 ± 2.3	0.31 ± 0.1	146.5 ± 8.5	16.5 ± 0.1	36.1 ± 2.3	0.6 ± 0.02	28.0 ± 0.5
Sudanell Blanco	82 ± 1.1	85.6 ± 6.3	14.3 ± 1.1	0.33 ± 0.12	180.2 ± 13.1	16.7 ± 0.3	41.9 ± 1.6	0.7 ± 0.06	23.7 ± 2.3
Sudanell GF	81 ± 0.7	123.1 ± 15.6	16.5 ± 5.3	0.69 ± 0.02	168.6 ± 15.1	14.8 ± 0.6	42.4 ± 1.3	0.6 ± 0.01	24.7 ± 1.2
Sudanell GF (2804)	81 ± 0.6	102.7 ± 8.5	42.5 ± 5.6	0.32 ± 0.04	167.4 ± 10.0	15.3 ± 0.7	30.8 ± 2.6	0.5 ± 0.08	30.9 ± 0.5
Suncling	81 ± 1.2	65.3 ± 6.5	21.7 ± 5.6	0.66 ± 0.03	220.7 ± 15.1	14.2 ± 0.6	31.0 ± 1.5	0.5 ± 0.06	27.2 ± 2.3
Super Crimson Gold	82 ± 0.7	81.4 ± 5.9	9.1 ± 0.8	0.22 ± 0.1	129.3 ± 13.0	13.4 ± 0.3	17.4 ± 1.0	0.7 ± 0.01	19.9 ± 1.0
Tebana	84 ± 0.7	106.9 ± 11.5	25.0 ± 2.5	0.46 ± 0.02	144.2 ± 12.5	14.4 ± 0.3	30.5 ± 1.2	0.4 ± 0.02	32.6 ± 2.1
Tempranillo de Aytona	84 ± 1.2	93.3 ± 6.5	13.0 ± 2.2	0.27 ± 0.04	142.7 ± 12.5	13.0 ± 0.9	23.4 ± 2.5	0.6 ± 0.07	20.7 ± 1.2
Tipo Campiel	84 ± 0.4	73.2 ± 8.6	8.6 ± 0.9	0.23 ± 0.02	181.5 ± 13.1	15.8 ± 0.3	45.9 ± 1.3	0.7 ± 0.02	23.7 ± 0.5
Venus	85 ± 0.5	134.0 ± 2.5	9.2 ± 1.1	0.10 ± 0.03	202.2 ± 14.2	14.0 ± 0.9	37.3 ± 1.0	0.7 ± 0.06	20.0 ± 1.0
Vesuvio	81 ± 0.9	71.3 ± 5.3	12.0 ± 2.3	0.33 ± 0.1	170.3 ± 10.0	13.4 ± 0.7	29.2 ± 2.6	0.5 ± 0.02	25.2 ± 1.1
Vivian	81 ± 1.2	77.4 ± 8.5	23.4 ± 2.5	0.60 ± 0.02	165.0 ± 12.5	16.9 ± 0.4	51.5 ± 1.3	0.7 ± 0.01	26.4 ± 0.5
Walgant	84 ± 0.4	96.3 ± 8.6	14.4 ± 1.1	0.29 ± 0.04	190.2 ± 15.1	16.3 ± 0.6	38.4 ± 4.2	0.6 ± 0.01	26.4 ± 2.3
Wiser	81 ± 0.5	76.9 ± 6.3	7.7 ± 0.8	0.20 ± 0.03	188.0 ± 13.1	16.8 ± 0.9	46.7 ± 3.6	0.8 ± 0.09	21.6 ± 1.1
Zaragozano	80 ± 0.3	108.5 ± 11.5	14.1 ± 1.1	0.26 ± 0.04	202.8 ± 15.1	15.8 ± 0.6	46.3 ± 5.3	0.7 ±0.02	22.8 ± 1.5
Zaragozano Amarillo	84 ± 0.4	80.4 ± 6.5	5.0 ± 0.05	0.12 ± 0.02	197.2 ± 14.2	17.2 ± 0.4	42.4 ± 2.5	0.7 ± 0.02	19.4 ± 1.5
Zaragozano Rojo	83 ± 1.2	136.3 ± 11.5	5.2 ± 0.05	0.07 ± 0.01	224.1 ± 15.1	15.7 ± 0.7	48.4 ± 2.1	0.5 ± 0.09	29.7 ± 0.5

11.1.2. Supplementary file 2. Sucrose, glucose, fructose, sorbitol and total sugars of the 94 cultivars studied.

	Sucrose	Glucose	Fructose	Sorbitol	Total sugars
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Adriática	74.5 ± 5.1	7.2 ± 2.6	9.0 ± 0.6	4.8 ± 0.5	95.5 ± 6.3
Alcañiz 1	50.5 ± 6.3	4.0 ± 0.02	6.4 ± 0.6	12.6 ± 1.1	75.5 ± 10.2
Alcañiz 2	80.9 ± 4.0	8.7 ± 1.1	9.7 ± 0.6	21.2 ± 0.3	120.5 ± 9.3
Alejandro Dumas	69.6 ± 5.3	9.1 ± 3.3	10.9 ± 1.3	15.6 ± 0.8	105.1 ± 5.6
Amarillo Calanda (131)	35.5 ± 5.1	10.4 ± 1.2	10.6 ± 0.6	12.5 ± 0.5	63.0 ± 15.3
Amarillo Calanda (2400)	69.9 ± 4.0	11.0 ± 1.2	13.8 ± 0.5	19.1 ± 0.3	113.8 ± 8.7
Amarillo de Gallur	79.8 ± 5.2	10.7 ± 2.4	11.1 ± 0.9	20.0 ± 3.6	121.5 ± 5.1
Andora	70.0 ± 4.0	7.7 ± 1.8	10.0 ± 0.9	18.5 ± 1.1	106.2 ± 9.3
Andross	76.6 ± 6.3	8.5 ± 2.5	9.9 ± 0.5	15.1 ± 0.8	110.1 ± 10.3
Baby Gold 5	72.4 ± 3.2	9.9 ± 2.2	11.6 ± 0.6	5.6 ± 0.5	99.5 ± 5.6
Baby Gold 6	83.3 ± 5.3	9.3 ± 3.6	10.6 ± 1.0	6.1 ± 0.6	109.3 ± 6.8
Baby Gold 7	71.9 ± 5.2	10.2 ± 0.5	12.0 ± 1.3	8.7 ± 0.2	102.8 ± 4.6
Baby Gold 8	83.8 ± 4.0	11.9 ± 1.2	12.4 ± 0.6	8.1 ± 0.5	116.2 ± 10.2
Baby Gold 9	71.0 ± 4.6	15.0 ± 1.2	14.0 ± 0.9	7.4 ± 0.2	109.3 ± 8.7
Baladin	74.0 ± 5.2	8.0 ± 0.8	8.6 ± 0.5	4.6 ± 0.5	95.2 ± 5.6
Benasque	67.1 ± 3.2	12.7 ± 0.7	2.0 ± 0.05	23.0 ± 1.1	105.6 ± 5.1
Big Top	86.8 ± 4.3	7.8 ± 0.8	9.1 ± 0.6	14.3 ± 0.8	118.0 ± 9.3
Bonet I	79.3 ± 5.1	10.9 ± 5.3	12.4 ± 1.3	15.3 ± 1.6	117.9 ± 6.3
Bonet II	73.7 ± 4.0	12.6 ± 1.1	12.3 ± 1.5	19.2 ± 1.1	117.8 ± 10.3
Bonet III	80.0 ± 6.3	12.2 ± 2.3	12.7 ± 1.0	35.0 ± 5.3	136.0 ± 5.6
Bonet IV	75.6 ± 8.2	14.5 ± 0.6	14.0 ± 2.3	21.1 ± 2.3	126.2 ± 10.2
Bonet V	79.9 ± 5.3	8.9 ± 0.7	11.8 ± 1.1	17.3 ± 1.3	118.0 ± 9.3
Borracho de Jarque	80.0 ± 4.0	9.2 ± 0.6	10.7 ± 1.1	23.9 ± 2.3	123.8 ± 10.3
Brasileño	75.4 ± 4.6	9.2 ± 0.9	10.7 ± 2.2	5.2 ± 0.5	100.4 ± 5.2
Calabacero	98.0 ± 9.1	13.6 ± 2.3	14.0 ± 1.5	7.6 ± 0.3	133.9 ± 15.6
Calanda San Miguel	88.5 ± 7.5	13.3 ± 1.2	12.2 ± 0.3	19.4 ± 1.1	133.5 ± 12.3
Calanda Tardío	74.4 ± 3.2	11.2 ± 2.6	12.4 ± 0.9	15.7 ± 0.9	113.8 ± 5.4
Campiel Rojo	74.3 ± 1.1	10.4 ± 3.5	11.9 ± 0.5	19.2 ± 1.1	115.8 ± 9.3
Carolyn	59.3 ± 5.2	10.3 ± 1.4	11.2 ± 0.3	20.6 ± 3.6	101.5 ± 5.1
Carson	74.6 ± 4.0	8.4 ± 0.5	9.4 ± 0.6	10.2 ± 0.8	102.7 ± 5.6
Catherina	81.2 ± 4.7	9.0 ± 0.5	10.5 ± 1.0	6.7 ± 0.2	107.4 ± 8.7
Del Gorro	77.4 ± 5.1	10.2 ± 0.3	11.1 ± 0.8	4.8 ± 0.5	103.5 ± 13.6
Diamante Amarillo	90.1 ± 2.4	8.7 ± 0.5	9.1 ± 0.6	17.9 ± 0.6	125.9 ± 10.3
Dixon	83.5 ± 5.6	10.7 ± 2.6	11.3 ± 0.3	6.7 ± 0.4	112.2 ± 10.2
Everst	70.4 ± 5.3	8.7 ± 0.5	10.2 ± 2.2	13.7 ± 1.2	103.0 ± 4.6
Fantasia	63.9 ± 2.4	13.6 ± 2.3	14.0 ± 0.9	7.5 ± 0.6	99.4 ± 6.3
Flamekist	60.5 ± 6.3	13.2 ± 1.2	13.0 ± 1.8	11.1 ± 0.5	97.8 ± 9.3
Flavortop	69.2 ± 3.2	11.9 ± 2.2	12.8 ± 1.1	8.0 ± 0.05	101.9 ± 10.0
Fortuna	71.1 ± 4.0	8.5 ± 0.5	10.1 ± 0.5	7.2 ± 0.4	96.9 ± 5.1
Fraga	74.0 ± 5.7	11.8 ± 0.7	11.7 ± 0.9	22.1 ± 1.2	119.6 ± 9.3
GF3	73.8 ± 5.2	10.1 ± 1.1	10.1 ± 1.0	4.9 ± 0.5	99.0 ± 8.1
Goiri	77.7 ± 6.3	4.0 ± 0.02	8.8 ± 0.6	8.3 ± 0.8	101.3 ± 5.6
Golden Queen	88.8 ± 4.6	9.9 ± 2.2	10.4 ± 2.2	10.4 ± 1.0	119.2 ± 7.3
Gomes	75.8 ± 5.1	10.1 ± 2.3	10.2 ± 0.9	22.1 ± 1.1	118.2 ± 9.5
Halford	71.9 ± 2.4	10.2 ± 1.1	9.9 ± 0.3	26.3 ± 1.0	118.3 ± 10.3
Infanta Isabel	86.6 ± 5.3	11.3 ± 1.1	13.5 ± 1.3	11.4 ± 0.5	122.8 ± 8.7
Jerónimo de Alfaro	82.1 ± 3.3	8.6 ± 0.5	11.1 ± 1.1	8.5 ± 0.3	110.3 ± 10.0
Jungerman	92.9 ± 5.3	9.4 ± 1.0	11.6 ± 0.5	6.7 ± 0.5	120.6 ± 10.2
Kakamas	76.0 ± 3.3	12.4 ± 2.2	13.2 ± 1.1	21.6 ± 2.0	123.3 ± 9.9
Keimoes	75.6 ± 3.2	10.3 ± 1.1	10.5 ± 1.0	24.0 ± 2.5	120.5 ± 5.6
Klamt	77.7 ± 4.0	8.2 ± 0.7	8.9 ± 0.6	8.9 ± 0.4	103.8 ± 5.6
Loadel	69.8 ± 6.3	8.2 ± 1.2	9.0 ± 0.6	6.1 ± 0.5	93.2 ± 6.3
Lovell	72.0 ± 5.2	7.3 ± 1.0	8.4 ± 0.3	18.3 ± 1.2	106.0 ± 5.1
Maluenda	76.1 ± 5.6	12.8 ± 2.3	12.6 ± 2.2	14.2 ± 1.6	115.8 ± 8.9
Maria Serena	75.0 ± 1.4	8.1 ± 1.0	8.5 ± 0.3	3.5 ± 0.3	95.1 ± 6.3
Maruja	73.8 ± 5.1	7.8 ± 0.5	10.6 ± 0.9	5.5 ± 0.3	97.8 ± 9.3
Maruja Porvenir	74.7 ± 5.6	8.9 ± 1.3	10.1 ± 0.7	9.7 ± 0.3	103.4 ± 10.3
Miraflores	72.3 ± 5.3	12.2 ± 2.2	12.2 ± 1.1	20.4 ± 2.6	117.2 ± 12.3
Mountaingold	76.9 ± 1.1	9.2 ± 0.7	10.1 ± 1.0	4.5 ± 0.8	100.6 ± 10.2
Nectar del Jalón	79.6 ± 4.0	10.5 ± 1.1	12.6 ± 0.5	11.3 ± 0.9	114.0 ± 9.3
NJC 97	78.9 ± 5.3	10.3 ± 0.6	11.3 ± 1.3	2.0 ± 0.05	102.9 ± 5.1

(continue)

	Sucrose	Glucose	Fructose	Sorbitol	Total sugars
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Nuevo	82.8 ± 3.2	11.4 ± 2.2	13.0 ± 0.5	16.7 ± 1.2	123.9 ± 8.7
Oropel	83.6 ± 6.3	11.2 ± 2.3	10.9 ± 0.7	15.9 ± 1.1	121.6 ± 5.6
Paloro A	78.4 ± 4.3	10.2 ± 1.2	10.8 ± 1.1	18.7 ± 1.2	118.2 ± 5.6
Paloro B	83.4 ± 5.2	13.1 ± 1.5	12.6 ± 0.9	19.2 ± 2.0	128.4 ± 9.6
Queen Giant	68.0 ± 3.7	9.6 ± 0.6	10.9 ± 0.8	3.2 ± 0.05	91.8 ± 6.3
Redhaven	65.6 ± 1.8	7.4 ± 0.5	8.6 ± 0.5	3.7 ± 0.4	85.3 ± 10.3
Rojo del Rito	82.5 ± 2.4	8.9 ± 1.1	10.8 ± 0.9	30.9 ± 4.8	133.1 ± 10.2
San Jaime	82.6 ± 6.3	8.2 ± 1.9	10.6 ± 1.0	8.6 ± 0.6	110.0 ± 9.3
San Lorenzo	82.2 ± 2.9	8.3 ± 0.6	10.2 ± 1.1	9.0 ± 0.8	109.7 ± 10.2
Sarell	69.1 ± 4.0	8.2 ± 0.7	10.4 ± 0.7	15.4 ± 0.9	103.1 ± 5.1
Selma	77.9 ± 5.3	9.0 ± 1.1	11.3 ± 1.1	6.1 ± 0.3	104.4 ± 10.3
Shasta	82.9 ± 2.6	8.7 ± 0.7	9.3 ± 0.6	12.2 ± 0.8	113.1 ± 10.2
Stanford	70.1 ± 6.6	7.7 ± 2.2	9.9 ± 0.9	8.1 ± 0.5	95.9 ± 5.6
Starn	79.9 ± 3.2	11.4 ± 2.3	10.9 ± 0.5	23.0 ± 1.2	125.2 ± 9.9
Sudanell 1	76.0 ± 6.4	10.3 ± 1.2	11.7 ± 1.0	14.4 ± 1.0	112.4 ± 5.6
Sudanell 2	70.7 ± 5.2	12.4 ± 1.7	13.4 ± 1.3	22.7 ± 1.1	119.2 ± 8.7
Sudanell 3	75.0 ± 4.0	10.3 ± 2.2	11.7 ± 1.2	11.0 ± 0.8	108.1 ± 5.1
Sudanell Blanco	69.2 ± 6.3	11.1 ± 1.1	10.0 ± 0.5	21.8 ± 2.2	112.2 ± 9.3
Sudanell GF	75.9 ± 1.2	12.4 ± 2.2	12.0 ± 1.1	11.7 ± 0.8	112.0 ± 5.6
Sudanell GF (2804)	75.6 ± 5.3	9.6 ± 0.6	10.6 ± 0.7	13.0 ± 0.9	108.9 ± 8.4
Suncling	81.0 ± 5.1	8.4 ± 0.7	9.1 ± 0.6	4.6 ± 0.4	103.1 ± 5.6
Super Crimson Gold	59.8 ± 3.2	9.2 ± 2.3	9.4 ± 0.9	2.5 ± 0.05	80.9 ± 10.9
Tebana	79.7 ± 6.3	8.5 ± 0.5	9.8 ± 0.5	6.0 ± 0.06	103.9 ± 7.8
Tempranillo de Aytona	74.2 ± 4.2	8.7 ± 0.6	8.4 ± 0.6	5.3 ± 0.3	96.6 ± 10.2
Tipo Campiel	65.3 ± 5.6	13.2 ± 1.2	12.5 ± 2.3	26.4 ± 3.6	117.3 ± 8.7
Venus	40.2 ± 5.1	11.1 ± 1.1	14.0 ± 0.8	5.7 ± 0.8	71.5 ± 12.5
Vesuvio	69.1 ± 3.6	10.0 ± 1.0	11.2 ± 1.1	5.0 ± 0.05	95.3 ± 6.3
Vivian	74.8 ± 4.0	9.9 ± 0.7	10.9 ± 0.9	27.4 ± 2.5	123.0 ± 9.3
Walgant	82.3 ± 2.1	8.7 ± 0.6	9.1 ± 1.0	20.9 ± 1.2	120.9 ± 10.3
Wiser	790 ± 3.2	11.7 ± 0.7	11.4 ± 1.3	21.9 ± 1.2	124.1 ± 5.6
Zaragozano	73.4 ± 5.2	11.3 ± 2.3	11.5 ± 0.9	16.2 ± 1.1	112.4 ± 8.7
Zaragozano Amarillo	76.7 ± 5.3	10.5 ± 1.2	10.6 ± 0.6	22.9 ± 3.6	120.7 ± 10.2
Zaragozano Rojo	73.0 ± 5.3	9.8 ± 0.5	11.9 ± 0.9	12.0 ± 1.0	106.7 ± 5.1

11.1.3. Supplementary file 3. Vitamin C, phenolics, flavonoids, anthocyanins and relative antioxidant capacity (RAC) of the 94 cultivars studied.

	Vitamin C	Phenolics	Flavonoids	Anthocyanins	RAC
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Adriática	15.5 ± 2.5	44.2 ± 3.7	7.0 ± 1.1	0.9 ± 0.06	646.0 ± 16.8
Alcañiz 1	12.1 ± 2.2	62.0 ± 2.8	50.3 ± 1.6	1.2 ± 0.05	784.2 ± 19.8
Alcañiz 2	19.5 ± 1.9	44.3 ±3.3	60.3 ± 2.5	2.5 ± 0.02	1146.4 ± 28.9
Alejandro Dumas	8.3 ± 1.6	47.8 ± 4.2	14.5 ± 0.9	2.5 ± 0.1	783.4 ± 19.5
Amarillo Calanda (131)	10.5 ± 1.8	52.4 ± 2.4	57.2 ± 2.5	2.1 ± 0.05	1152.3 ± 27.5
Amarillo Calanda (2400)	9.5 ± 0.3	46.7 ± 3.6	45.0 ± 1.1	2.0 ± 0.06	1068.4 ± 23.5
Amarillo de Gallur	19.2 ± 2.2	49.2 ± 3.7	37.5 ± 0.8	7.6 ± 0.1	1036.3 ± 16.8
Andora	9.1 ± 1.6	32.6 ± 4.2	5.6 ± 0.5	0.7 ± 0.06	503.2 ± 17.8
Andross	19.9 ± 2.3	45.1 ± 2.8	24.1 ± 1.6	1.1 ± 0.2	733.4 ± 19.8
Baby Gold 5	8.3 ± 2.5	45.6 ± 3.3	16.1 ± 0.9	3.4 ± 0.05	659.3 ± 17.8
Baby Gold 6	16 ± 1.9	44.9 ± 3.6	10.1 ± 2.5	2.2 ± 0.3	837.3 ± 16.5
Baby Gold 7	10.8 ± 1.1	38.4 ± 1.3	12.0 ± 0.9	1.8 ±0 .5	818.5 ± 23.5
Baby Gold 8	15.2 ± 1.8	46.4 ± 3.7	13.4 ± 1.2	1.1 ± 0.02	885.5 ± 17.6
Baby Gold 9	14.8 ± 2.2	41.6 ± 4.2	28.8 ± 1.1	2.4 ± 0.02	538.2 ± 14.3
Baladin	12.2 ± 1.0	37.7 ± 2.4	4.7 ± 0.5	0.9 ± 0.05	530.8 ± 16.8
Benasque	15.4 ± 2.1	45.9 ± 1.5	35.7 ± 3.3	4.9 ± 0.1	1028.6 ± 25.6
Big Top	3.4 ± 0.6	22.2 ± 2.8	3.0 ± 0.5	4.8 ± 0.1	186.0 ± 10.9
Bonet I	16.1 ± 2.4	47.6 ± 1.3	28.5 ± 1.2	1.8 ± 0.1	930.6 ± 15.7
Bonet II	11.9 ± 2.5	46.3 ± 1.2	31.1 ± 2.5	1.4 ± 0.01	950.2 ± 17.8
Bonet III	18.1 ± 1.8	47.5 ± 3.3	37.2 ± 1.6	1.7 ± 0.06	1184.0 ± 26.7
Bonet IV	14.3 ± 1.6	46.8 ± 3.6	20.5 ± 0.9	4.3 ± 0.3	1016.6 ± 23.5
Bonet V	8.9 ± 2.1	48.3 ± 1.0	30.9 ± 2.2	2.9 ± 0.2	979.6 ± 24.5
Borracho de Jarque	11.1 ± 1.6	47.6 ± 1.6	31.1 ± 2.5	7.0 ± 1.1	949.7 ± 16.7
Brasileño	13.3 ± 2.2	46.7 ± 1.4	16.2 ± 2.2	7.9 ± 0.1	963.3 ± 19.8
Calabacero	15.2 ± 2.6	40.8 ± 3.7	21.1 ± 1.4	1.0 ± 0.05	686.1 ± 14.6
Calanda San Miguel	9.0 ± 1.9	51.8 ± 2.9	40.4 ± 1.1	3.2 ± 0.5	971.8 ± 17.4
Calanda Tardío	12.2 ± 1.0	51.3 ± 2.0	39.1 ± 4.2	1.7 ± 0.01	1098.2 ± 26.5
Campiel Rojo	12.7 ± 1.1	50.2 ± 2.2	42.9 ± 1.6	3.1 ± 0.3	1107.5 ± 24.8
Carolyn	9.3 ± 0.6	44.8 ± 2.8	14.0 ± 2.5	1.5 ± 0.01	829.3 ± 12.7
Carson	16.2 ± 1.6	47.3 ± 3.3	8.2 ± 0.5	1.1 ± 0.2	799.4 ± 16.8
Catherina	11.8 ± 1.3	42.6 ± 1.7	14.0 ± 0.9	0.9 ± 0.06	770.1 ± 23.5
Del Gorro	11.9 ± 2.1	45.3 ± 2.4	16.2 ± 1.4	1.8 ± 0.1	805.1 ± 17.8
Diamante Amarillo	12.2 ± 2.2	50.9 ± 4.2	44.9 ± 2.5	2.1 ± 0.02	979.6 ± 23.6
Dixon	12.3 ± 1.8	45.7 ± 3.7	13.6 ± 1.0	1.5 ± 0.1	817.1 ± 24.9
Everst	9.3 ± 0.6	47.4 ± 3.6	23.5 ± 2.2	1.4 ± 0.05	859.6 ± 14.9
Fantasia	4.9 ± 1.9	42.2 ± 1.3	7.9 ± 0.5	6.8 ± 0.3	630.7 ± 13.6
Flamekist	6.8 ± 0.2	47.4 ± 1.9	17.8 ± 1.6	1.8 ± 0.06	884.5 ± 11.6
Flavortop	9.5 ± 1.0	44.5 ± 5.2	13.8 ± 1.2	12 ± 0.9	877.3 ± 10.3
Fortuna	13.6 ± 2.5	41.3 ± 3.2	10.3 ± 1.1	0.8 ± 0.02	661.8 ± 19.8
Fraga	12.3 ± 0.9	48.4 ± 2.8	43.2 ± 4.5	2.7 ± 0.1	1014.8 ± 15.6
GF3	11.8 ± 1.6	36.1 ± 1.8	10.4 ± 0.9	3.1 ± 0.6	554.3 ± 17.8
Goiri	19.3 ± 2.2	40.1 ± 4.5	9.4 ± 0.5	0.8 ± 0.01	615.1 ± 18.7
Golden Queen	13.1 ± 2.1	48.5 ± 2.4	36.0 ± 2.0	1.2 ± 0.02	1047.3 ± 26.3
Gomes	13.9 ± 1.7	45.9 ± 4.3	33.5 ± 3.0	2.1 ± 0.05	1005 ± 24.9
Halford	14.0 ± 1.8	48.4 ± 3.3	31.7 ± 1.3	1.9 ± 0.1	1080.6 ± 24.6
Infanta Isabel	17.4 ± 1.5	45.0 ± 3.7	18.8 ± 2.5	1.0 ± 0.06	940.1 ± 16.8
Jerónimo de Alfaro	10.8 ± 1.6	47.2 ± 3.6	18.4 ± 1.1	1.7 ± 0.2	862.3 ± 18.7
Jungerman	11.6 ± 1.0	48.1 ± 4.2	10.2 ± 1.6	2.0 ± 0.01	802.6 ± 24.9
Kakamas	11.1 ± 1.6	48.1 ± 2.3	38.0 ± 2.3	1.2 ± 0.2	1007.2 ± 21.5
Keimoes	12.4 ± 1.9	46.3 ± 3.7	28.3 ± 0.9	1.1 ± 0.2	913.0 ± 23.6
Klamt	13.8 ± 2.1	42.1 ± 1.2	14.1 ± 1.1	0.9 ± 0.1	764.1 ± 19.8
Loadel	12.8 ± 2.5	25.5 ± 2.8	5.2 ± 0.5	0.7 ± 0.05	635.5 ± 17.8
Lovell	16.3 ± 2.2	47.0 ± 5.2	21.8 ± 1.1	1.3 ± 0.06	768.7 ± 24.9
Maluenda	12.8 ± 2.5	48.3 ± 4.5	31.8 ± 2.3	3.9 ± 0.3	975.4 ± 23.5
Maria Serena	8.2 ± 2.1	30.2 ± 2.4	5.9 ± 0.5	0.9 ± 0.01	390.2 ± 11.0
Maruja	14.7 ± 1.6	42.7 ± 2.8	16.6 ± 2.5	0.9 ± 0.02	818.6 ± 13.2
Maruja Porvenir	14.5 ± 1.8	44.7 ± 3.3	12.2 ± 1.2	1.0 ± 0.09	708.7 ± 15.6
Miraflores	8.3 ± 0.6	51.9 ± 3.6	34.9 ± 1.6	2.1 ± 0.01	954.3 ± 16.8
Mountaingold	10.5 ± 1.0	44.5 ± 2.4	12.6 ± 0.9	2.2 ± 0.08	880.5 ± 18.7
Nectar del Jalón	16.8 ± 2.6	36.3 ± 4.2	11.1 ± 1.1	3.9 ± 0.09	551.2 ± 17.8
NJC 97	9.2 ± 1.6	41.4 ± 3.7	9.4 ± 0.5	1.0 ± 0.06	689.4 ± 12.3

(continue)

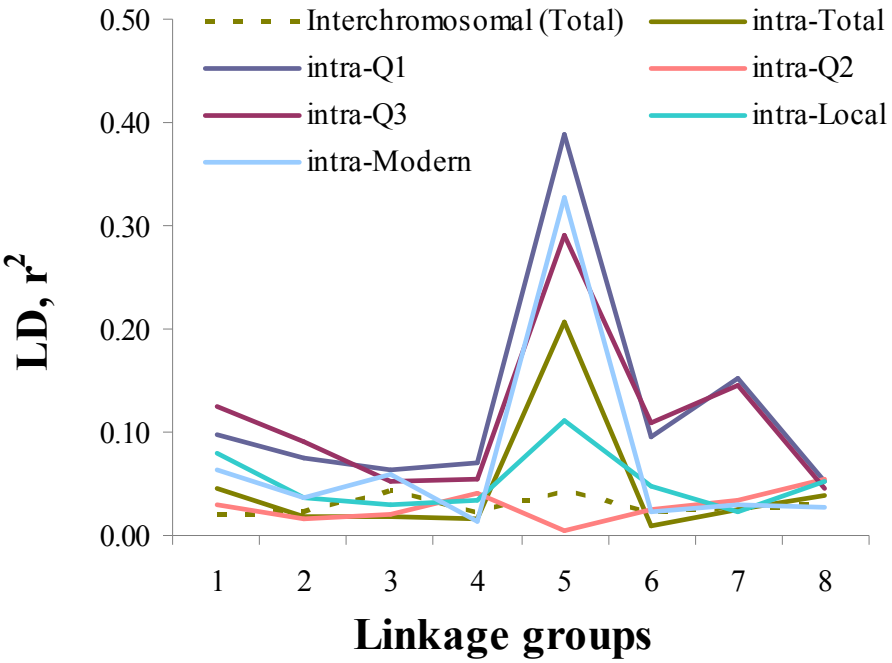
	Vitamin C	Phenolics	Flavonoids	Anthocyanins	RAC
	Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Nuevo	18.2 ± 2.1	48.7 ± 5.6	63.0 ± 5.6	2.5 ± 0.05	1052.4 ± 24.9
Oropel	15.8 ± 3.6	45.8 ± 1.3	39.1 ± 2.4	1.8 ± 0.2	972.4 ± 23.5
Paloro A	14.6 ± 1.9	50.3 ±3 .3	38.1 ± 2.5	4.6 ± 0.02	1009.6 ± 22.5
Paloro B	15.1 ± 2.2	48.5 ± 4.3	34.0 ± 2.5	5.1 ± 0.1	1070.6 ± 21.0
Queen Giant	4.3 ± 0.6	43.0 ± 2.8	5.2 ± 0.5	5.6 ± 0.6	571.6 ± 19.8
Redhaven	6.3 ± 0.6	34.8 ± 3.6	7.4 ± 1.1	1.5 ± 0.05	674.3 ± 10.2
Rojo del Rito	11.0 ± 2.5	48.3 ± 2.4	28.6 ± 0.9	10 ± 4.2	923.9 ± 24.3
San Jaime	15.9 ± 1.8	43.4 ± 3.3	15.2 ± 2.1	1.1 ± 0.06	917.2 ± 24.9
San Lorenzo	18.1 ± 1.0	41.2 ± 3.7	17.6 ± 1.7	1.2 ± 0.3	757.6 ± 16.8
Sarell	17.5 ± 1.6	42.2 ± 2.3	12.9 ± 1.6	0.8 ± 0.06	704.5 ± 20.3
Selma	12.9 ± 2.1	46.8 ± 2.7	17.7 ± 1.1	1.1 ± 0.02	871.6 ± 18.7
Shasta	27.8 ± 1.9	38.9 ± 1.5	7.3 ± 0.5	0.9 ± 0.05	591.4 ± 21.2
Stanford	11.1 ± 1.6	46.3 ± 4.6	24.9 ± 1.8	1.8 ± 0.01	767.6 ± 17.8
Starn	11.8 ± 1.0	47.0 ± 2.8	27.5 ± 2.5	2.0 ± 0.02	958.9 ± 15.6
Sudanell 1	13.0 ± 2.2	46.8 ± 4.3	28.6 ± 1.9	1.9 ± 0.05	1011.9 ± 23.5
Sudanell 2	11.9 ± 0.9	40.2 ± 3.3	30.6 ± 0.9	2.0 ± 0.02	951.4 ± 21.5
Sudanell 3	17.7 ± 2.1	46.1 ± 1.2	24.3 ± 1.1	3.2 ± 0.02	966.4 ± 19.8
Sudanell Blanco	15.1 ± 1.0	50.7 ± 3.6	40.1 ± 2.6	1.1 ± 0.06	1084.7 ± 24.7
Sudanell GF	10.2 ± 2.5	50.2 ± 4.2	36.3 ± 2.4	1.1 ± 0.01	954.8 ± 21.4
Sudanell GF (2804)	9.9 ± 0.6	45.5 ± 5.3	25.8 ± 3.6	1.1 ± 0.2	858.8 ± 24.9
Suncling	9.4 ± 1.9	41.6 ± 3.7	17.5 ± 1.1	1.1 ± 0.05	790.1 ± 16.8
Super Crimson Gold	5.6 ± 0.6	31.8 ± 2.4	4.2 ± 0.5	3.0 ± 0.1	598.7 ± 23.5
Tebana	14.7 ± 2.2	32.7 ± 4.8	6.1 ± 0.5	1.0 ± 0.06	696.3 ± 12.3
Tempranillo de Aytona	11.7 ± 1.8	38.4 ± 2.8	4.3 ± 0.5	3.2 ± 0.1	691.0 ± 18.7
Tipo Campiel	12.0 ± 2.5	47.8 ± 3.8	47.1 ± 1.6	3.2 ±0 .01	1064 ± 24.1
Venus	2.9 ± 0.5	18.0 ± 3.6	4.5 ± 0.5	2.3 ± 0.01	367.8 ± 11.1
Vesuvio	10.8 ± 2.2	45.8 ± 3.3	13.9 ± 2.5	1.7 ± 0.05	605.3 ± 17.8
Vivian	15.3 ± 1.8	48.6 ± 2.9	32.4 ± 0.9	7.9 ± 0.1	1040.8 ± 23.5
Walgant	12.3 ± 1.6	46.1 ± 4.2	12.8 ± 1.1	1.0 ± 0.02	624.3 ± 24.1
Wiser	11.6 ± 2.2	46.0 ± 3.7	34.0 ± 0.9	1.4 ± 0.06	949.6 ± 16.8
Zaragozano	14.5 ± 2.5	47.6 ± 2.8	19.5 ± 1.1	4.4 ± 0.1	717.3 ± 19.8
Zaragozano Amarillo	17.9 ± 1.9	47.9 ± 1.3	56.2 ± 1.6	3.0 ± 0.05	1132.4 ± 23.5
Zaragozano Rojo	12.3 ± 2.5	50.5 ± 3.7	28.8 ± 2.5	2.8 ± 0.02	992.2 ± 16.8

11.2. Material suplementario correspondiente al capítulo 4

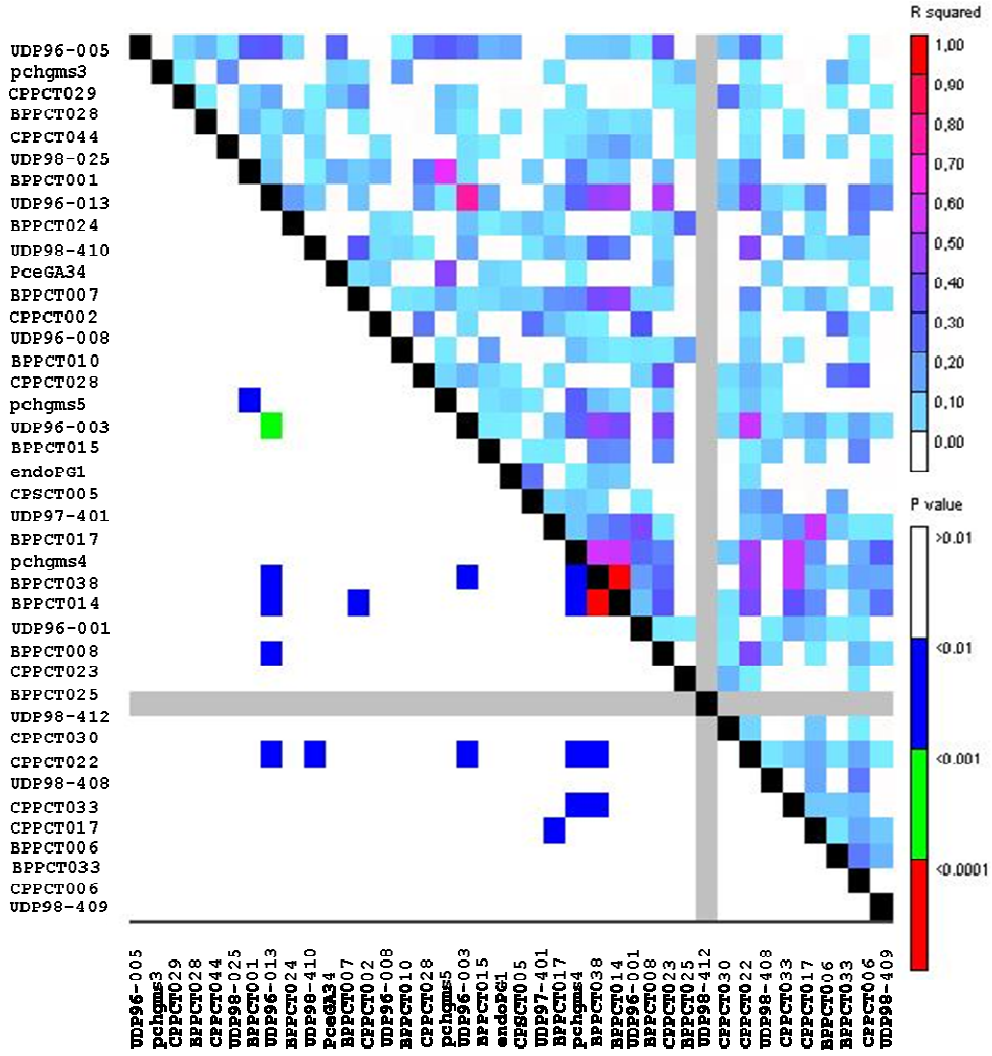
11.2.1 Supplementary file 1. Mean estimated values for different genetic parameters of the 94 peach/nectarine cultivars based on 40 SSR loci.

SSR	A	Ae	Ho	He	Fis	I	PD
BPPCT001	7.00	3.77	0.30	0.74	0.59	1.40	0.69
BPPCT006	7.00	3.61	0.43	0.73	0.41	1.55	0.70
BPPCT007	5.00	1.41	0.18	0.29	0.38	0.64	0.29
BPPCT008	4.00	2.56	0.32	0.61	0.47	1.78	0.61
BPPCT010	3.00	1.14	0.13	0.12	-0.08	0.26	0.12
BPPCT014	2.00	1.06	0.06	0.06	0.00	0.13	0.06
BPPCT015	10.0	5.20	0.69	0.81	0.15	1.90	0.73
BPPCT017	7.00	1.46	0.22	0.32	0.31	0.74	0.32
BPPCT024	4.00	2.01	0.75	0.51	-0.47	0.78	0.50
BPPCT025	11.00	4.85	0.97	0.80	-0.21	1.84	0.69
BPPCT028	5.00	1.67	0.26	0.40	0.35	0.82	0.40
BPPCT033	5.00	2.74	0.98	0.64	-0.53	1.17	0.64
BPPCT038	6.00	1.52	0.23	0.34	0.32	0.75	0.34
CPPCT002	3.00	1.62	0.31	0.38	0.18	0.68	0.38
CPPCT006	4.00	1.54	0.28	0.35	0.20	0.68	0.35
CPPCT017	4.00	2.18	0.74	0.54	-0.37	0.95	0.54
CPPCT022	8.00	2.58	0.81	0.62	-0.31	1.36	0.61
CPPCT023	2.00	1.18	0.17	0.16	-0.06	0.29	0.16
CPPCT028	7.00	3.49	0.68	0.72	0.06	1.45	0.72
CPPCT029	6.00	3.21	0.92	0.69	-0.33	1.29	0.69
CPPCT030	5.00	1.35	0.28	0.27	-0.04	0.60	0.26
CPPCT033	2.00	1.37	0.17	0.27	0.37	0.44	0.27
CPPCT044	8.00	3.93	0.97	0.75	-0.29	1.52	0.69
CPSCT005	2.00	1.69	0.26	0.41	0.37	0.60	0.41
endoPG1	5.00	2.01	0.76	0.51	-0.49	0.82	0.51
PceGA34	5.00	2.92	0.53	0.66	0.20	1.20	0.66
pchgms3	5.00	1.95	0.17	0.49	0.65	0.88	0.49
pchgms4	2.00	1.41	0.08	0.12	0.33	0.24	0.12
pchgms5	2.00	1.16	0.13	0.14	0.07	0.27	0.14
UDP96-001	9.00	3.19	0.95	0.69	-0.38	1.39	0.69
UDP96-003	7.00	2.01	0.38	0.51	0.25	1.09	0.50
UDP96-005	2.00	1.99	0.29	0.50	0.42	0.69	0.50
UDP96-008	5.00	3.14	0.66	0.69	0.04	1.30	0.68
UDP96-013	6.00	1.87	0.31	0.47	0.34	0.89	0.47
UDP97-401	2.00	1.35	0.12	0.26	0.54	0.42	0.26
UDP98-025	4.00	3.59	0.98	0.73	-0.34	1.32	0.68
UDP98-408	3.00	1.24	0.15	0.20	0.25	0.37	0.19
UDP98-409	6.00	2.32	0.98	0.57	-0.72	0.99	0.57
UDP98-410	5.00	3.51	0.97	0.74	-0.31	1.40	0.70
UDP98-412	8.00	4.78	0.94	0.80	-0.18	1.70	0.69
Mean	5.10	2.39	0.48	0.49	0.05	0.96	0.47
All loci	203						
Mean local cultivars	4.41	2.26	0.54	0.45	-0.20	0.85	0.45
All loci for local cultivars	172						

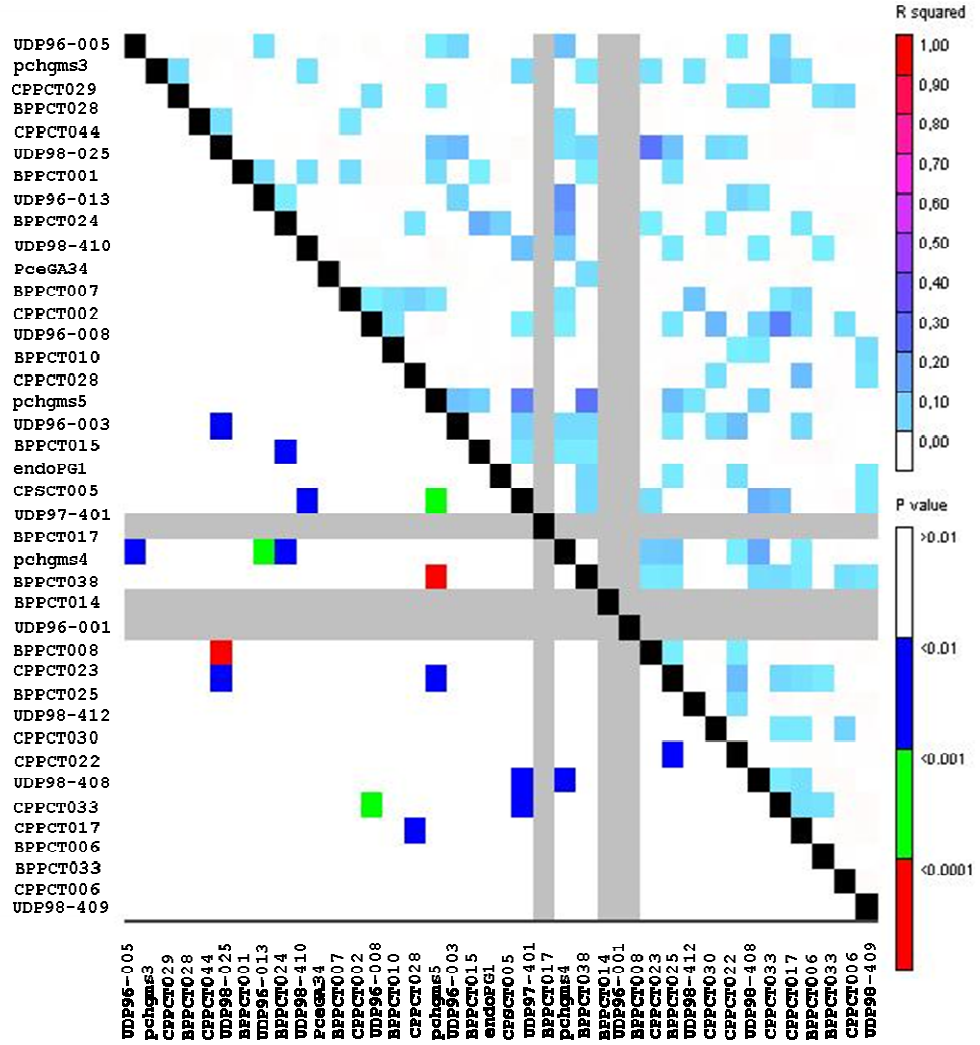
A observed number of alleles per locus, A_e effective number of alleles per locus, H_o observed heterozygosity, H_e expected heterozygosity, F_{is} Wright's fixation index, *I* Shannon's information index, *PD* power of discrimination.



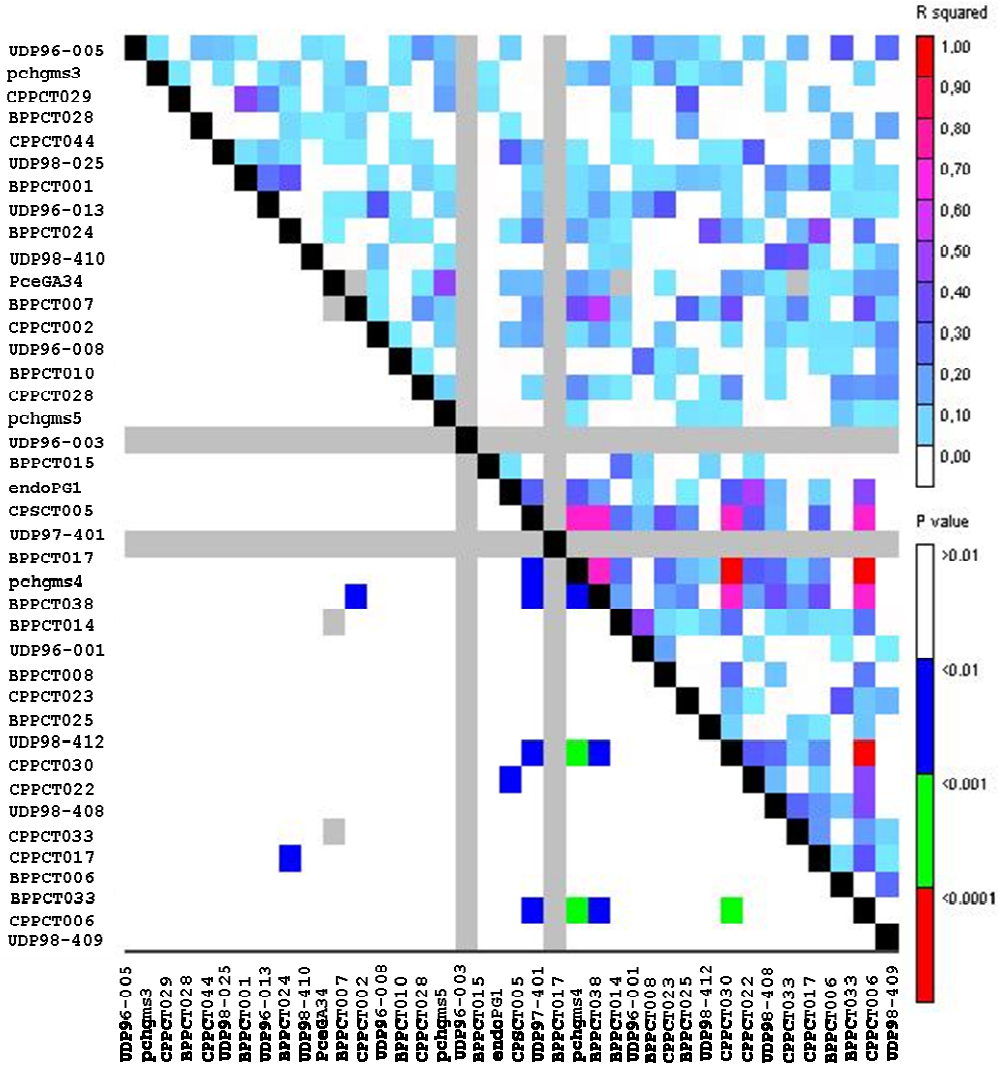
11.2.2. Supplementary file 2. Linkage disequilibrium scores (r^2), averaged across chromosomes and germplasm groups, according to the analysis with software STRUCTURE (Q1-Q3), and to previous knowledge of the varieties (local and modern).



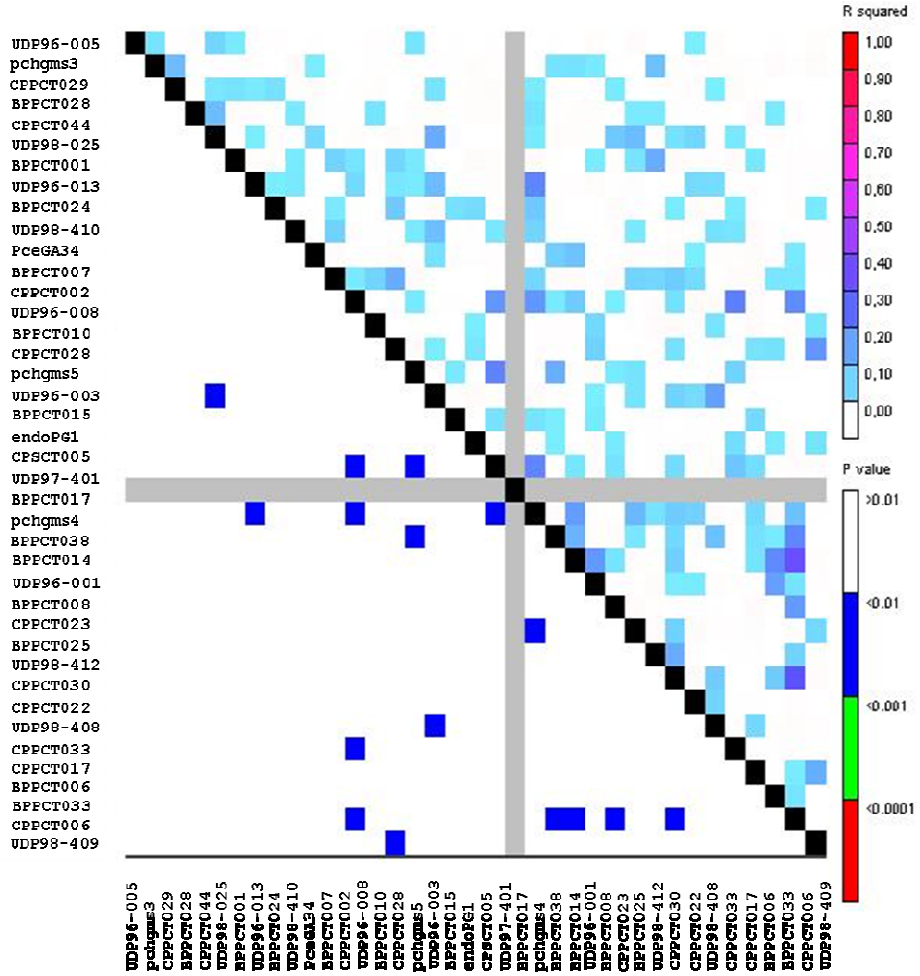
11.2.3. Supplementary file 3. Linkage disequilibrium plot based on Q1 analysis obtained from STRUCTURE software screened in 94 peach/nectarine cultivars. At the right side are represented the r^2 values and at the left side the *p*-values, according the colors of the legend.



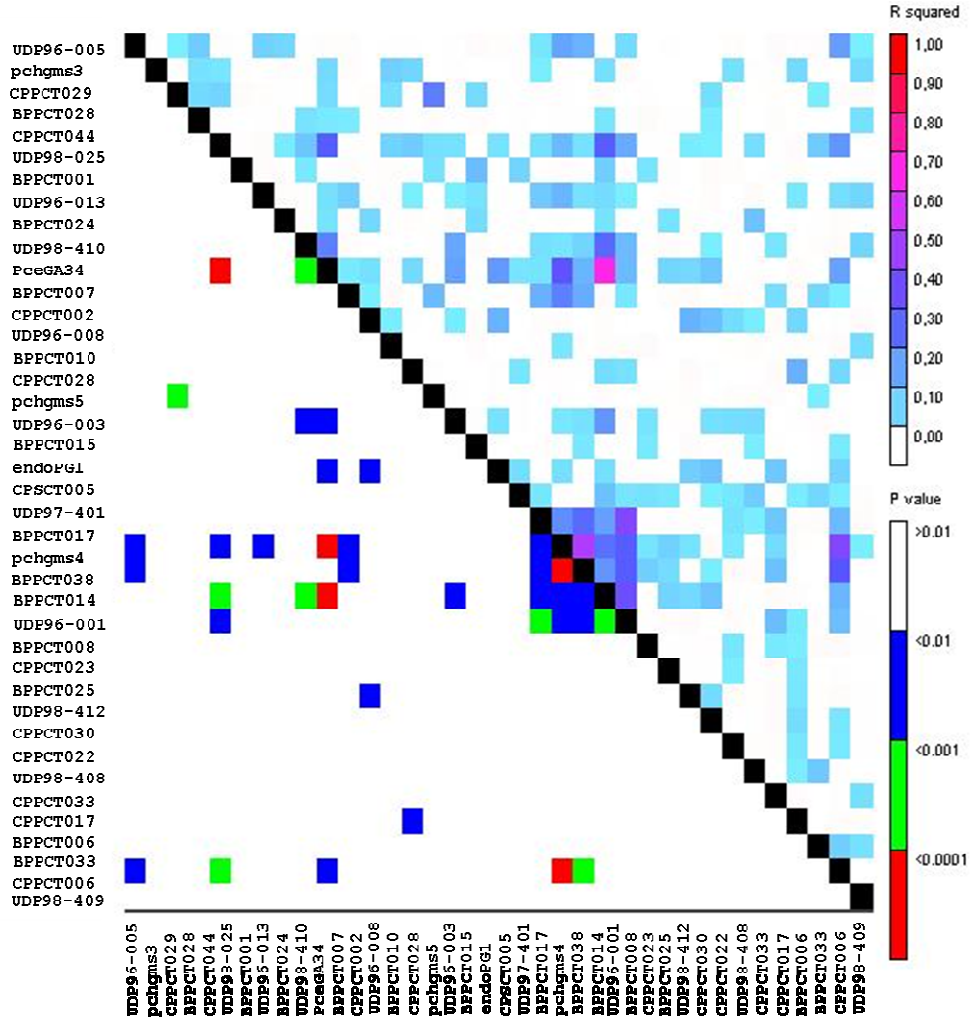
11.2.4. Supplementary file 4. Linkage disequilibrium plot based on Q2 analysis obtained from STRUCTURE software screened in 94 peach/nectarine cultivars. At the right side are represented the r^2 values and at the left side the *p*-values, according the colors of the legend.



11.2.5. Supplementary file 5. Linkage disequilibrium plot based on Q3 analysis obtained from STRUCTURE software screened in 94 peach/nectarine cultivars. At the right side are represented the r^2 values and at the left side the *p*-values, according the colors of the legend.



11.2.6. Supplementary file 6. Linkage disequilibrium plot based on local cultivars screened in 94 peach/nectarine cultivars. At the right side are represented the r^2 values and at the left side the *p*-values, according the colors of the legend.



11.2.7. Supplementary file 7. Linkage disequilibrium plot based on modern cultivars screened in 94 peach/nectarine cultivars. At the right side are represented the r^2 values and at the left side the *p*-values, according the colors of the legend.

11.3. Material suplementario correspondiente al capítulo 5

11.3.1 Lista completa de las 347 asociaciones entre los marcadores de tipo SNPs y algunos caracteres pomológicos del fruto.

Caracteres	Marcador	Scaffold	Posición	p-value
Fecha de floración	SNP_IGA_37843	1	12641440	0,0000025655
	SNP_IGA_365780	3	20635992	0,0000009567
	SNP_IGA_430583	4	15574015	0,0000010476
	SNP_IGA_431437	4	15743804	0,0000000116
	SNP_IGA_441507	4	18422944	0,0000012142
	SNP_IGA_441749	4	18496230	0,0000005332
	SNP_IGA_441887	4	18520777	0,0000012142
	SNP_IGA_441904	4	18522596	0,0000012142
Fecha de cosecha	SNP_IGA_46754	1	14980305	0,0000000088
	SNP_IGA_48586	1	15234386	0,0000000023
	SNP_IGA_55903	1	15890555	0,0000000088
	SNP_IGA_56163	1	15907025	0,0000000088
	SNP_IGA_56198	1	15908997	0,0000000088
	SNP_IGA_57051	1	16186175	0,0000000114
	SNP_IGA_57241	1	16202261	0,0000000114
	SNP_IGA_57544	1	16389065	0,0000000088
	SNP_IGA_57793	1	16426046	0,0000000088
	SNP_IGA_63566	1	18553050	0,0000017881
	SNP_IGA_63603	1	18560713	0,0000017881
	SNP_IGA_63638	1	18571836	0,0000017881
	SNP_IGA_63776	1	18603486	0,0000017881
	SNP_IGA_63825	1	18621833	0,0000002418
	SNP_IGA_63833	1	18622159	0,0000002418
	SNP_IGA_63847	1	18623934	0,0000002418
	SNP_IGA_64918	1	18762086	0,0000002418
	SNP_IGA_65002	1	18809420	0,0000002418
	SNP_IGA_65286	1	18911503	0,0000017881
	SNP_IGA_86245	1	25343182	0,0000017344
	SNP_IGA_86252	1	25343795	0,0000017344
	SNP_IGA_111017	1	35798416	0,0000014258
	SNP_IGA_132155	1	44936042	0,0000000769
	SNP_IGA_137253	2	461255	0,0000006505
	SNP_IGA_137307	2	474944	0,0000000497
	SNP_IGA_137745	2	512699	0,0000006505
	SNP_IGA_137839	2	529491	0,0000000043
	SNP_IGA_138882	2	654242	0,0000000043
	SNP_IGA_139270	2	692014	0,0000006795
	SNP_IGA_139612	2	713093	0,0000000372
	SNP_IGA_140089	2	732572	0,0000003632
	SNP_IGA_140105	2	733147	0,0000000372
	SNP_IGA_140371	2	745074	0,0000000372
	SNP_IGA_140441	2	748755	0,0000006795
	SNP_IGA_140557	2	754089	0,0000000037
	SNP_IGA_140573	2	754603	0,0000000372
	SNP_IGA_140599	2	755371	0,0000000037
	SNP_IGA_140823	2	775785	0,0000000040
	SNP_IGA_140857	2	776722	0,0000000040

(continue)

Caracteres	Marcador	Scaffold	Posición	p-value
Fecha de cosecha	SNP_IGA_140864	2	776885	0,0000000601
	SNP_IGA_140922	2	778085	0,0000000040
	SNP_IGA_140933	2	778300	0,0000000601
	SNP_IGA_140938	2	778744	0,0000000037
	SNP_IGA_140951	2	779117	0,0000000040
	SNP_IGA_140965	2	779808	0,0000000040
	SNP_IGA_140990	2	780479	0,0000000037
	SNP_IGA_141600	2	879987	0,0000000026
	SNP_IGA_141607	2	880882	0,0000000026
	SNP_IGA_141612	2	881016	0,0000000026
	SNP_IGA_141624	2	881365	0,0000000003
	SNP_IGA_141858	2	911846	0,0000004242
	SNP_IGA_141868	2	913067	0,0000006834
	SNP_IGA_142214	2	941180	0,0000006834
	SNP_IGA_142231	2	942619	0,0000000548
	SNP_IGA_142473	2	961984	0,0000003145
	SNP_IGA_142518	2	964244	0,0000000037
	SNP_IGA_143002	2	1011959	0,0000000548
	SNP_IGA_143150	2	1023786	0,0000004899
	SNP_IGA_143346	2	1034154	0,0000002139
	SNP_IGA_143360	2	1035269	0,0000002139
	SNP_IGA_143796	2	1052865	0,0000000037
	SNP_IGA_143809	2	1053489	0,0000000040
	SNP_IGA_143907	2	1057628	0,0000000293
	SNP_IGA_143917	2	1057806	0,0000000040
	SNP_IGA_143925	2	1058060	0,0000000040
	SNP_IGA_144878	2	1116293	0,0000002139
	SNP_IGA_144902	2	1117840	0,0000004605
	SNP_IGA_144913	2	1118746	0,0000000227
	SNP_IGA_144919	2	1119192	0,0000000227
	SNP_IGA_144961	2	1122279	0,0000002139
	SNP_IGA_145447	2	1149743	0,0000000336
	SNP_IGA_145505	2	1157565	0,0000000227
	SNP_IGA_145514	2	1161102	0,0000002139
	SNP_IGA_145601	2	1166250	0,0000002139
	SNP_IGA_145717	2	1174968	0,0000000336
	SNP_IGA_146220	2	1190084	0,0000002139
	SNP_IGA_146377	2	1200921	0,0000000336
	SNP_IGA_146426	2	1202511	0,0000000115
	SNP_IGA_146706	2	1221424	0,0000000227
	SNP_IGA_146765	2	1223419	0,0000004605
	SNP_IGA_147219	2	1254917	0,0000000537
	SNP_IGA_147233	2	1255423	0,0000000537
	SNP_IGA_147360	2	1276270	0,0000000040
	SNP_IGA_147371	2	1276757	0,0000000003
	SNP_IGA_147378	2	1277029	0,0000000003
	SNP_IGA_147687	2	1291153	0,0000000345
	SNP_IGA_147716	2	1292259	0,0000000040
	SNP_IGA_147782	2	1295620	0,0000000345
	SNP_IGA_148471	2	1346254	0,0000000003
	SNP_IGA_148492	2	1346931	0,0000000040
	SNP_IGA_148528	2	1348523	0,0000000601

(continue)

Caracteres	Marcador	Scaffold	Posición	p-value
Fecha de cosecha	SNP_IGA_148537	2	1348663	0,0000000043
	SNP_IGA_148598	2	1351253	0,0000000074
	SNP_IGA_148760	2	1363655	0,0000000296
	SNP_IGA_148770	2	1364391	0,0000000774
	SNP_IGA_148777	2	1364858	0,0000000296
	SNP_IGA_149868	2	1459484	0,0000000774
	SNP_IGA_150539	2	1519059	0,0000000013
	SNP_IGA_150673	2	1531581	0,0000000912
	SNP_IGA_150678	2	1531768	0,0000000077
	SNP_IGA_150857	2	1552639	0,0000000774
	SNP_IGA_150874	2	1553038	0,0000000774
	SNP_IGA_151067	2	1564832	0,0000003349
	SNP_IGA_151613	2	1643232	0,0000003349
	SNP_IGA_152082	2	1699475	0,0000009262
	SNP_IGA_152111	2	1702004	0,0000003349
	SNP_IGA_152301	2	1715209	0,0000003349
	SNP_IGA_152320	2	1716750	0,0000003236
	SNP_IGA_152439	2	1725935	0,0000000017
	SNP_IGA_152976	2	1761256	0,0000000015
	SNP_IGA_153388	2	1781488	0,0000000015
	SNP_IGA_153443	2	1784370	0,0000000001
	SNP_IGA_153556	2	1796649	0,0000000032
	SNP_IGA_153672	2	1802827	0,0000004393
	SNP_IGA_153686	2	1804532	0,0000000032
	SNP_IGA_153785	2	1814837	0,0000004393
	SNP_IGA_154038	2	1827831	0,0000004393
	SNP_IGA_154259	2	1850663	0,0000000024
	SNP_IGA_154354	2	1887418	0,0000000032
	SNP_IGA_154368	2	1887989	0,0000000028
	SNP_IGA_154383	2	1888630	0,0000000005
	SNP_IGA_154391	2	1888715	0,0000000003
	SNP_IGA_155412	2	1929182	0,0000000037
	SNP_IGA_155433	2	1929742	0,0000000037
	SNP_IGA_155673	2	1947776	0,0000000015
	SNP_IGA_155680	2	1949477	0,0000000037
	SNP_IGA_156002	2	1972009	0,0000000032
	SNP_IGA_156313	2	2008443	0,0000000004
	SNP_IGA_156742	2	2037199	0,0000000121
	SNP_IGA_156774	2	2043178	0,0000000032
	SNP_IGA_157433	2	2094415	0,0000000001
	SNP_IGA_157529	2	2114745	0,0000021898
	SNP_IGA_157556	2	2119637	0,0000000015
	SNP_IGA_157644	2	2134115	0,0000000037
	SNP_IGA_157737	2	2145946	0,0000000015
	SNP_IGA_157889	2	2167137	0,0000000638
	SNP_IGA_158011	2	2186582	0,0000000517
	SNP_IGA_158017	2	2186975	0,0000000638
	SNP_IGA_158157	2	2204335	0,0000024599
	SNP_IGA_158810	2	2237722	0,0000004660
	SNP_IGA_158884	2	2239970	0,0000000226
	SNP_IGA_158919	2	2241727	0,0000004660
	SNP_IGA_159649	2	2307132	0,0000003725
	SNP_IGA_159881	2	2326322	0,0000022712

(continue)

Caracteres	Marcador	Scaffold	Posición	p-value
Fecha de cosecha	SNP_IGA_161939	2	2478864	0,0000012589
	SNP_IGA_163292	2	2570445	0,0000010416
	SNP_IGA_163588	2	2601927	0,0000010416
	SNP_IGA_163716	2	2607969	0,0000001724
	SNP_IGA_163725	2	2608462	0,0000002265
	SNP_IGA_164374	2	2673561	0,0000000483
	SNP_IGA_164863	2	2722646	0,0000006335
	SNP_IGA_164886	2	2727676	0,0000004773
	SNP_IGA_171044	2	3134298	0,0000007101
	SNP_IGA_171092	2	3136025	0,0000007101
	SNP_IGA_171112	2	3137552	0,0000007101
	SNP_IGA_171141	2	3139384	0,0000007101
	SNP_IGA_173418	2	3241279	0,0000007361
	SNP_IGA_174917	2	3310222	0,0000007361
	SNP_IGA_228997	2	9066783	0,0000000097
	SNP_IGA_234881	2	9868018	0,0000000040
	SNP_IGA_236039	2	10125154	0,0000000712
	SNP_IGA_236207	2	10134167	0,0000000344
	SNP_IGA_236409	2	10144711	0,0000000712
	SNP_IGA_236414	2	10145001	0,0000000712
	SNP_IGA_236666	2	10160089	0,0000000712
	SNP_IGA_287687	2	25227906	0,0000004773
	SNP_IGA_287700	2	25228844	0,0000000637
	SNP_IGA_303724	3	4002228	0,0000002418
	SNP_IGA_309349	3	5659770	0,0000020747
	SNP_IGA_363719	3	19759990	0,0000017344
	SNP_IGA_403353	4	8996802	0,0000008379
	SNP_IGA_403613	4	9041129	0,0000003850
	SNP_IGA_442063	4	18548028	0,0000000025
	SNP_IGA_442235	4	18585658	0,0000000025
	SNP_IGA_442267	4	18590365	0,0000000025
	SNP_IGA_442526	4	18655371	0,0000000384
	SNP_IGA_443304	4	18742047	0,0000000254
	SNP_IGA_443441	4	18770127	0,0000002878
	SNP_IGA_444204	4	18861832	0,0000000254
	SNP_IGA_444291	4	18881545	0,0000000254
	SNP_IGA_446390	4	19397033	0,0000019947
	SNP_IGA_448998	4	19896554	0,0000000008
	SNP_IGA_449007	4	19897176	0,0000000008
	SNP_IGA_449112	4	19905501	0,0000000000
	SNP_IGA_450711	4	20165259	0,0000000008
	SNP_IGA_543247	5	276220	0,0000005675
	SNP_IGA_543786	5	467067	0,0000007578
	SNP_IGA_543942	5	481014	0,0000007578
	SNP_IGA_544157	5	521864	0,0000007578
	SNP_IGA_566064	5	4919872	0,0000017344
	SNP_IGA_566086	5	4921618	0,0000017344
	SNP_IGA_566097	5	4922376	0,0000017344
	SNP_IGA_567030	5	4971864	0,0000017344
	SNP_IGA_567045	5	4972718	0,0000017344
	SNP_IGA_569241	5	5175645	0,0000017344
	SNP_IGA_569485	5	5202012	0,0000017344
	SNP_IGA_569773	5	5232302	0,0000017344

(continue)

Caracteres	Marcador	Scaffold	Posición	p-value
Fecha de cosecha	SNP_IGA_600691	5	14995466	0,0000000497
	SNP_IGA_619807	6	4759496	0,0000000040
	SNP_IGA_630302	6	8238299	0,0000019075
	SNP_IGA_636280	6	10477001	0,0000020777
	SNP_IGA_700469	6	28045174	0,0000005271
	SNP_IGA_746619	7	7470226	0,0000015070
	SNP_IGA_746629	7	7471270	0,0000015070
	SNP_IGA_748434	7	7699845	0,0000001297
	SNP_IGA_749366	7	7830289	0,0000001297
	SNP_IGA_776214	7	14886166	0,0000001375
	SNP_IGA_776348	7	14946644	0,0000001927
	SNP_IGA_779276	7	16145401	0,0000010686
	SNP_IGA_783950	7	18388591	0,0000000924
	SNP_IGA_792898	7	22673209	0,0000000005
	SNP_IGA_797680	8	1271540	0,0000020747
	SNP_IGA_806528	8	2986511	0,0000000009
	SNP_IGA_806534	8	2986700	0,0000000040
	SNP_IGA_806539	8	2986891	0,0000000009
	SNP_IGA_806544	8	2987023	0,0000000040
	SNP_IGA_806557	8	2987480	0,0000000040
	SNP_IGA_806575	8	2987760	0,0000000040
	SNP_IGA_806585	8	2988121	0,0000000009
	SNP_IGA_806590	8	2988272	0,0000000040
	SNP_IGA_864149	8	13756987	0,0000011382
	SNP_IGA_878210	8	17931190	0,0000000420
	SNP_IGA_878717	8	18085149	0,0000000002
	SNP_IGA_878831	8	18117446	0,0000019385
	SNP_IGA_878981	8	18179927	0,0000000009
	SNP_IGA_879061	8	18219533	0,0000000009
	SNP_IGA_879131	8	18245683	0,0000000009
	SNP_IGA_879224	8	18309578	0,0000000702
Índice de madurez	SNP_IGA_784373	7	18510773	0,0000009434
	SNP_IGA_784792	7	18648990	0,0000008821
	SNP_IGA_784825	7	18680865	0,0000008821
	SNP_IGA_785228	7	18757412	0,0000009434
	SNP_IGA_785447	7	18842085	0,0000008821
	SNP_IGA_786333	7	19334370	0,0000017596
	SNP_IGA_786464	7	19405833	0,0000017596
	SNP_IGA_786805	7	19508227	0,0000017596
	SNP_IGA_786882	7	19522278	0,0000017596
	SNP_IGA_786935	7	19542449	0,0000017596
Flavonoides	SNP_IGA_82861	1	23722082	0,0000024454
	SNP_IGA_93589	1	27651740	0,0000001972
	SNP_IGA_93646	1	27677094	0,0000012306
	SNP_IGA_93768	1	27715921	0,0000012306
	SNP_IGA_94024	1	27815137	0,0000007409
	SNP_IGA_94057	1	27821450	0,0000007409
	SNP_IGA_95319	1	28342277	0,0000020014
	SNP_IGA_96046	1	28534000	0,0000020014
	SNP_IGA_96144	1	28547153	0,0000025583
	SNP_IGA_96544	1	28663689	0,0000025583
	SNP_IGA_96555	1	28677236	0,0000025583

(continue)

Caracteres	Marcador	Scaffold	Posición	p-value
Flavonoides	SNP_IGA_110227	1	35577807	0,0000004035
	SNP_IGA_110232	1	35578084	0,0000004035
	SNP_IGA_110413	1	35617714	0,0000001371
	SNP_IGA_111017	1	35798416	0,0000003610
	SNP_IGA_111259	1	36067680	0,0000005569
	SNP_IGA_111329	1	36098186	0,0000005569
	SNP_IGA_112690	1	36758815	0,0000001680
	SNP_IGA_628833	6	7901344	0,0000000213
	SNP_IGA_629027	6	7914628	0,0000000213
	SNP_IGA_629062	6	7918349	0,0000000213
	SNP_IGA_629558	6	8000787	0,0000000221
	SNP_IGA_630243	6	8227954	0,0000000784
	SNP_IGA_630266	6	8232664	0,0000003114
	SNP_IGA_630302	6	8238299	0,0000002706
	SNP_IGA_630550	6	8337946	0,0000000735
	SNP_IGA_630662	6	8369151	0,0000000735
	SNP_IGA_636280	6	10477001	0,0000018901
	SNP_IGA_637857	6	10733784	0,0000017553
	SNP_IGA_637861	6	10734009	0,0000017553
	SNP_IGA_638036	6	10823366	0,0000017814
	SNP_IGA_638783	6	11004159	0,0000017814
	SNP_IGA_638859	6	11016846	0,0000017814
Antocianinas	SNP_IGA_53531	1	15750283	0,0000000002
	snp_1_15750387	1	15750387	0,0000000002
	SNP_IGA_54637	1	15844751	0,0000000002
	SNP_IGA_96167	1	28550473	0,0000000002
	SNP_IGA_181444	2	3800271	0,0000000002
	SNP_IGA_392956	4	5689470	0,0000000002
	SNP_IGA_393060	4	5694032	0,0000000002
	SNP_IGA_393507	4	5726360	0,0000000002
	SNP_IGA_395202	4	6168570	0,0000000002
Capacidad antioxidante (RAC)	SNP_IGA_48586	1	15234386	0,0000004932
	SNP_IGA_63825	1	18621833	0,0000007245
	SNP_IGA_63833	1	18622159	0,0000007245
	SNP_IGA_63847	1	18623934	0,0000007245
	SNP_IGA_64918	1	18762086	0,0000007245
	SNP_IGA_65002	1	18809420	0,0000007245
	SNP_IGA_65816	1	19085285	0,0000017499
	SNP_IGA_110413	1	35617714	0,0000011894
	SNP_IGA_112690	1	36758815	0,0000007554
	SNP_IGA_303724	3	4002228	0,0000007245
Sorbitol	SNP_IGA_152976	2	1761256	0,0000006341
	SNP_IGA_153388	2	1781488	0,0000006341
	SNP_IGA_153443	2	1784370	0,0000006341
	SNP_IGA_153556	2	1796649	0,0000001038
	SNP_IGA_153686	2	1804532	0,0000001038
	SNP_IGA_154354	2	1887418	0,0000001038
	SNP_IGA_154391	2	1888715	0,0000008157
	SNP_IGA_155673	2	1947776	0,0000006341
	SNP_IGA_156002	2	1972009	0,0000001038
	SNP_IGA_156313	2	2008443	0,0000001038
	SNP_IGA_156774	2	2043178	0,0000001038

(continue)

Caracteres	Marcador	Scaffold	Posición	p-value
Sorbitol	SNP_IGA_157529	2	2114745	0,0000020355
	SNP_IGA_157556	2	2119637	0,0000006341
	SNP_IGA_157737	2	2145946	0,0000006341
	SNP_IGA_158884	2	2239970	0,0000015331
	SNP_IGA_164886	2	2727676	0,0000023958
	SNP_IGA_287687	2	25227906	0,0000023958
	SNP_IGA_287700	2	25228844	0,0000023958
	SNP_IGA_442063	4	18548028	0,0000000966
	SNP_IGA_442235	4	18585658	0,0000000966
	SNP_IGA_442267	4	18590365	0,0000000966
	SNP_IGA_443304	4	18742047	0,0000001460
	SNP_IGA_444204	4	18861832	0,0000001460
	SNP_IGA_444291	4	18881545	0,0000001460
	SNP_IGA_448998	4	19896554	0,0000003412
	SNP_IGA_449007	4	19897176	0,0000003412
	SNP_IGA_449112	4	19905501	0,0000000126
	SNP_IGA_450711	4	20165259	0,0000003412
	SNP_IGA_700469	6	28045174	0,0000009341
	SNP_IGA_878717	8	18085149	0,0000000297
	SNP_IGA_878981	8	18179927	0,0000002387
	SNP_IGA_879061	8	18219533	0,0000002387
	SNP_IGA_879131	8	18245683	0,0000002387
	SNP_IGA_879224	8	18309578	0,0000007114
Azúcares totales	SNP_IGA_442063	4	18548028	0,0000009786
	SNP_IGA_442235	4	18585658	0,0000009786
	SNP_IGA_442267	4	18590365	0,0000009786
	SNP_IGA_443304	4	18742047	0,0000017609
	SNP_IGA_444204	4	18861832	0,0000017609
	SNP_IGA_444291	4	18881545	0,0000017609
	SNP_IGA_449112	4	19905501	0,0000007096
	SNP_IGA_636024	6	10460202	0,0000002198
	SNP_IGA_636280	6	10477001	0,0000000365
	SNP_IGA_636292	6	10477315	0,0000002198
	SNP_IGA_637345	6	10604025	0,0000002198
	SNP_IGA_637355	6	10606410	0,0000002198
	SNP_IGA_870629	8	15787171	0,0000023052
	SNP_IGA_879224	8	18309578	0,0000015839

11.4. Artículos publicados, aceptados o enviados en revistas internacionales (SCI) durante el transcurso de esta tesis doctoral

- **Font i Forcada C, Oraguzie N, Igartua E, Moreno MA, Gogorcena Y** (2012a) Population structure and marker-trait associations for pomological traits in peach and nectarine cultivars. *Tree Genet Genomes* (DOI: 10.1007/s11295-012-0553-0) **(Capítulo 4)**.
- **Font i Forcada C, Gogorcena Y, Moreno MA** (2012b) Agronomical and fruit quality traits of two peach cultivars on peach-almond hybrid rootstocks growing on Mediterranean conditions. *Sci Hortic* 140:157-163 **(Capítulo 6)**.
- **Font i Forcada C, Gradziel TM, Gogorcena Y, Moreno MA**. Phenotypic diversity of local Spanish and modern peach and nectarine [*Prunus persica* (L.) Batsch] cultivars **(en fase final de revisión, Capítulo 3)**.
- **Font i Forcada C, Gogorcena Y, Moreno MA**. Fruit sugar and phytochemical constituents of peach and nectarine cultivars on almond x peach hybrid rootstocks **(en fase final de revisión, Capítulo 7)**.
- **Font i Forcada C, Gogorcena Y, Moreno MA**. Agronomical parameters, sugar profile and antioxidant compounds of Catherine peach cultivar influenced by different plum rootstocks **(en fase final de revisión, Capítulo 8)**.

11.5. Otros artículos SCI publicados en revistas internacionales:

- **Font i Forcada C, Kodad O, Juan T, Estopañán G, Socias i Company R** (2011) Genetic variability and pollen effect on the transmission of the chemical components of the almond kernel. *SJAR* 9(3):781-789.
- **Font i Forcada C*, Fernández i Martí A*, Socias i Company R** (2012) Mapping quantitative trait loci for kernel composition in almond. *BMC Genetics* 13:47 (DOI:10.1186/1471-2156-13-47) (*both authors contributed equally).
- **Fernández i Martí A*, Font i Forcada C*, Socias i Company R** (2012) Genetic analysis for physical nut traits in almond. *Tree Genet Genomes* (DOI 10.1007/s11295-012-0566-8) (*both authors contributed equally).
- **Fernández i Martí A, Athanson B, Koepke T, Font i Forcada C, Dhingra A, Oraguzie N** (2012) Genetic diversity and relatedness of sweet cherry (*Prunus avium* L.) cultivars based on single nucleotide polymorphic (SNP) markers. *Frontiers Plant Sci* 3:116 (DOI:10.3389/fpls.2012.00116).