ESTUDIO AGRONÓMICO Y DE LA CALIDAD DEL FRUTO DEL MELOCOTONERO [*P. persica* (L.) Batsch] EN DIFERENTES POBLACIONES DE MEJORA PARA LA SELECCIÓN DE NUEVOS CULTIVARES

TESIS DOCTORAL

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Estudio agronómico y de la calidad del fruto del melocotonero [*P. persica* (L.) Batsch] en diferentes poblaciones de mejora para la selección de nuevos cultivares

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ESTUDIO AGRONÓMICO Y DE LA CALIDAD DEL FRUTO DEL MELOCOTONERO [*P. persica* (L.) Batsch] EN DIFERENTES POBLACIONES DE MEJORA PARA LA SELECCIÓN DE NUEVOS CULTIVARES

Memoria presentada por **Celia M. Cantín Mardones**,
Licenciada en Biología, para optar al grado de Doctor
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CERTIFICAN

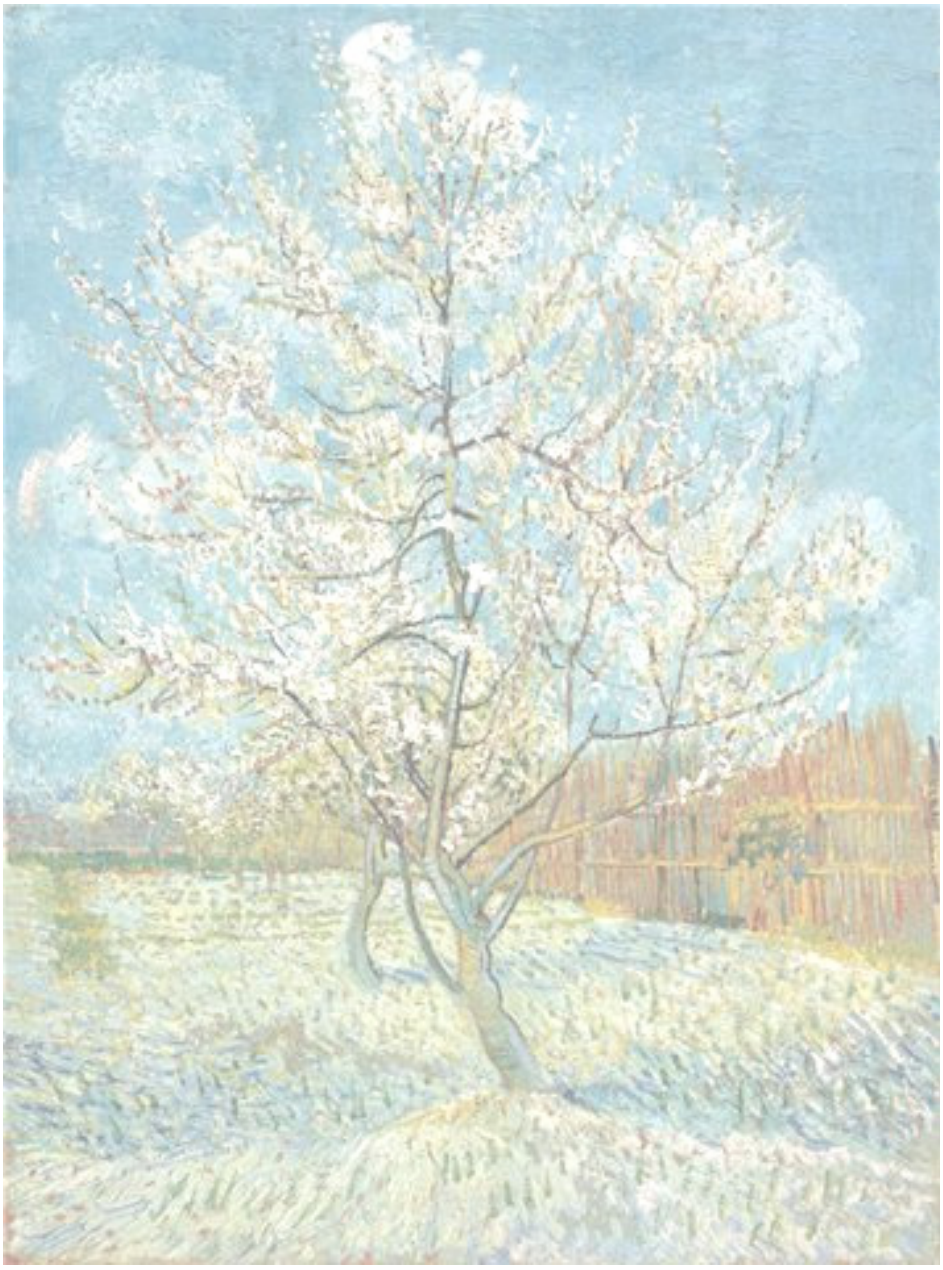
Que la Tesis Doctoral titulada "**Estudio agronómico y de la calidad del fruto del melocotonero [*P. persica* (L.) Batsch] en diferentes poblaciones de mejora para la selección de nuevos cultivares**", ha sido realizada por la Licenciada en Biología Dña. CELIA Mª CANTÍN MARDONES, en el Departamento de 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 en Ciencias.

Zaragoza, Mayo de 2009

Fdo. Mª Ángeles Moreno Sánchez

Fdo. Yolanda Gogorcena Aoiz

A mis padres, por mostrarme el camino y
tenderme su ayuda para caminar
A Jesús R., por ser mi luz de guía
a lo largo del recorrido



Melocotonero en flor **(1988) Vincent Van Gogh**

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La leyenda de Momotarô *(Folklore japonés)*

Hace mucho tiempo, en una aldea lejana de Japón, vivían un anciano y una anciana, que nunca habían podido tener hijos y por eso se sentían muy solos y tristes.

Un día, mientras el anciano recogía leña en la montaña, la anciana fue al río a lavar la ropa. Al cabo de un rato observó que por el río descendía un extraño objeto que le llamó la atención. Sorprendida, comprobó que era un melocotón gigante, el más grande que había visto nunca. Lo sacó del agua y lo llevó a su casa, pensando dárselo a su marido para cenar.

Al llegar a casa, la mujer le dijo al anciano: "Mira que melocotón tan hermoso he traído para tu cena". Y el anciano respondió: "Córtalo por la mitad, y nos lo comeremos entre los dos". La mujer trajo un gran cuchillo, y se disponía a partir el melocotón en dos mitades, cuando de repente, se oyó una voz desde su interior. "¡Esperad, por favor! ¡No me cortéis!" Ante el asombro de los dos ancianos, el melocotón comenzó a abrirse lentamente por sí solo y de su interior salió un niño. "No os asustéis", les dijo el niño. "El Dios de los cielos vio lo solos que estabais, y ha decidido enviarme para ser a partir de ahora vuestro hijo".

Ilustración: Mikele

El anciano y la anciana, con gran alegría, adoptaron al niño, y como había nacido de un melocotón, decidieron llamarle Momotarô (el niño melocotón).

Se sintieron muy complacidos y felices de poder criar por fin al niño que siempre habían deseado tener, y le educaron para que llegase a ser un buen muchacho. Pasó el tiempo, y un día, cuando Momotarô cumplió los 19 años, se acercó a sus padres adoptivos y les habló de la siguiente manera: "Padre, madre, habéis sido muy amables conmigo y me habéis cuidado muy bien. Ahora que me he hecho mayor, debo agradecéroslo de alguna forma. A lo lejos, en algún lugar del océano, se encuentra la Isla de los Ogros. Allí viven muchos ogros malvados que a menudo vienen a las aldeas de los alrededores para robar a la gente. He decidido ir a esa isla y acabar con los ogros. Por favor, padre, dame tu permiso".

Momotarô se convirtió en uno de los más nobles y semi-históricos héroes del Japón, luchó con el diablo oni y afrontó muchas aventuras.

Convénzame de que usted
tiene una semilla…
y estoy preparado para
esperar maravillas

H. D. Thoreau, 1860

RESUMEN

El melocotonero [*Prunus persica* (L.) Bastch] es uno de los frutales más tecnificado y difundido en todo el mundo y es la tercera especie frutal más producida a escala mundial. España es el tercer productor mundial y el segundo a nivel Europeo, con una producción de más de un millón de toneladas. Sin embargo, las variedades comercializadas proceden en su mayoría de los programas de mejora y selección de Estados Unidos, Italia y Francia, lo que provoca una excesiva dependencia de las obtenciones extranjeras en el mercado español. Por otra parte, los problemas actuales de muchos cultivares en la calidad de la fruta, junto con la consecuente pérdida de competitividad a nivel internacional, potencian el desarrollo de programas nacionales de mejora y selección para la búsqueda de nuevos cultivares, con buena calidad de fruto, y adaptados a las condiciones de cultivo mediterráneas. Este es el caso del programa que se desarrolla en la Estación Experimental de Aula Dei (CSIC) en el que se enmarca esta Tesis Doctoral.

El objetivo general de este estudio fue la selección de nuevos genotipos de melocotón y nectarina para el sector frutícola español, adaptados a las condiciones mediterráneas del Valle Medio del Ebro. Los objetivos específicos de mejora de este programa incluyen la extensión del calendario de cosecha, la obtención de nuevos tipos de fruto y la mejora de la calidad de los mismos (forma, color de la piel y la pulpa, firmeza, sabor, contenido en azúcares y compuestos bioactivos, etc.). La mayoría de los cruzamientos realizados se enfocaron a la obtención de genotipos con frutos de tipo melocotón de pulpa amarilla, con buen tamaño, coloración, firmeza y sabor. Otros cruzamientos fueron encaminados a la obtención de genotipos con fruto tipo melocotón de pulpa blanca, nectarina y paraguayo.

Por todo ello, en este trabajo se evaluó el comportamiento agronómico y la calidad organoléptica, nutricional y poscosecha del fruto de 1111 genotipos, procedentes de quince poblaciones de mejora de melocotonero, durante tres años consecutivos. Las evaluaciones llevadas a cabo mostraron una amplia variabilidad fenotípica para todos los parámetros evaluados, en particular, para las fechas de floración y cosecha, producción, tamaño y forma del fruto y contenido en sólidos solubles. Igualmente, se encontraron correlaciones significativas entre los parámetros agronómicos y de calidad del fruto, cuyos coeficientes variaron según de la población de mejora estudiada. El análisis de componentes principales permitió la simplificación de las variables estudiadas y la agrupación de las distintas progenies de acuerdo a sus características agronómicas y de calidad de fruto. Este estudio facilitó la selección de aquellos genotipos con la combinación de atributos de calidad de fruto más interesante.

Hasta el momento, la calidad organoléptica y nutricional ha recibido un papel secundario en los programas de mejora. Sin embargo, hoy en día, la calidad del fruto es un objetivo prioritario en la mejora genética de frutales, ya que condiciona la aceptación por parte del consumidor, y por tanto, el éxito de los nuevos cultivares. Entre los atributos de la calidad organoléptica y nutricional del fruto, se analizó el perfil de azúcares de 205 genotipos de la población de mejora. Los resultados mostraron un efecto significativo del cruzamiento y de los caracteres cualitativos (tipo de fruto, color de la pulpa y adherencia del hueso a la pulpa) sobre el perfil de azúcares de melocotones y nectarinas. Los frutos tipo nectarina de pulpa blanca mostraron el mayor nivel de azúcares entre los tipos de frutos evaluados. También se observó una variación en los niveles de azúcares individuales de los frutos (sacarosa, glucosa, fructosa y sorbitol) a lo largo de diferentes años de estudio, aunque su perfil se mantuvo estable. Los atributos relacionados con el perfil de azúcares se correlacionaron significativamente entre sí y con otros parámetros de la calidad del fruto como la fecha de cosecha, la producción, el peso del fruto o la acidez. Se observó una variación de los coeficientes de estas correlaciones dependiendo del cruzamiento evaluado.

También se analizó el contenido de compuestos bioactivos (fenoles totales, flavonoides, antocianinas, vitamina C) y la capacidad antioxidante del fruto en 218 genotipos de la población de mejora, con el objetivo de obtener cultivares con frutos enriquecidos en compuestos beneficiosos para la salud. Los flavonoides y los compuestos fenólicos totales resultaron ser los principales compuestos antioxidantes en melocotones y nectarinas. Se observó una gran variabilidad en el

perfil antioxidante entre los genotipos evaluados, demostrando una alta contribución del cruzamiento a cada uno de los parámetros nutricionales analizados. El perfil nutricional varió también con los caracteres cualitativos (tipo de fruto y color de la pulpa). Los frutos tipo melocotón de pulpa blanca mostraron un mayor poder antioxidante que el resto de tipos de frutos analizados. Además, no se observó un efecto significativo del año en el perfil fitoquímico de la fruta.

Por último, se evalúo la calidad poscosecha de la progenie de una de las poblaciones de mejora ('Venus' x 'Big Top'), en cuanto a la susceptibilidad a los daños por frío y los mecanismos genéticos que controlan este problema poscosecha. Los resultados demostraron la fuerte influencia que el genotipo ejerce en dicha susceptibilidad y la importancia que tiene la duración del período de almacenamiento a bajas temperaturas (15 ó 30 días) en el desarrollo y la severidad de este daño. Sin embargo, no se observaron diferencias significativas entre los distintos años de estudio. Por otra parte, para abordar el estudio del control genético de este desorden fisiológico, se construyó un mapa parcial del grupo de ligamiento 4 con un total de 38 SSRs y genes candidatos. Se localizaron distintos QTLs implicados en el control de los síntomas más importantes de este desorden fisiológico (harinosidad visual y sensorial, y enrojecimiento de la pulpa).

La amplia variabilidad genética, junto con el elevado número de genotipos evaluados en esta tesis, constituye una herramienta muy útil para futuros programas de mejora y selección de cultivares de melocotón y nectarina. Los resultados de este trabajo contribuirán de manera significativa a la comprensión de los mecanismos genéticos que controlan la calidad de la fruta de esta especie. Finalmente, este trabajo ha permitido la preselección de 26 genotipos con la mejor combinación de atributos agronómicos y de calidad del fruto en las condiciones de cultivo del Valle Medio del Ebro.

SUMMARY

Peach [*Prunus persica* (L.) Bastch] is one of most dynamic and widespread fruit crop over the world and the third most produced fruit worldwide. Spain is the second producer in Europe, and the third in the world, with a production of more than one million tons. However, the new released cultivars, mainly coming from breeding programs of EEUU, Italy and France, have produced an excessive and not desirable dependency on the foreign countries. Additionally, the inconsistent or poor fruit quality of some cultivars, together with the subsequent lost of competitiveness into the international plane, boost to the development of national breeding and selection programs for the selection of cultivars with higher fruit quality and adapted to the Mediterranean area. This is the case of the breeding program developed in the Estación Experimental de Aula Dei (CSIC) in which this thesis has been included.

The final objective of this study was the selection of outstanding peach and nectarine genotypes for the Spanish industry, with good adaptation to Mediterranean conditions when grown in the Ebro Valley. The specific breeding goals of this program were the extension of the harvest period, the release of new fruit types and the improvement of the fruit quality (shape, skin and flesh color, firmness, flavor, sugars and bioactive compounds content, etc.). Most crosses were aimed at developing improved yellow peaches with emphasis on red skin color, good size and firmness, and enhanced flavor. A few crosses were focused to the development of improved white fleshed peaches, nectarines, and flat shape fruits.

Because of that, the agronomic performance and organoleptic, nutritional and postharvest fruit quality of 1111 genotypes, coming from fifteen breeding populations, were evaluated over three consecutive years. These evaluations showed a great phenotypic variance for all the studied traits, such as the flowering and harvesting dates, yield, fruit weight, and soluble solids content. On the other hand, significant correlations were found between the agronomic and quality traits, whose coefficients varied depending on the evaluated progeny. The principal component analysis allowed the simplification of the studied variables and the grouping of the different progenies according to the agronomic and fruit quality traits. This study assisted the selection of outstanding genotypes with the most interesting fruit quality attributes.

The organoleptic and nutritional quality have been secondary objectives in the former fruit breeding strategies. Nevertheless, these quality attributes have become interesting targets of breeding programs in recent years, since it determines the acceptance of peach and nectarine cultivars by consumers, and therefore, the exit of new released cultivars in the market. Among the organoleptic and nutritional fruit quality attributes, the sugar profile of 205 genotypes from the breeding population was analyzed. The results showed a significant effect of cross and qualitative traits (fruit type, flesh color, and stone adherence) on the peach and nectarine sugar profile. White fleshed nectarines showed the highest sugar level among the evaluated fruit types. A variation in the level of individual sugar levels (sucrose, glucose, fructose, and sorbitol) was also observed, although the sugar profile remained stable throughout the time. All sugar traits were significantly correlated between them and with other fruit quality traits such as harvesting date, yield, fruit weight or acidity. A variation of the coefficients was observed depending on the evaluated cross.

The content of bioactive compounds (total phenolics, flavonoids, anthocyanins, and vitamin C) and the antioxidant capacity of the fruit have been also analyzed in 218 genotypes from the breeding program in order to obtain functional genotypes with enhanced health properties fruit. The flavonoids and total phenolic compounds were the main antioxidant compounds in peaches and nectarines. A great variability in the antioxidant profile among the evaluated genotypes was also found, showing a great contribution of cross to every nutritional trait. The nutritional profile varied also with qualitative traits (fruit type and flesh color). White fleshed peaches showed higher antioxidant capacity than the other evaluated fruit types. In addition, no significant effect of year on the fruit phytochemical profile was observed.

Finally, the postharvest fruit quality of one of the breeding progenies (‘Venus’ x ‘Big Top’) has also been studied, attending to the susceptibility to chilling injury and the genetic control of this

postharvest problem. These studies showed a high influence of genotype on the chilling injury susceptibility and the importance of the storage period (15 or 30 days) at low temperatures on the development and severity of this disorder affecting postharvest quality of peaches and nectarines. However, no significant differences among different years were observed. To deal with the genetic control of this postharvest problem, a partial map of the linkage group 4 was constructed with 38 SSRs and candidate genes. Several QTLs involved in the genetic control of the most important symptoms of this physiological disorder (mealiness, graininess, and bleeding) were localized in this linkage group.

The large genetic variability, together with the high number of evaluated genotypes on this thesis, will constitute a helpful tool for future peach and nectarine breeding and selection strategies, and it will contribute to improve the knowledge of the genetic studies on this crop. Finally, the results obtained in this work allowed the pre-selection of 26 outstanding genotypes with the best combination of agronomic and fruit quality attributes when growing in the Ebro Valley conditions.

Índice General

Abreviaturas

AFLP	"amplified fragment length polimorphism"
ANOVA	análisis de varianza
AsA	ácido ascórbico
BAC	"bacterial artificial chromosome"
BI	"bleeding"
C3GE	equivalentes de cianidin-3-glucósido
CD	diametro polar
CE	equivalentes de catequina
CG	genes candidatos
CI	daños por frío
cM	centiMorgan
CMF	"clingstone melting flesh"
CNMF	"clingstone non melting flesh"
DF	grados de libertad
DHAA	ácido dehidroascórbico
DNA	ácido desoxirribonucléico
dNTP	desoxiribonucleótidos
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDTA	ácido etilendiaminotetraacético
EST	"expressed sequence tag"
FMF	"freestone melting flesh"
Fw	"fruit weight"
GAE	equivalentes de ácido gálico
GC	cromatografía de gases
Gr	"graininess"
H	altura
ha	hectárea
HPLC	cromatografía líquida de alta resolución
LD	desequilibrio de ligamiento
LDOX	"leucoanthocyanidin dioxygenase"
LG	grupo de ligamiento
LOD	"logarithm of the odds"
MAS	selección asistida por marcadores
Mea	"mealiness"
MS	suma de cuadrados
MSE	error estándar de la media
N	Newton
NIRS	"near infrared reflectance spectroscopy"
NS	no significativo
PAL	fenilalanin-amonia-liasa
PC	componente principal
PCA	análisis de componentes principales
PCR	reacción en cadena de la polimerasa
PF	peso fresco
PG	poligalacturonasa
PME	pectin metilesterasa

QTL	"quantitative trait loci"
RAC	capacidad antioxidante relativa
RFLP	"restriction fragment length polimorphism"
RH	humedad relativa
RI	índice de madurez
ROS	especies reactivas de oxígeno
SD	diámetro de sutura
SD	desviación estándar
SDI	"suture deformation index"
SRAP	"sequence-related amplified polymorhism"
SSC	contenido de sólidos solubles
SSR	"single sequence repeat"
TA	acidez valorable
TCA	tricloroacético

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Tienes, baturrica mía,
algo de melocotón:
de terciopelo la piel
y un hueso por corazón

Lasierra Rigal, 1983

Capítulo 1

Introducción general

Introducción general

1.1. ORIGEN Y EXPANSIÓN DEL MELOCOTONERO

Algunos autores de la antigüedad de los que existen referencias escritas sobre el melocotonero, como Teofrasto (s. I a.C.), Columela y Plinio (s. I d.C.) (Fig. 1.1), pensaban que el origen del melocotonero se encontraba en Persia (el actual Irán). Sin embargo, su verdadero origen parece estar en el oeste de China, donde podría cultivarse desde hace 4000 años (Scorza y Okie, 1990; Wang, 1985). Allí, la especie presenta la mayor diversidad genética (Vavilov, 1951), y dispone de las mayores colecciones de melocotonero (Wang et al., 2002). Desde China, el cultivo se expandió hacia Persia, siguiendo las rutas de comercio, y desde allí se introdujo en el mundo griego, entre los años 400 y 300 a.C. Posteriormente, en los siglos I y II d.C., su cultivo se extendió por el Imperio Romano (Hancock et al., 2008).

Desde la zona del Mediterráneo, en los siglos XVI, XVII y XVIII, durante la época de colonización, el melocotonero se extendió a América del Norte, Sudamérica, Sur de África, Australia y Nueva Zelanda (Hancock et al., 2008; Scorza y Okie, 1990).

Figura 1.1. Plantación de melocotonero en la Estación Experimental de Aula Dei, con árboles en fase de floración.

El nombre de melocotón, antiguamente llamado 'manzana persa', procede de *melo* (manzana) y *cotonum* (algodón), destacando así la particularidad aterciopelada de su piel. Este fruto es una *drupa* de gran tamaño con una epidermis delgada (piel), un mesocarpo carnoso (pulpa) y un endocarpo (hueso) que contiene la semilla. El fruto del melocotonero presenta una gran variedad de formas (redonda o plana), colores y vellosidad de la epidermis (glabra o pubescente), texturas (fundente o no fundente) y colores de la pulpa (blanca o amarilla; teñida o no de rojo alrededor del hueso),

diferencias en el hueso (forma y adherencia o no a la carne) y grado de acidez (baja, media o alta).

Se reconocen 5 especies distintas de melocotonero: *P. persica, P davidiana* (Carr.) Franch., *P. mira* Koehne, *P. kansuensis* Rehd. y *P. ferganensis* (Kost. & Rjab) Kov. & Kost (Hancock et al., 2008). Sin embargo, solo la especie *P. persica* se cultiva por su fruta, con la excepción de algún caso de uso local de *P. ferganensis* y *P. mira*. Todas estas especies son compatibles con *P. persica*, por lo que suelen utilizarse para la obtención de híbridos (Hancock et al., 2008; Scorza y Okie, 1990).

1.2. IMPORTANCIA ECONÓMICA Y DISTRIBUCIÓN GEOGRÁFICA DEL MELOCOTONERO

1.2.1. Importancia del cultivo del melocotonero a nivel mundial

El melocotonero es uno de los frutales más tecnificado y más difundido en todo el mundo y es la tercera especie frutal más producida a escala mundial, después del manzano y del peral. Las principales áreas de producción están en Asia, Europa y América, aunque su cultivo se da en los cinco continentes, entre las latitudes 30º y 45º Norte y Sur (Hancock et al., 2008; Scorza y Okie, 1990).

En los últimos 25 años, la producción de melocotonero se ha incrementado en un 230% (Fig. 1.2) pasando de 7.500 miles de t en 1980 a 17.500 miles de t en 2007. En las zonas tradicionales de cultivo, este aumento se debe fundamentalmente a la renovación de las plantaciones, al incremento de la superficie en regadío y a la mejora de las técnicas de cultivo (FAOSTAT, 2007). En otras zonas, este incremento ha sido más acusado en algunas áreas cálidas de inviernos suaves y con pocas horas de frío, como Chile, Argentina, Méjico, sur de Estados Unidos y sur de Italia, debido a la introducción de nuevos cultivares con pocos requerimientos de horas frío o *low chilling*, originarios principalmente de Estados Unidos (Byrne, 2003; Carbó y Iglesias, 2002).

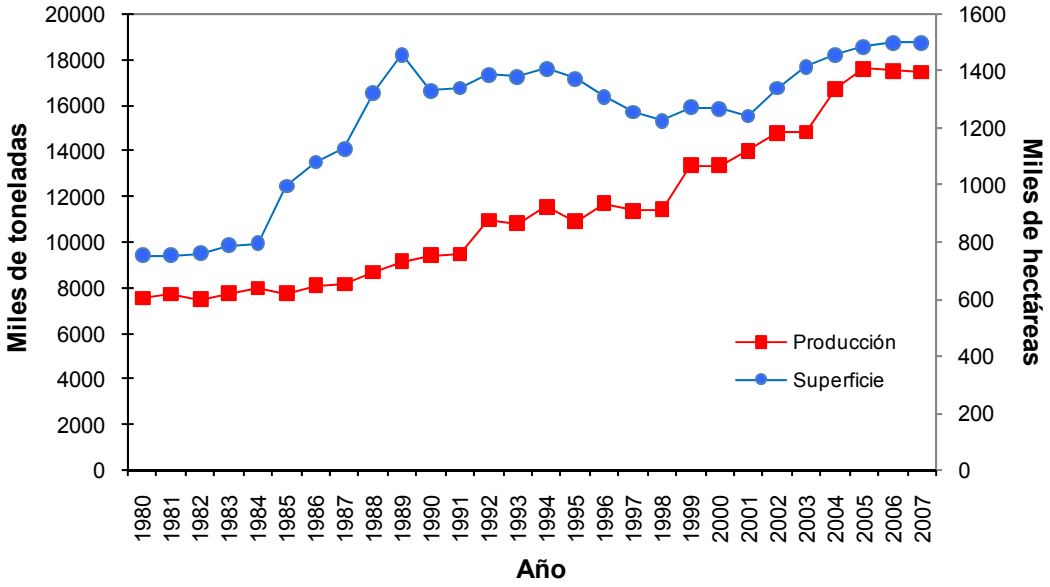

Figura 1.2. Evolución de la superficie cultivada y de la producción mundial de melocotonero en el período 1980-2007 (FAOSTAT, 2007).

En el año 2007, la producción mundial estuvo alrededor de 17.000 miles de t (FAOSTAT, 2007). De esta producción, España aportó 1.149 miles de tm, por detrás de China (8.032 miles de tm) e Italia (1.718 miles de tm), y por delante de Estados Unidos (1.009 miles de tm). Otros países con una producción significativa a nivel mundial son Grecia, Turquía y Francia.

De la producción total, solamente el 8% es objeto de intercambios comerciales. Los exportadores más importantes a nivel mundial son la Unión Europea (UE) (80% de las exportaciones mundiales), Chile (7%) y Estados Unidos (6%). Alemania es el principal importador a nivel mundial, con 270 miles de t al año, seguido por Francia con 140 miles de t al año (FAOSTAT, 2007).

1.2.2. Importancia del cultivo del melocotonero en la Unión Europea

En el año 2007 en la UE, el melocotonero ocupaba el segundo lugar en importancia después del manzano, tanto en superficie con unas 260.000 ha, como en producción, con 4.150 miles de t (FAOSTAT, 2007). La producción se concentra casi exclusivamente en los países del área mediterránea: Italia, España, Grecia y Francia, ya que el riesgo de heladas es menor que en los países del norte de la UE, donde su cultivo es menos importante y se destina al mercado interno.

La superficie y la producción de melocotonero en la UE se han mantenido estables o han disminuido ligeramente en los últimos años (Fig. 1.3). El descenso

observado en la producción del año 2003 se debió a la disminución de la producción en Italia, Grecia y Francia.

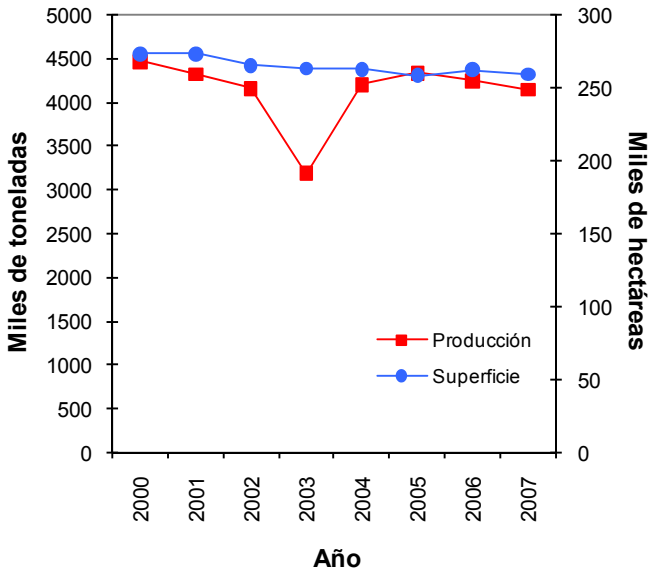

Figura 1.3. Evolución de la superficie cultivada y de la producción del melocotonero en la Unión Europea en el período 2000-2007 (FAOSTAT, 2007).

En cuanto a los intercambios extracomunitarios, las cantidades exportadas por la UE son superiores a las importadas, con oscilaciones importantes entre años. La cantidad anual media exportada (2006) fue de 1.118 miles de tm, y la importada de 982 miles de tm. En ese año, España fue el primer exportador del mundo con 545 miles de t (FAOSTAT, 2007). Los principales países destinatarios son los países de Europa Central y Oriental, seguidos de Suiza y Rusia.

1.2.3. Importancia del cultivo del melocotonero en España

España es actualmente el segundo productor de melocotón de la UE y el tercero del mundo con más de un millón de toneladas (FAOSTAT, 2007). Según datos del 2007, el melocotonero, con unas 81.000 ha, ocupa el segundo lugar en superficie total dedicada al cultivo de los frutales no cítricos, por detrás del almendro (650.000 ha) y por delante del manzano (37.500 ha) y del peral (32.000 ha) (FAOSTAT, 2007).

La superficie de cultivo del melocotonero en España aumentó en un 200% desde 1980 hasta 2005. Desde el año 1990 al 2001, la superficie de cultivo se mantuvo oscilando en torno a las 75.000 ha, aumentando sustancialmente en los últimos años (Fig. 1.4). Por otra parte, la producción aumentó en un 297% desde 1980 hasta 2007, con

descensos puntuales debidos a fuertes heladas o descensos térmicos acusados en las primaveras de algunos años (FAOSTAT, 2007).

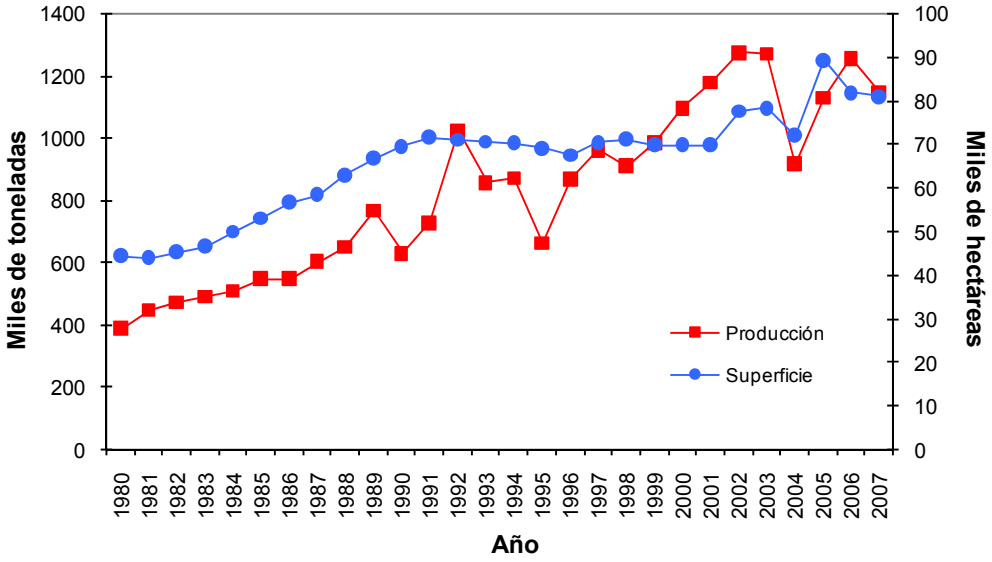

Figura 1.4. Evolución de la superficie cultivada y de la producción de melocotonero en España (FAOSTAT, 2007).

También la exportación de melocotón y nectarina en España ha aumentado progresivamente en los últimos 15 años, como puede observarse en la Fig. 1.5. Los cultivares más exportados son los más tempranos, y los principales países destinatarios son Reino Unido, Alemania y Francia (MARM, 2007). Las importaciones son de escasa relevancia.

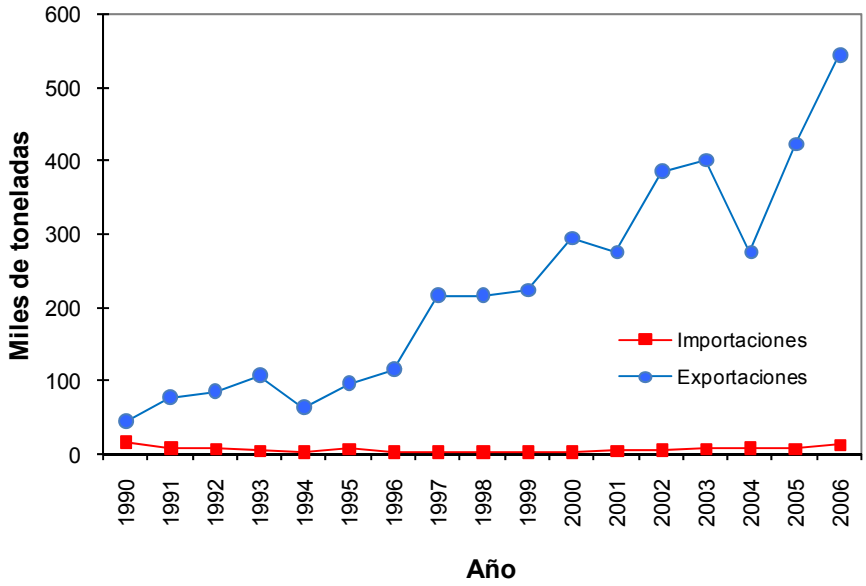

Figura 1.5. Evolución de las importaciones y de las exportaciones de melocotón y nectarina en España en el período 1990-2006 (MARM, 2007).

La producción de melocotón en España se da mayoritariamente en las regiones de clima seco y caluroso, por la menor incidencia de enfermedades y heladas de primavera. En el año 2007, Cataluña, con un 26,6% de la producción total, seguida por Aragón (23,8%) y la Región de Murcia (18,9%), fueron las Comunidades Autónomas con mayor contribución (Fig. 1.6) a la producción total española (1.149.500 tm) (MARM, 2007).

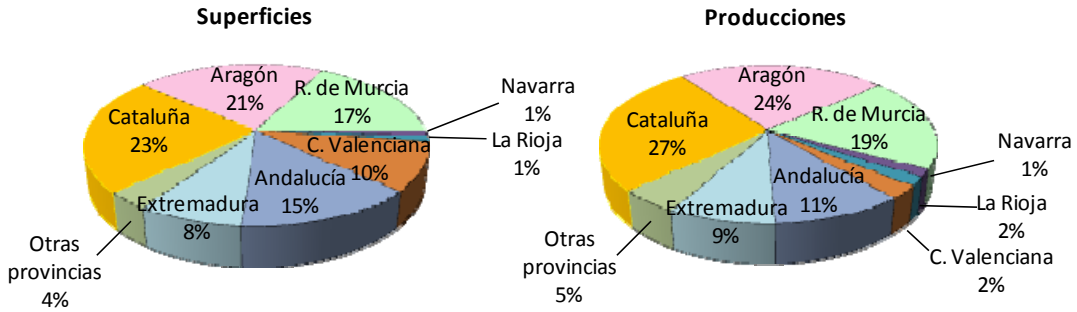

Figura 1.6. Distribución de las superficies y producciones de melocotón en 2006 por Comunidades Autónomas (MARM, 2007).

Las diferencias de las condiciones climáticas entre las diferentes regiones productoras dan lugar a importantes variaciones, especialmente en lo que respecta a disponibilidad de horas frío y época de maduración. En el Valle del Ebro (Fig. 1.7) el riesgo de heladas es más alto que en otras regiones, pero es también donde se dispone

de mayor número de horas frío (> 800 horas) que hacen posible el uso de cultivares más exigentes en reposo invernal. En las regiones situadas más al sur (Andalucía, Murcia y Valencia) la disponibilidad de horas frío es menor (200-600 horas) y los cultivares más empleados son aquellos con bajos requerimientos en horas frío (*low chilling* o *mid chilling*) y de recolección más temprana. Andalucía es la región con maduración más temprana, mientras La Rioja, Navarra y Aragón son las más tardías. Estas diferencias dan como resultado un amplio calendario de maduración en nuestro país, que comprende desde finales de abril hasta finales de octubre (Rodríguez y Cos, 1998).

Figura 1.7. Plantación de melocotonero en la Estación Experimental de Aula Dei, con árboles en fase de maduración del fruto.

En cuanto a la distribución de la producción según los tipos de fruto, la producción de melocotones o duraznos es predominante, con un 65% del total, seguido de las nectarinas, con el 33%, y los paraguayos, con el 2% (Llácer et al., 2009).

1.3. GRUPOS POMOLÓGICOS DEL MELOCOTONERO

La especie *Prunus persica* está dividida habitualmente por los botánicos en diferentes *variedades botánicas* (International Organization for Plant Information, IOPI) según sus frutos tengan piel pubescente o lisa (melocotón o nectarina), y forma globular o plana (Fig. 1.8). Tanto la nectarina como el melocotón plano o achatado, han sido originadas por mutaciones y constituyen diferentes grupos pomológicos.

Figura 1.8. Frutos de las diferentes variedades botánicas de *P. persica*. De izquierda a derecha: melocotón, nectarina y paraguayo.

La clasificación botánica según los distintos tipos de fruto no ha estado exenta de controversia. Para clarificar la situación, actualmente se acepta mayoritariamente la siguiente clasificación (Fuente: IOPI, http://www.iopi.org):

- Melocotón: *Prunus persica* (L.) Batsch var. *persica*. Incluye a las variedades de melocotón ya sean de carne amarilla o blanca, de hueso libre o adherente. Dentro de este grupo, algunos autores denominan *pavía* al melocotón de hueso adherente. Este grupo de frutos también recibe la denominación de *duraznos*.
- Nectarina de hueso libre: *Prunus persica* (L.) Batsch var. *nectarina* (Aiton) Maxim. Incluye a las nectarinas de hueso libre, bien sean de carne blanca o amarilla.
- Nectarina de hueso adherente: *Prunus persica* (L.) Batsch var. *nucipersica* (Borkh.) Schneider. Incluye a las nectarinas de hueso adherente bien sean de carne blanca o amarilla. Este tipo de fruto recibe también denominaciones como *brugnon, briñón* o *pavía*.
- Paraguayo: *Prunus persica* (L.) Batsch var. *platycarpa* L.H. Bailey. Incluye las variedades de forma plana o achatada, bien sean con piel de melocotón o nectarina, carne blanca o amarilla.

En sentido botánico, el termino *var*. indica *variedad*. Sin embargo, desde el punto de vista genético, la separación en variedades y formas no tiene sentido dado que el origen del carácter distintivo es una mutación. Así, lo que se conoce en un contexto agronómico como *variedades*, son en sentido estricto *cultivares*, aunque a menudo se utilizan indistintamente. A lo largo de este trabajo, ambos términos se utilizarán en su sentido estricto.

1.4. LA CALIDAD EN EL FRUTO DEL MELOCOTONERO

1.4.1. Definición de calidad en la fruta

La palabra *calidad* proviene del latín *qualitas*, que significa atributo, propiedad o naturaleza básica de un objeto. Según el Diccionario de la Real Academia Española de la Lengua en su primera acepción, "calidad es la propiedad o conjunto de propiedades inherentes a algo, que permiten juzgar su valor" (RAE, 2005). Kramer y Twigg (1966) definieron la calidad como "aquellas características químicas y físicas que determinan su aceptación por parte del consumidor". Aquí se introduce un carácter subjetivo de la calidad, ya que distintos consumidores juzgarán un mismo producto de acuerdo con sus preferencias personales.

En los últimos años se ha constatado un mayor interés de los productores, comercializadores y consumidores por la búsqueda de la calidad en todos los productos agrícolas. Para facilitar su estudio, la calidad de un producto vegetal puede dividirse en cuatro componentes principales:

- calidad organoléptica o sensorial
- calidad nutricional
- calidad poscosecha
- calidad sanitaria

Dentro de la *calidad organoléptica* o *sensorial* se encuentran la apariencia (tamaño, color, forma, defectos, etc.), la textura (firmeza, jugosidad, fibrosidad, etc.) y el flavor (sabor y aromas) (Arthey, 1975; Bruhn et al., 1991; Crisosto et al., 2001a; Colaric et al., 2005).

En la *calidad nutricional* encontramos todos los componentes nutritivos de la fruta como carbohidratos, proteínas, lípidos, vitaminas y minerales. En los últimos años ha aumentado el interés por los alimentos funcionales y nutraceúticos, que son aquellos que poseen propiedades beneficiosas adicionales para la salud humana. Así, los antioxidantes derivados de las plantas, como la vitamina E, vitamina C y polifenoles se están convirtiendo, poco a poco, en importantes factores de la dieta humana (Prior y Cao, 2000; Kaur y Kapoor, 2001; Tomás-Barberán y Espín, 2001; Lule y Xia, 2005).

La *calidad poscosecha* es aquella que incluye la capacidad de conservación y la resistencia a la manipulación y al transporte de la fruta. Estos atributos son especialmente importantes en los países del hemisferio sur (Chile, Argentina, Australia, Sudáfrica), más alejados de las principales áreas de consumo (Anderson, 1979; Lill et al., 1989; Kader, 2002).

La *calidad sanitaria* tiene en cuenta la presencia o ausencia de tóxicos naturales, contaminantes y/o microorganismos patógenos, que puedan dar lugar a una acción tóxica

(Cámara, 2006). Es un componente importante de la calidad del producto vegetal, aunque no va ser objeto a tratar en esta tesis.

Todos estos componentes de la calidad de la fruta están determinados por distintos factores pre- y poscosecha (Goldman et al., 1999; Lee y Kader, 2000). Entre los factores precosecha, encontramos los factores genéticos (cultivar y pautas de maduración), el estado de madurez del fruto en el momento de cosecha (Kader, 1999), y otros factores extrínsecos a la planta como las condiciones climáticas, la fertilidad y capacidad de riego del terreno, el abonado, el control de plagas y enfermedades, y otras prácticas culturales. La cosecha marca el fin del cultivo y el comienzo del periodo poscosecha (almacenamiento, preparación para el mercado, distribución y venta), que tendrá también una influencia notable en la calidad final del producto una vez que llegue a la mesa del consumidor.

1.4.2. Desarrollo, madurez y calidad del fruto

El fruto del melocotonero y de otros frutales de hueso se caracteriza por tener un patrón de crecimiento del tipo doble sigmoideo (Connors, 1919) con una primera fase temprana de división celular (I), un período intermedio de crecimiento lento (II), y una fase final de expansión celular (III), caracterizada por un rápido crecimiento del fruto hasta que alcanza prácticamente su tamaño final (Fig. 1.9).

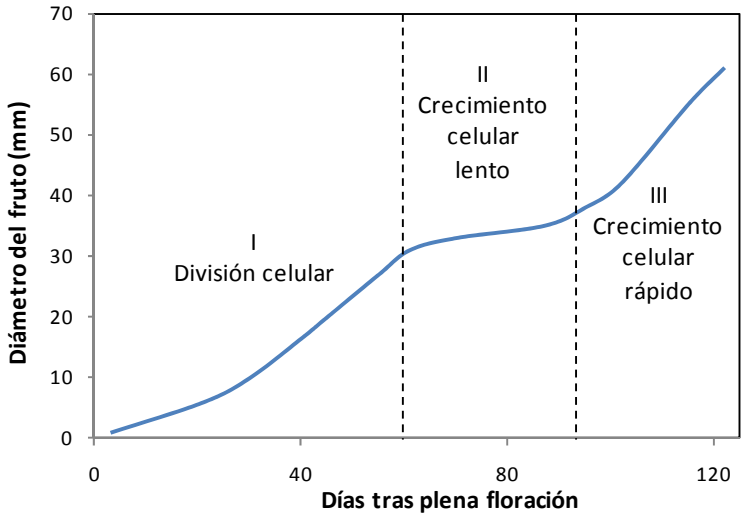

Figura 1.9. Curva de crecimiento (tipo doble sigmoidea) del fruto del melocotonero (Connors, 1919) en la que pueden observarse las tres etapas características de su crecimiento.

Sin embargo, en la fase final (III), la fruta todavía necesita de un proceso de maduración para desarrollar un color, textura y sabor más agradables para el consumidor. En este estado, denominado de *madurez fisiológica*, el fruto no está listo para ser consumido, pero ya ha adquirido la capacidad de llegar a su calidad óptima o *madurez de consumo*. Por lo tanto, los términos madurez fisiológica (*mature*) y madurez de consumo (*ripe*) denotan diferentes estados de desarrollo del fruto (Fig. 1.10) (Watada et al., 1984).

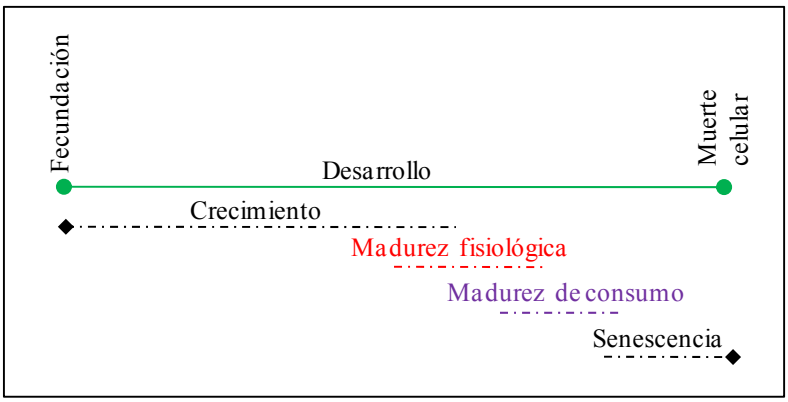

Figura 1.10. Etapas del desarrollo del fruto y madurez desde la fecundación hasta la muerte celular (adaptado de Watada et al., 1984).

La madurez fisiológica es aquel estado en el cual un fruto ha alcanzado un estado de desarrollo suficiente para que, después de la cosecha y periodo poscosecha, su calidad sea, al menos, la mínima aceptable para el consumidor final (Crisosto et al., 1995). La madurez de consumo es el estado de desarrollo en el que el fruto ha alcanzado su máxima calidad organoléptica y nutricional, que lo hacen apto para el consumo humano inmediato.

Para cada fruto y cultivar, el momento óptimo de recolección depende de lo que se denomina madurez de recolección o *madurez comercial* y es determinante para obtener una buena calidad final para el consumidor (Kader, 1999; Remorini et al., 2008; Nunes et al., 2009). La madurez comercial es un estado de desarrollo en el que ya se ha alcanzado la madurez fisiológica, e influye no solo en la calidad organoléptica y nutricional del fruto, sino también en su calidad poscosecha y en la duración de su vida útil (Kader et al., 1982; Infante et al., 2008b). Si el melocotón se cosecha antes de que su desarrollo fisiológico sea el adecuado, no podrá completar la evolución climatérica durante su conservación, será más susceptible a los daños mecánicos y su calidad será deficiente (Ju et al., 2000; Lill et al., 1989). El fruto evolucionará perdiendo firmeza pero ya no aparecerán ni el aroma ni el sabor característicos (Lleó et al., 1999). Por el

contrario, si se recolecta demasiado maduro, su vida útil se acortará y el fruto será más susceptible a desarrollar problemas de harinosidad.

El melocotón es un *fruto climatérico* y como tal, evoluciona una vez separado del árbol hasta adquirir la madurez óptima de consumo, siempre y cuando haya sido recolectado en un estado adecuado de madurez fisiológica. Los cambios ocurridos durante la maduración fisiológica del fruto, hasta llegar a su madurez de consumo, requieren energía que es suministrada por la respiración. Al iniciarse la maduración fisiológica, la respiración aumenta hasta llegar a un máximo denominado *pico climatérico* (Fig. 1.11), característico de frutos como el melocotón, la ciruela, la pera y la manzana. Simultáneamente al aumento en la respiración, se produce un brusco incremento en la producción de etileno que acelera el proceso de maduración del fruto.

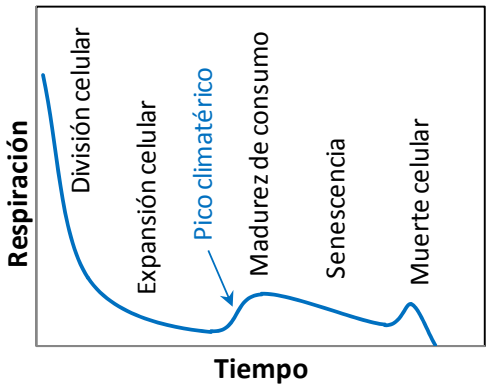

Figura 1.11. Patrón de respiración desde el cuajado hasta la senescencia y muerte celular de un fruto climatérico (Tromp et al., 2005).

En los frutos no climatéricos como cítricos, cereza, uva o fresa, la actividad respiratoria es baja durante todo su desarrollo, e incluso disminuye durante el proceso de maduración. En estos frutos, el inicio de la maduración fisiológica es difícil de identificar (Tromp et al., 2005).

Para decidir el momento óptimo de recolección se utilizan medidas objetivas como los índices de madurez (Kader, 1999; Nunes et al., 2009). Estos estándares deben ser sencillos, realizables en campo, baratos, objetivos, correlacionados con la calidad y, a ser posible, no destructivos (Crisosto, 1994). Algunos de los índices de madurez más utilizados son:

- Cronológicos: días tras plena floración.
- Físicos: color, tamaño, forma, características superficiales, firmeza.
- Químicos: sólidos solubles (SSC), acidez, relación SSC/acidez.
- Fisiológicos: actividad respiratoria, emisión de etileno.

El cambio de color de los frutos es el parámetro más utilizado para determinar la madurez fisiológica del fruto. Además, es el aspecto externo más fácilmente evaluado por el consumidor, que lo percibe como un indicador de la madurez, y por tanto, de la calidad organoléptica. En la mayor parte de los cultivares, la fecha de recolección se determina por cambios en el color de fondo de la piel (color dominante de toda la superficie de la fruta), de verde a amarillo o rojo. Los cambios en el color de fondo del fruto no dependen de la luz solar, por lo tanto son aptos para su uso como índices de madurez (Crisosto, 1994).

El tamaño y la forma del fruto son otros de los indicadores del momento de cosecha. Sin embargo, no pueden ser utilizados de forma independiente puesto que dependen de diversos factores como la producción del árbol, las condiciones climatológicas y/o las prácticas culturales (Crisosto, 1994). Cuando la sutura y los hombros de los melocotones y nectarinas están bien desarrollados, se consideran maduros. Sin embargo, para que sean un indicador fiable, deben combinarse con otros parámetros como el color de la piel (Kader, 1999).

La firmeza es otro de los parámetros más utilizados para determinar el momento de cosecha, y su valor, en el momento de recolección, influye directamente en la susceptibilidad a los daños mecánicos durante el manejo poscosecha (Crisosto et al., 2001c). El ablandamiento del fruto durante la maduración, se debe fundamentalmente a la solubilización enzimática de las pectinas insolubles de la pared celular, que provoca una reducción de la cohesión celular y con ello el ablandamiento del tejido (Redgwell y Fischer, 2002). Sin embargo, la firmeza no es siempre un indicador adecuado, dado que varía con diferentes factores como el tamaño del fruto, las condiciones climatológicas y/o las prácticas culturales (Crisosto, 1994). La firmeza puede medirse mediante métodos destructivos (penetrómetro) (Fig. 1.12) o no destructivos (durómetro, equipos de impacto y espectroscopía de reflectancia en el infrarrojo cercano o *near infrared reflectance spectroscopy*, NIRS) que registran la fuerza necesaria para una determinada deformación o resistencia a la penetración (Bureau et al., 2009b; Lu, 2004; Subedi y Walsh, 2009; Valero et al., 2007).

Figura 1.12. Penetrómetro (método destructivo) utilizado para la medida de la firmeza del melocotón.

El contenido de sólidos solubles (SSC, *soluble solids content*) aumenta durante la maduración del fruto hasta alcanzar un nivel máximo para cada cultivar. Este es un parámetro ampliamente utilizado como índice de madurez debido a la buena relación de esta medida con la palatabilidad del fruto y su aceptación por el consumidor (Colaric et al., 2005; Crisosto y Crisosto, 2005). El SSC puede medirse también mediante métodos destructivos (refractometría) o no destructivos (NIRS) (Lu, 2001; Bureau et al., 2009b).

La acidez depende del cultivar y de distintos factores precosecha, por lo que no suele usarse de forma independiente como índice de madurez (Lill et al., 1989). La acidez puede medirse mediante una sencilla valoración ácido-base (TA, *titratable acidity*) o mediante NIRS (Bureau et al., 2009b; Nicolai et al., 2007).

La relación SSC/TA (RI, *ripening index*) es uno de los índices de madurez más utilizados y resulta especialmente útil en frutas como el melocotón en las que el equilibrio dulce-ácido es clave para su aceptación (Ferrer et al., 2005). Se ha descrito que este parámetro está mejor relacionado con la calidad del fruto que el SSC o la TA por sí solos (Baldwin et al., 1998; Crisosto y Crisosto, 2005; Kader, 1999; Malundo et al., 2001a).

Los cambios fisiológicos como actividad respiratoria, concentración de metabolitos intermediarios y evolución del etileno son también útiles, aunque su determinación resulta más complicada y, por tanto, menos práctica.

En la mayoría de los casos, la fecha de recolección del melocotón se determina mediante la combinación de varios índices de madurez (Crisosto, 1994). El fruto suele recolectarse cuando su tamaño deja de aumentar, el color de fondo vira de verde a amarillo o rojo, la firmeza disminuye (50-60 N) (Crisosto, 1994), y el fruto se separa fácilmente del árbol (Crisosto et al., 1995). Estos han sido los criterios utilizados en este trabajo.

1.4.3. Calidad organoléptica

La calidad organoléptica es aquella que capta el consumidor directamente con sus sentidos, y se refiere al color, forma, tamaño, sabor, textura y aroma (Cámara, 2006).

El color de la fruta del melocotonero tiene un impacto importante en la aceptación del consumidor y el éxito en el mercado (Badenes et al., 2006; Liverani, 2002; Scorza y Sherman, 1996). La forma del fruto influye también en la aceptación del consumidor y en el manejo poscosecha. En melocotón y nectarina, los consumidores prefieren las formas globulares sin protuberancias ni irregularidades (Badenes et al., 2006). Por otro lado, las suturas y cicatrices pistilares pronunciadas pueden provocar daños mecánicos en la fruta, durante el manejo y transporte poscosecha. La ausencia de defectos, conjuntamente con la frescura y la uniformidad, son los principales componentes de la apariencia y, por lo tanto, influyen en la decisión primaria de compra. Los daños por frío y los efectos del etileno son un tipo de defectos poscosecha que aparecen en los cultivares sensibles, como respuestas fisiológicas a condiciones de conservación inadecuadas.

En frutas y hortalizas, el sabor se expresa normalmente en términos de la combinación de principios dulces y ácidos (Malundo et al., 1995; Malundo et al., 2001a; Sweeney et al., 1970). El dulzor de la fruta se debe principalmente a los azúcares solubles (Brooks et al., 1993), aunque se ha descrito una mejor correlación de la percepción del dulzor con el índice RI y el nivel de ácidos orgánicos, que solo con los azúcares totales (Colaric et al., 2005; Moing et al., 1998). La acidez es otro de los componentes más importante del sabor y del aroma del fruto (Colaric et al., 2005; Moing et al., 1998) y se debe principalmente a los ácidos orgánicos (málico, cítrico, oxálico, tartárico) (Wu et al., 2005b). Otros componentes del sabor del fruto incluyen el amargor y la astringencia, que dependen directamente de compuestos como los flavonoides, taninos y otros compuestos fenólicos. Además, la interacción química entre los distintos componentes de la fruta, puede afectar tanto a la intensidad como a la percepción del sabor (Malundo et al., 1995).

Uno de los objetivos más importantes en el estudio de la calidad organoléptica del fruto, es la determinación de correlaciones entre atributos sensoriales y medidas de firmeza, color u otros parámetros nutricionales susceptibles de ser medidos, que permitan determinar de forma objetiva la calidad organoléptica del fruto (Colaric et al., 2005; Crisosto y Crisosto, 2005; Esti et al., 1997; Génard et al., 1994; Karakurt et al., 2000; Robertson et al., 1989; Ruiz y Egea, 2008).

1.4.4. Calidad nutricional

La calidad nutricional es aquella relacionada con la capacidad de los alimentos de proporcionar los elementos nutritivos que favorezcan una buena salud y eviten la aparición de enfermedades (Cámara, 2006). Incluye a la calidad nutraceútica, que se refiere a la presencia de sustancias antioxidantes que actúan como protectoras frente al cáncer y enfermedades cardiovasculares (Lee, 2007; Lule y Xia, 2005; Smith-Warner et al., 2002; Smith-Warner et al., 2001). Así, la calidad nutritiva de los productos vegetales depende de la cantidad y calidad de los macro (proteínas, carbohidratos y lípidos) y micronutrientes (vitaminas y elementos minerales) que forman parte de su composición. El creciente interés sobre los alimentos funcionales y nutraceúticos, ha convertido a las frutas y verduras en la gran expectativa de la nutrición humana (Kaur y Kapoor, 2001; Prior y Cao, 2000; Sun et al., 2002; Szeto et al., 2002).

El descubrimiento de que determinados alimentos poseían compuestos biológicamente activos y beneficiosos para la salud más allá de la nutrición básica, abrió una nueva etapa en la ciencia de la nutrición. Estos compuestos, denominados funcionales o bioactivos, ayudan a prevenir enfermedades como el cáncer, tienen un efecto protector ante problemas cardiovasculares, son neutralizantes de los radicales libres, reducen el colesterol y la hipertensión y previenen la trombosis, entre otros efectos beneficiosos (Lule y Xia, 2005; Prior y Cao, 2000; Rossi et al., 2008; Smith-Warner et al., 2002; Sun et al., 2002). También se denominan alimentos funcionales, farmacoalimentos o nutracéuticos, a aquellos alimentos que contienen estos metabolitos. Como la mayor parte de estos compuestos son de origen vegetal, muchos autores los denominan fitoquímicos. Las frutas y hortalizas son particularmente ricas en estos fitoquímicos (García-Alonso et al., 2004; Kalt et al., 1999; Kaur y Kapoor, 2001), entre los que se encuentran los compuestos fenólicos (fenoles, flavonoides, antocianos, carotenoides) y las vitaminas A y C. Los compuestos fenólicos, incluyendo las antocianinas, se han identificado como potentes quelantes de radicales libres, lo que ha llevado a investigadores y productores a estudiar las frutas según su potencial antioxidante (Cevallos-Casals y Cisneros-Zevallos, 2003; Gil et al., 2002; Ruiz et al., 2005; Rupasinghe et al., 2006).

Además, muchos de estos compuestos, como las vitaminas A, C, E y K, o los aminoácidos aromáticos que forman parte de la estructura de los compuestos fenólicos, son nutrientes esenciales que no pueden ser sintetizados *in vivo* y deben obtenerse a través de la dieta, mediante la ingesta de frutas, hortalizas o complementos dietéticos (Kaur y Kapoor, 2001; Prior y Cao, 2000). Estos compuestos son los responsables de explicar la famosa ‘paradoja francesa’, que relaciona una dieta alta en grasas saturadas y

colesterol con una baja mortalidad por enfermedades coronarias. El alto consumo de frutas y hortalizas, especialmente aceitunas, aceite de oliva y vino en las regiones mediterráneas, proporciona antioxidantes que protegen de los efectos nocivos de una dieta alta en grasas (Oliver, 1997).

1.4.5. Componentes del melocotón con incidencia en su calidad organoléptica y nutricional

Desde el punto de vista nutricional, las frutas son productos ricos en agua, pobres en proteínas (1-3%) y lípidos (entre 0,1 y 0,2%, excepto en aguacates, olivas y frutos secos) y con un amplio rango de valores en lo que a carbohidratos se refiere, que suelen situarse entre el 10% y el 25%. Además, son ricos en micronutrientes: vitaminas (particularmente vitamina C) y minerales (Knee, 2002; Rangana, 1986).

1.4.5.1. Carbohidratos

Los carbohidratos son compuestos derivados de la fotosíntesis, que influyen de forma notable en la calidad organoléptica y nutricional del melocotón (Colaric et al., 2005; Crisosto y Crisosto, 2005; Esti et al., 1997; Génard et al., 1994; Versari et al., 2002). Influyen en el sabor y el aroma del fruto, por el equilibrio que se establece entre dulzor y acidez; en el color, mediante los derivados glicosilados de las antocianinas; y en la textura, porque constituyen los polisacáridos estructurales de la pared celular de las células del fruto.

Conforme el fruto madura, el contenido de azúcares solubles totales aumenta. La última etapa de crecimiento celular (III) se caracteriza por la expansión celular y la acumulación de carbohidratos en el mesocarpo del fruto (Lo Bianco y Rieger, 2002b). Los carbohidratos más abundantes en el melocotón maduro son la sacarosa (20-90 mg g^{-1} peso fresco), glucosa y fructosa (2-20 mg g^{-1} peso fresco), seguidos por el sorbitol (< 10 mg g^{-1} peso fresco) (Albás et al., 2004; Lo Bianco y Rieger, 2002b; Morandi, 2008; Moriguchi et al., 1990b; Vizzotto et al., 1996; Wu et al., 2005b). De forma minoritaria, el fruto contiene otros azúcares como manosa, galactosa, xilosa y mioinositol, cuyas distintas proporciones contribuyen a las diferencias de sabor entre cultivares. El análisis individualizado de los azúcares de la fruta requiere de la utilización de la cromatografía líquida de alta resolución (HPLC) (método utilizado en este trabajo) o la cromatografía de gases (GC), mediante la obtención previa de derivados volátiles.

El aumento en los niveles de sacarosa, que ocurre en el fruto en la última fase de crecimiento (Fig. 1.13) (Liverani y Cangini, 1991; Lo Bianco y Rieger, 2002a; Lo Bianco et

al., 1999), se atribuye al descenso de la actividad invertasa ácida (encargada de hidrolizar la sacarosa) en las células del mesocarpo, junto a la síntesis *de novo* como resultado de un aumento de la actividad de la sacarosa sintasa (Moriguchi et al., 1990a) y sacarosa fosfato sintasa (Hubbard et al., 1991). Por otro lado, al ser el azúcar mayoritario en el fruto del melocotón, la alta correlación entre el contenido de sacarosa y los azúcares totales (Dirlewanger et al., 1999; Gurrieri et al., 2001; Ledbetter et al., 2006) permite utilizar el contenido en sacarosa como un indicador del contenido total de azúcares. Además, 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).

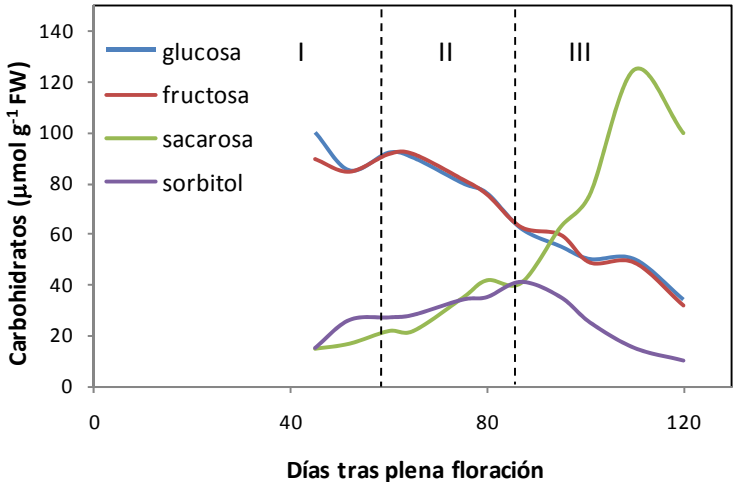

Figura 1.13. Evolución de los niveles de azúcares solubles más abundantes durante el desarrollo y la maduración del melocotón (adaptado de Vizzotto et al., 1996).

La concentración de sorbitol en el mesocarpo se mantiene baja durante todo el desarrollo del fruto (Lo Bianco y Rieger, 2002b; Moriguchi et al., 1990b; Vizzotto et al., 1996), debido a la inmediata conversión en fructosa y glucosa llevada a cabo por las enzimas sorbitol deshidrogenasa y sorbitol oxidasa, respectivamente. A pesar de ser un azúcar minoritario, existe un interés creciente por el contenido de sorbitol en la fruta, debido a las propiedades de este azúcar alcohol y su contribución a la calidad nutricional y poscosecha de la fruta. El sorbitol puede utilizarse como un sustituto de la glucosa, apto para diabéticos, y como edulcorante natural alternativo a la sacarosa, debido a su menor aporte calórico y a su efecto positivo en el sabor del melocotón (Colaric et al., 2005; Forni et al., 1992; Rapaille et al., 2003). Además, se ha descrito un control del sorbitol sobre el pardeamiento y la conservación de la fruta deshidratada, y un efecto antioxidante que ayuda a la conservación de los sabores del fruto (Forni et al., 1992). Por todo ello, la

obtención de frutas ricas en sorbitol es actualmente uno de los objetivos perseguidos en distintos programas de mejora (Ledbetter et al., 2006).

Además de su contribución al sabor y al color de las frutas, los carbohidratos tienen un papel fundamental en su textura. Los polisacáridos estructurales forman parte de las paredes celulares y tienen un papel decisivo en el ablandamiento de la pulpa debido a la solubilización, despolimerización, desesterificación y pérdida de azúcares neutros de las cadenas laterales (Seymour y Gross, 1996).

A pesar de que el perfil de azúcares suele ser bastante estable (Bassi et al., 1996), existen variaciones en los contenidos de los distintos azúcares y sus índices de relación según cultivares (Gurrieri et al., 2001; Usenik et al., 2008), genotipos (Dirlewanger et al., 1999; Wu et al., 2003), grado de madurez (Chapman y Horvat, 1990; Usenik et al., 2008; Wu et al., 2005a), condiciones de cultivo (Ledbetter et al., 2006) o manejo poscosecha. Además de los contenidos de azúcares, las relaciones existentes entre ellos son importantes a la hora de identificar perfiles de interés. Por ejemplo, son interesantes los valores bajos en las relaciones glucosa/fructosa o sacarosa/fructosa, dado el alto poder endulzante de la fructosa (1,75) frente a la glucosa (0,75) y la sacarosa (1) (Pangborn, 1963). También un valor bajo en la relación azúcares totales/sorbitol (alto porcentaje de sorbitol), es interesante para la identificación de frutas ricas en sorbitol.

El contenido en sólidos solubles (SSC) es una medida muy utilizada para la estimación del contenido de azúcares, debido a la rapidez y sencillez de su medida y a la correlación existente entre el SSC y el contenido de azúcares totales (Dirlewanger et al., 1999; Gurrieri et al., 2001; Ledbetter et al., 2006). Por ello, es una medida utilizada comúnmente en los programas de mejora para dirigir la selección hacia fruta más dulce (Harker et al., 2002b).

El SSC se utiliza también como un índice de calidad de la fruta, debido a la asociación entre la aceptación del consumidor y un alto SSC descrita en varios trabajos (Crisosto y Crisosto, 2005; Kader, 1999; Malundo et al., 2001b; Robertson y Meredith, 1988). Por otra parte, los criterios de calidad para melocotón dependen de la fecha de recolección. Así, en Europa, en los cultivares precoces se admiten valores entre 9 y 11 ºBrix, mientras que en cultivares más tardíos, el SSC deber ser mayor de 11 ºBrix (Badenes et al., 2006). Sin embargo, en estudios sobre la aceptación de distintos cultivares de melocotón por parte de los consumidores, se ha demostrado que el índice de calidad basado exclusivamente en el SSC no es suficiente, ya que la relación entre el SSC y el grado de aceptación por parte del consumidor es dependiente del tipo de cultivar, y por lo tanto, no existe un valor de SSC que asegure la satisfacción del consumidor para cualquier tipo de melocotón o nectarina (Crisosto y Crisosto, 2005). Como solución a este problema, Crisosto et al. (2006a) propusieron una clasificación de

los cultivares de melocotón en grupos de calidad organoléptica similar (cultivares dulces, ácidos, aromáticos y/o equilibrados), sugiriendo la creación de índices mínimos de calidad, establecidos independientemente para cada uno de los grupos organolépticos.

1.4.5.2. Ácidos orgánicos

Los ácidos orgánicos son los principales responsables de la acidez del fruto (Bassi y Selli, 1990; Colaric et al., 2005; Génard et al., 1994), e intervienen, junto con los azúcares, en la percepción del dulzor y el aroma del melocotón (Colaric et al., 2005; Moing et al., 1998). La acidez es, junto con el dulzor, uno de los principales responsables del sabor de los frutos y de la aceptación del consumidor (Colaric et al., 2005; Crisosto y Crisosto, 2005; Esti et al., 1997; Harker et al., 2002a; Kader, 1999). La acidez es tan importante que incluso determina las preferencias de distintos grupos etnogeográficos. En general, los consumidores asiáticos prefieren cultivares de melocotón y nectarina de baja acidez (Wen y Sherman, 2002), mientras que en Europa y América se prefieren cultivares más ácidos (Badenes et al., 1998a; Badenes et al., 1998c).

A diferencia de los carbohidratos, los ácidos orgánicos proceden de las reacciones del ciclo de los ácidos tricarboxílicos, a partir de precursores glicolíticos (Wills et al., 1983). Pueden encontrarse disueltos en la parte acuosa de la célula como ácidos, ésteres, glicósidos o formando cristales. El ácido orgánico mayoritario en el melocotón maduro es el málico (4-6 mg g^{-1} peso fresco), seguido de los ácidos cítrico (0,5-3 mg g^{-1} peso fresco), quínico (0,5-2 mg g^{-1} peso fresco) y succínico (< 0,5 mg g^{-1} peso fresco) (Moing et al., 1998; Sweeney et al., 1970; Wills et al., 1983; Wu et al., 2005b). Las concentraciones de estos ácidos, y con ellos la acidez valorable (TA), alcanzan un máximo durante el desarrollo del melocotón (fase III) y luego disminuyen durante la madurez y el periodo poscosecha (Fig. 1.14) (Chapman y Horvat, 1990; Chapman et al., 1991; Moing et al., 1998; Usenik et al., 2008; Wu et al., 2005b), debido a su utilización en la respiración del fruto y su conversión a azúcares. Además del grado de madurez, el cultivar y el genotipo también influyen en la acidez del fruto (Day et al., 1997; Robertson et al., 1990; Wu et al., 2003; 2005b). Así, melocotones y nectarinas suelen clasificarse como de alta o baja acidez según su valor de TA en el momento de la cosecha (Crisosto y Crisosto, 2005; Hilaire, 2003).

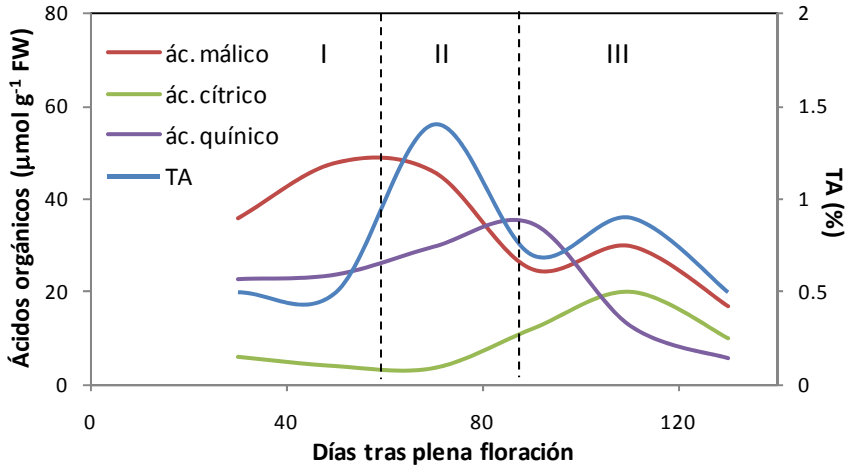

Figura 1.14. Evolución de la acidez valorable (TA) y los niveles de los ácidos orgánicos más abundantes, durante el desarrollo y la maduración del melocotón (adaptado de Moing et al., 1998).

1.4.5.3. Compuestos fenólicos

Los compuestos fenólicos son un grupo heterogéneo de metabolitos secundarios que comprende más de 10.000 compuestos, entre los que se incluyen los ácidos fenólicos, los flavonoides, y las antocianinas, que son un tipo de flavonoides. Pueden encontrarse en los vegetales de forma aislada o unidos a azúcares. Su característica básica es la presencia de al menos un grupo fenol (un anillo aromático hidroxilado) en su estructura química (Fig. 1.15), que parece ser responsable de conferirles la capacidad de neutralizar los radicales libres (Benavente-García et al., 2000; Fukumoto y Mazza, 2000; Lule y Xia, 2005).

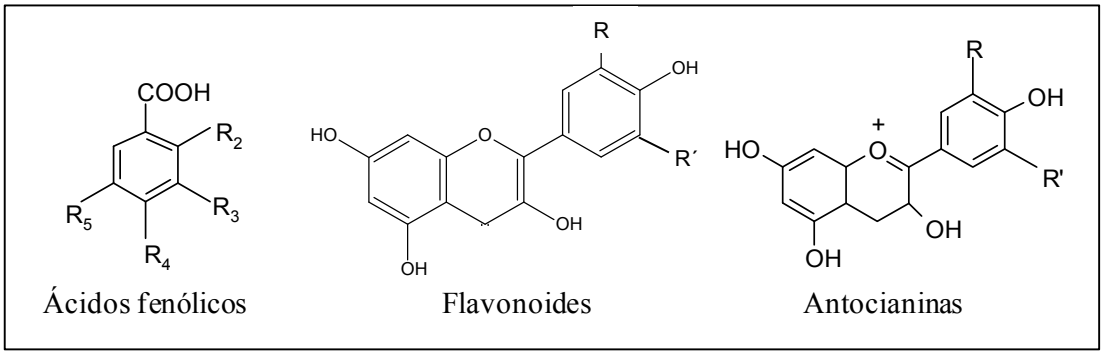

Figura 1.15. Estructura básica de ácidos fenólicos, flavonoides y antocianinas, en la que se observa la presencia de al menos un grupo fenol (Vasco et al., 2009).

Los compuestos fenólicos en plantas son sintetizados principalmente a través de dos rutas del metabolismo secundario (Fig. 1.16): la vía del ácido shikímico y la ruta del ácido malónico (Robards y Antolovich, 1997). La vía del ácido shikímico es responsable de la biosíntesis de la mayoría de fenoles en plantas. Esta ruta convierte los precursores de carbohidratos procedentes de la glicólisis y la ruta de las pentosas fostato en aminoácidos aromáticos. La ruta del ácido shikímico está presente en plantas, hongos y bacterias, pero no en animales. Los animales no son capaces de sintetizar los aminoácidos aromáticos fenilalanina, triptófano y tirosina, por lo que dependemos de la ingesta para la obtención de estos nutrientes esenciales.

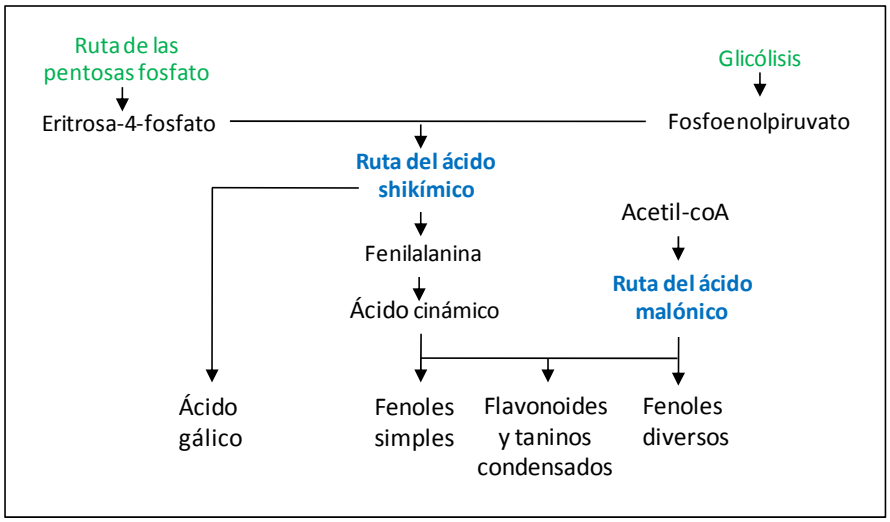

Figura 1.16. Rutas metabólicas para la biosíntesis de los compuestos fenólicos en plantas (Taiz y Zeiger, 2006).

Los flavonoides son el grupo más diverso y numeroso dentro de los compuestos fenólicos. Incluye a más de 5.000 compuestos ampliamente distribuidos en las plantas, a los que se atribuye una contribución significativa en la capacidad antioxidante de frutas y verduras (Prior y Cao, 2000). Dentro de este grupo se encuentran, entre otros, flavanoles, flavonoles, flavonas, isoflavonas, taninos y antocianinas (Robards y Antolovich, 1997). Las antocianinas son pigmentos naturales ampliamente distribuidos entre flores, frutas y vegetales, responsables de sus brillantes colores rojos, violetas y azules, aunque el color rojo o anaranjado de algunas frutas como la naranja o el tomate, se debe a los carotenoides más que a las antocianinas. Además de sus propiedades colorantes, tienen una potente actividad antioxidante modulada por las diferentes glicosilaciones e hidroxilaciones de sus moléculas (Rice-Evans et al., 1996; Wang et al., 1997). Las principales antocianinas en melocotón son la cianidin-3-glucósido y la cianidin-3-rutinosido (Tomás-Barberán et al., 2001; Vasco et al., 2009; Wu et al., 2005b).

El contenido en polifenoles influye en distintos aspectos de la calidad organoléptica y nutricional de las frutas como el sabor, el color y el aroma (Kim et al., 2003a). Se ha descrito que el mayor contenido de fenoles en un fruto suele ir asociado con una mejora en el sabor de éste (Robertson y Meredith, 1988). Además, muchos de estos compuestos fenólicos han demostrado tener una importante actividad fisiológica en humanos como agentes antioxidantes, anticarcinógenos, antimicrobianos, antialérgicos, antimutagénicos y antiinflamatorios (Kalt et al., 1999; Kaur y Kapoor, 2001; Kim et al., 2003b). De hecho, la mayoría de los autores indican que los polifenoles son los contribuyentes principales a la capacidad antioxidante en las frutas no cítricas (Cevallos-Casals et al., 2006; Chang et al., 2000; Drogoudi et al., 2008; Gil et al., 2002; Leontowicz et al., 2002; Tulipani et al., 2008; Vizzotto et al., 2007), mientras la vitamina C es el principal antioxidante en frutas cítricas y bayas (fresas, frambuesas, moras, etc.) (Gardner et al., 2000; Pedersen et al., 2000). Los flavonoides totales o el contenido en antocianinas también muestran correlaciones significativas con la capacidad antioxidante, aunque estas correlaciones son menores a las encontradas para el contenido en fenoles totales. Estas sustancias actúan como antioxidantes, mediante la neutralización de radicales libres implicados en la mayoría de las enfermedades degenerativas. Los radicales libres o especies reactivas de oxígeno (ROS) son liberados por el estrés oxidativo que se produce en los organismos aerobios, causando daño oxidativo a moléculas como lípidos, proteínas y ácidos nucléicos, provocando finalmente la desorganización y la muerte celular (Rice-Evans et al., 1996).

La cantidad y composición de los compuestos fenólicos en las frutas dependen de muchos factores como el genotipo (Kalt et al., 2001b; Scalzo et al., 2005; Tulipani et al., 2008; Vizzotto et al., 2007), el cultivar (Dalla Valle et al., 2007; Gil et al., 2002; Kim et al., 2003b; Ruiz et al., 2005; Tavarini et al., 2008), las condiciones climáticas (Kataota et al., 1984; Tomás-Barberán y Espín, 2001; Tromp et al., 2005), las prácticas culturales (2001a; Kalt et al., 2000b), el origen geográfico y las condiciones poscosecha (Asami et al., 2003; Di Vaio et al., 2008; Gil et al., 2000; Gonçalves et al., 2007; Rickman et al., 2007). Además, estos metabolitos secundarios no se encuentran distribuidos uniformemente en los tejidos del fruto, sino que se concentran principalmente en la piel (Asami et al., 2003; Cevallos-Casals et al., 2006; Rupasinghe et al., 2006). La concentración de fenoles varía también con la madurez del fruto, siendo mayor en la primera fase de crecimiento y decreciendo conforme éste va madurando (Bureau et al., 2009a; Díaz-Mula et al., 2008; Kalt et al., 2000a; Serrano et al., 2005). La pulpa del melocotón maduro contiene un rango de polifenoles totales que oscila de 10 a 80 mg GAE (equivalentes de ácido gálico) por 100 g de peso fresco, flavonoides en un rango de 2-30 mg 100 g^{-1} CE (equivalentes catequina), antocianinas en un rango de 0-40 mg

C3GE (equivalentes de cianidin-3-glucósido) por kg dependiendo de la pigmentación del fruto (Fig. 1.17) y una capacidad antioxidante que varía entre 200-700 μg Trolox g^{-1} de peso fresco (Asami et al., 2003; Dalla Valle et al., 2007; Di Vaio et al., 2008; Gil et al., 2002; Tavarini et al., 2008; Tomás-Barberán et al., 2001).

Figura 1.17. Homogenados de pulpa de melocotones y nectarinas evaluados en esta tesis, en los que pueden observarse las distintas coloraciones obtenidas de los frutos.

1.4.5.4. Vitamina C

La vitamina C es uno de los factores más importantes de la calidad nutricional de frutas y hortalizas y es un importante micronutriente de la dieta humana, ya que lleva a cabo importantes actividades biológicas. La vitamina C previene el escorbuto y ayuda a mantener sana la piel, las encías y los vasos sanguíneos (Davey et al., 2000). Además, participa en la formación de colágeno y en la absorción de hierro, reduce el nivel de colesterol, potencia el sistema inmunitario y tiene un papel destacado como agente antioxidante (Proteggente et al., 2002). La mayor parte de los vertebrados son capaces de sintetizar ácido ascórbico (AsA), pero algunos mamíferos entre los que se encuentran primates y humanos, han perdido esa capacidad y necesitan el AsA como un componente esencial de la dieta. Las frutas y hortalizas constituyen la fuente principal de vitaminas en la dieta humana, constituyendo más del 90% del total de vitamina C ingerido (Lee y Kader, 2000). La vitamina C es el antioxidante principal en frutas cítricas y bayas (Gardner et al., 2000; Pedersen et al., 2000). Sin embargo, su contribución a la capacidad antioxidante de frutas no cítricas, como el melocotón, es menor que la de los compuestos fenólicos (Cevallos-Casals et al., 2006; Chang et al., 2000; Gil et al., 2002; Leontowicz et al., 2002; Tulipani et al., 2008). Su capacidad antioxidante se debe a su facilidad para ceder electrones, actuando como agente reductor de muchas especies reactivas de oxígeno (ROS). Debido a su carácter hidrosoluble, protege a las moléculas disueltas en la

porción acuosa de las células y tejidos, y reduce los radicales libres de tocoferol de nuevo a sus formas activas en las membranas celulares (Kaur y Kapoor, 2001).

El contenido total de vitamina C en frutas consiste en la suma de AsA y su forma oxidada, el ácido dehidroascórbico (DHAA). Ambas formas son interconvertibles entre sí, siendo la forma reducida la que posee propiedades antioxidantes, y la que se presenta principalmente en los tejidos de las plantas (Davey et al., 2000; Wills et al., 1983). El contenido en vitamina C de frutas y hortalizas depende de muchos factores, como el genotipo y el cultivar (Gil et al., 2002; Lee y Kader, 2000; Nelson et al., 1972; Tulipani et al., 2008; Vizzotto et al., 2007), las condiciones climáticas (intensidad de luz y temperatura) (Lee y Kader, 2000; Nelson et al., 1972), las prácticas de cultivo (Lee y Kader, 2000), el estado de madurez (Nagy, 1980), los métodos de cosecha y el manejo poscosecha (Rickman et al., 2007; Smoot y Nagy, 1980; Wright y Kader, 1997b, a). La concentración de vitamina C varía significativamente dependiendo de la especie y el cultivar, encontrando un amplio rango de valores, en torno a 80 mg 100 g^{-1} de peso fresco en la naranja y el limón, 60 mg 100 g^{-1} en la fresa y el kiwi, 3-15 mg 100 g^{-1} en melocotón y 0-2 mg 100 g^{-1} en manzana (Agar et al., 1997; Davey et al., 2000; Odriozola-Serrano et al., 2007; Tavarini et al., 2008; Wills et al., 1983). Aunque la concentración de vitamina C disminuye durante la maduración de la fruta, el contenido total tiende a aumentar dado que el tamaño del fruto aumenta (Lee y Kader, 2000).

1.4.6. Calidad poscosecha

La calidad poscosecha es aquella que incluye la capacidad de conservación y la resistencia a la manipulación y al transporte de la fruta. Se estima que las pérdidas poscosecha en frutas y hortalizas oscilan entre un 5 y un 25% en los países desarrollados, y de un 20 a un 50% en los países en desarrollo, según producto, cultivar y condiciones de manejo (Kader, 2002).

El grado de deterioro de los productos cosechados es generalmente proporcional a su tasa respiratoria. Según la tasa de respiración y producción de etileno (C_2H_4) durante la maduración, las frutas se dividen en climatéricas y no climatéricas. Las frutas climatéricas, como el melocotón y la nectarina, muestran un fuerte incremento en la producción de CO_2 y C_2H_4 durante la maduración, que no se observa en las frutas no climatéricas (Fig. 1.11).

Las frutas y hortalizas continúan viviendo después de su cosecha: respiran, transpiran y están sujetas a continuos cambios, la mayor parte de ellos no deseables, que deterioran la calidad interna y externa del producto. La velocidad de este deterioro depende del tipo de producto, condiciones de cultivo y otros factores (Kader et al., 1982),

pero principalmente de las condiciones en las que se almacenan: temperatura, humedad relativa, movimiento y composición del aire, etc. (Gogorcena et al., 2009; Kader, 2002). Los cambios que ocurren durante la poscosecha no pueden evitarse, pero sí retrasarse dentro de ciertos límites. El control de la temperatura es la herramienta más efectiva para alargar la vida poscosecha de la fruta, debido a la ralentización del metabolismo que provocan las bajas temperaturas. Cada 10ºC de disminución de la temperatura de conservación, se reduce de 2 a 4 veces la tasa respiratoria celular (Thomson et al., 2002).

Entre las causas de pérdida de calidad poscosecha están la deshidratación, los desórdenes fisiológicos, los daños mecánicos y las infecciones por patógenos (Kader, 2002). La pérdida de agua debida a la transpiración afecta a la apariencia, textura y calidad nutricional de la fruta. Se controla mediante el uso de ceras, plásticos u otro tipo de recubrimientos de superficie, y sobre todo mediante el control de la temperatura y la humedad relativa durante el almacenaje del producto.

Los desórdenes fisiológicos poscosecha están causados principalmente por la exposición a temperaturas de almacenamiento no deseables. Entre ellos, encontramos los daños por congelación (tras almacenamiento por debajo de la temperatura de congelación), daños por frío (en productos susceptibles tras almacenamiento a temperaturas bajas por encima del punto de congelación) y daños por calor (por exposición directa al sol o altas temperaturas).

Distintos tipos de daños mecánicos (daños en superficie, pardeamiento por impacto o por vibración, etc.) aceleran la pérdida de agua, proporcionan lugares de entrada para los patógenos, y estimulan la producción de CO_2 y C_2H_4.

Las podredumbres son otros de los síntomas más obvios y comunes de deterioro de la fruta. La mayoría de los hongos y bacterias que afectan a frutas y hortalizas después de la cosecha, son patógenos que invaden los tejidos tras daños mecánicos o fisiológicos. La resistencia natural de las frutas al ataque de patógenos va disminuyendo con la maduración y la senescencia.

1.4.6.1. Daños por frío

Los daños por frío (*chilling injury*, CI) son el principal problema que afecta a la calidad poscosecha de melocotones y nectarinas. Se trata de un desorden fisiológico que afecta a aquellas frutas susceptibles, tras su almacén a temperaturas bajas por encima del punto de congelación. Melocotones y nectarinas maduran y se deterioran rápidamente a temperatura ambiente, debido a su naturaleza climatérica y a sus tasas de respiración y producción de etileno. Para ralentizar ese proceso y alargar su vida útil, se

lleva a cabo el almacenamiento a bajas temperaturas, limitado por la aparición de los daños por frío. Estos daños aparecen antes y de forma más intensa cuando la fruta se almacena en el rango de 2,2 a 7,6 ºC (Lurie y Crisosto, 2005), que cuando se almacena a la temperatura óptima para melocotón y nectarina (de -0,5 ºC a 0 ºC) (Crisosto y Mitchell, 2002). Los síntomas se hacen evidentes tras el almacenamiento en frío, normalmente cuando llega al consumidor (Bruhn et al., 1991; Crisosto, 2006; Fernández-Trujillo et al., 1998; Infante et al., 2009), provocando un acusado efecto negativo en su grado de satisfacción. Esta fisiopatía afecta a todos los parámetros de calidad del fruto, desde la apariencia, a causa del pardeamiento de la piel; la textura, debido a la rotura de la estructura celular de la pulpa; y el sabor y jugosidad, ya que pierden los jugos y se desarrollan sabores desagradables (Crisosto et al., 1999; Lurie y Crisosto, 2005).

La harinosidad (*mealiness*) es uno de los síntomas de daños por frío con mayor incidencia en la calidad del melocotón (Fig. 1.18). Algunos estudios estructurales han demostrado que la causa de la harinosidad de la pulpa es la separación que se produce entre las células del parénquima del fruto (Luza et al., 1992). Este proceso es similar al que ocurre de forma normal durante el ablandamiento de la fruta en la maduración. La diferencia es que mientras en la fruta no harinosa estas células están recubiertas por una fina capa de jugo, en las frutas harinosas esta capa de jugo no existe (Harker y Sutherland, 1993) debido a una alteración en la capacidad de retención de agua de los polisacáridos de las paredes celulares (Sonego et al., 1995). El desarrollo de la harinosidad en melocotones y nectarinas almacenados a 0 ºC se ha atribuido al aumento de la actividad de la pectin metilesterasa (PME) y/o a la inhibición de la endo-poligalacturonasa (endo-PG) a bajas temperaturas (Ben-Arie y Sonego, 1980; González-Aguero et al., 2008; Zhou et al., 2000; Zhou et al., 1999). Esto lleva a la formación de largas moléculas de pectinas con baja esterificación y de alto peso molecular, que confieren una desagradable textura de gel en la pulpa (Ben-Arie y Lavee, 1971; Lurie et al., 2003). Los efectos positivos de distintos tratamientos poscosecha sobre el control de la harinosidad parecen deberse a un aumento en la actividad de la endo-PG (Crisosto et al., 2004a; Wang, 1995; Zhou et al., 2001a; Zhou et al., 2000).

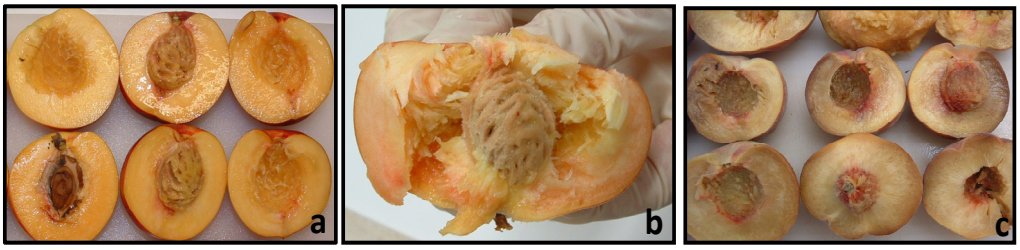

Figura 1.18. Distintos síntomas de daño por frío: fruta sana (a), con harinosidad (b) y con pardeamiento de la pulpa (c).

La textura correosa (*leatheriness*) es otro de los síntomas de daños por frío, similar a la harinosidad en cuanto a la textura seca de la pulpa. Sin embargo, la harinosidad se produce en frutas blandas, mientras la textura correosa aparece en frutas más firmes, que no se han ablandado durante el proceso de maduración (Brummell et al., 2004; Luza et al., 1992). Los tratamientos con aplicación de etileno exógeno parecen aliviar este síntoma de daño por frío, favoreciendo el ablandamiento normal del fruto (Crisosto et al., 2001b; Dong et al., 2001; Palou et al., 2003).

El pardeamiento interno de la pulpa (*flesh browning*) (Fig. 1.18) parece estar relacionado con cambios en la permeabilidad de la membrana, debido al deterioro del tejido. Los compuestos fenólicos y la enzima polifenol oxidasa, que normalmente se encuentran en compartimentos celulares separados, entran en contacto y producen el pardeamiento de la pulpa (Kader y Chordas, 1984). La coloración antociánica de la pulpa de algunos melocotones y nectarinas, que es considerada como una característica atractiva del fruto, parece estar relacionada con una mayor incidencia de este síntoma de daños por frío tras almacenamiento en frío (Ogundiwin et al., 2009a; Ogundiwin et al., 2008b).

El enrojecimiento de la pulpa (*bleeding*) es el síntoma menos estudiado de los daños por frío. La coloración antociánica roja que algunos melocotones y nectarinas exhiben de forma natural alrededor del hueso parece intensificarse, e incluso extenderse por toda la pulpa, tras una exposición larga a bajas temperaturas. Sin embargo, se ha sugerido que este enrojecimiento puede ser consecuencia de la senescencia del tejido, o de la respuesta a condiciones generales de estrés durante el periodo poscosecha, incluyendo, entre ellas, el almacenamiento prolongado a bajas temperaturas (Lurie y Crisosto, 2005; Manganaris et al., 2008b). La aparición de este enrojecimento se ha asociado a un aumento de la actividad fenilalanin-amonia-liasa (PAL) y a un aumento en la acumulación de antocianinas (Manganaris et al., 2008b).

La susceptibilidad a los daños por frío depende del *background* genético (Crisosto et al., 1999; Ogundiwin et al., 2007; Peace et al., 2006), el tamaño y la madurez del fruto

(Ju et al., 2000; Kader et al., 1982; Von Mollendorf, 1987), y distintos factores precosecha como la fertilización, irrigación, posición del fruto en el árbol y carga de cosecha (Lill et al., 1989; Von Mollendorf, 1987). La gran variabilidad en la susceptibilidad a este desorden entre los diferentes cultivares de melocotón y nectarina (Crisosto et al., 1999; Kader, 1985) es importante para la selección de cultivares tolerantes en los programas de mejora. En general, los melocotones son más susceptibles que las nectarinas, y entre ellos, los de carne fundente son más susceptibles que los de carne no fundente (Brovelli et al., 1999a; Crisosto et al., 1999; Manganaris et al., 2006a).

Distintos tratamientos pre o poscosecha han logrado reducir y retrasar los diferentes síntomas de este desorden fisiológico. Entre ellos están el pre-acondicionamiento a altas temperaturas previo al almacenamiento en frío (Anderson, 1979; Crisosto et al., 2004a; Fernández-Trujillo y Artés, 1997; Manganaris et al., 2008b; Murray et al., 2007; Nanos y Mitchell, 1991; Wang et al., 1992; 2001b; Zhou et al., 2000), la aplicación interrumpida de calor durante el almacenamiento en frío (Anderson, 1979; Ben-Arie et al., 1970; Girardi et al., 2005; Lill, 1985; Nanos y Mitchell, 1991; Zhou et al., 2001a), los tratamientos pre o poscosecha con reguladores de crecimiento (Jin et al., 2009; Ju et al., 1999; Manganaris et al., 2006c; Zilkah et al., 1997), el almacenamiento en atmósfera controlada (Anderson, 1979; Cantín et al., 2008a; Girardi et al., 2005; Murray et al., 2007; Zhou et al., 2000), la aplicación de etileno exógeno durante el almacenamiento (Crisosto et al., 2001b; Dong et al., 2001; Palou et al., 2003; Zhou et al., 2001a), o los tratamientos pre o poscosecha con inhibidores de etileno (Candan et al., 2007, 2008; Hayama et al., 2008; Ju et al., 1999; Larrigaudière et al., 2009; Manganaris et al., 2008b; Zhou et al., 2001a).

1.5. MEJORA Y CONTROL GENÉTICO DE ATRIBUTOS DE CALIDAD DEL MELOCOTÓN

1.5.1. Mejora genética de la calidad del fruto del melocotonero

Según el Diccionario de Ciencias Hortícolas (SECH, 1999), la mejora genética de la calidad de los productos agrícolas es la aplicación de técnicas genéticas a la obtención de productos con propiedades que acrecienten su valor. Según el pionero genetista soviético N.I. Vavilov a principios del s. XX, la mejora genética vegetal es la evolución de las plantas dirigida por el hombre (Crow, 2001).

La mejora genética es posible siempre que exista a priori una variabilidad, bien sea natural o bien provocada mediante diversas técnicas (Sánchez-Monge, 1993). El

método más utilizado para generar nueva variabilidad es la mejora clásica o convencional mediante la realización de cruzamientos.

El melocotonero es una de las especies con una mayor gama de cultivares entre los frutales caducifolios, apareciendo nuevas selecciones cada año (Badenes, 2000; Byrne, 2002). Así, el número de nuevos cultivares registrados en la década de los 90 fue superior a 500 (Fideghelli et al., 1998). La disponibilidad de cultivares de melocotón es actualmente muy elevada y continúa aumentando, aunque la mayoría de ellos tiene un origen común a partir de un reducido número de cultivares comerciales, si se compara con la gran diversidad genética existente.

A partir de unas pocas introducciones procedentes de Asia Central y China se desarrollaron los cultivares europeos, que han sido, a su vez, fuente de germoplasma para otras zonas productoras como América del Norte y del Sur, Sudáfrica, Australia y Nueva Zelanda (Hancock et al., 2008). Estos cultivares se han adaptado a los gustos del consumidor europeo, consiguiendo frutos uniformes y de gran calibre, con buena apariencia y con mayor acidez que los asiáticos (Byrne, 2003; Day et al., 1997). En el ámbito europeo, Italia y Francia lideran la creación varietal, aunque muy por detrás de Estados Unidos, en cuanto al número de cultivares incluidos en el registro único. Como en otros países productores de melocotón de Europa, nuestro país se encuentra bajo una fuerte dependencia de los cultivares de origen extranjero, principalmente de Estados Unidos (Badenes et al., 1998a; Llácer, 2005). En las últimas décadas, se han introducido en España nuevos cultivares procedentes de Asia con nuevas texturas, *stony hard*, formas de fruto achatado y carácter subácido (Badenes et al., 2006).

Los objetivos específicos de cada programa de mejora dependen de las necesidades en cada una de las zonas de cultivo, y en los últimos años se han introducido nuevos caracteres de acuerdo a las exigencias de los consumidores. Los programas de mejora en China y en el resto de Asia, tienen como objetivo la obtención de cultivares con menores exigencias en horas frío y adaptados a los gustos del mercado oriental, por lo que se han seleccionado sobre todo cultivares subácidos, con elevado contenido en azúcares y de carne blanca (Byrne y Boonprakob, 2008; Wang et al., 2002; Wen y Sherman, 2002). Sin embargo, en Europa del Este y Rusia se buscan cultivares resistentes al frío, con mayores valores de acidez (Dumitru et al., 2003). En Italia, Francia y España, el objetivo es alargar la campaña de recolección y producir cultivares de calidad con mayor vida poscosecha y resistencia al manejo (Cantín et al., 2006; Hilaire, 2003; Liverani, 2002; Martínez-Calvo et al., 2006; Moreno, 2005). Entre los programas de selección y mejora desarrollados en España, cabe destacar su inicio con la selección de la variedad población Sudanell, en la Estación de Aula Dei (CSIC) por el Dr. M. Cambra (1979). Esta selección dio lugar a tres clones de Sudanell, con maduración escalonada,

que todavía se cultivan y tienen una gran importancia económica. Posteriormente, como consecuencia de varias prospecciones de melocotonero en Aragón, se seleccionaron otras variedades locales: 'Montaced' (melocotón de carne blanca) y 'Montejota' (paraguayo) por el Dr. M. Carrera del Centro de Investigación y Tecnología Agroalimentaria de la DGA, así como tres clones del melocotonero tardío de Calanda: 'Jesca', 'Calante' y 'Evaisa', por J.L. Espada del Centro de Técnicas Agrarias de la DGA (Moreno, 2005). Más recientemente, cabe citar los programa llevados a cabo en Valencia (Martínez-Calvo et al., 2006), Cataluña, Murcia, Andalucía (Llácer et al., 2009) y Zaragoza, en la Estación Experimental de Aula Dei (Cantín et al., 2006; Moreno, 2005), en el que se enmarca el desarrollo de esta Tesis Doctoral. En países como Nueva Zelanda, Australia y Sudáfrica, debido a la lejanía de los mercados principales, la prioridad son los cultivares de larga vida poscosecha, resistentes a los daños por frío. A pesar de las peculiaridades de cada programa, no cabe duda de que los objetivos de mejora de la calidad organoléptica, nutricional y poscosecha del fruto van a ocupar en el futuro un lugar cada vez más relevante en los programas de mejora genética de frutales (Elorriaga de Bonis, 2005; Infante et al., 2008a; Reggidori, 2005). De hecho, en los últimos Programas-Marco de la Unión Europea, la mejora de la calidad de la fruta y los estudios médicos encaminados a demostrar las ventajas del consumo de fruta, han sido líneas prioritarias (Fuente: SCADPlus).

La mejora genética de plantas, aplicando técnicas convencionales o de ingeniería molecular, ha tenido como objetivos principales el aumento de la productividad, la resistencia a plagas y enfermedades o la mejora de características agronómicas (Nuez y Llácer, 2001). La mejora genética de la calidad en plantas es un objetivo relativamente reciente, que surgió en el momento en el que la superproducción de algunos cultivos en países desarrollados hizo que la calidad fuese un factor decisivo en el éxito de un nuevo cultivar en el mercado. Actualmente, los programas de mejora cada vez tienen más en cuenta los aspectos de calidad de fruto (Byrne, 2002, 2003; Martínez-Calvo et al., 2006), ya que la baja calidad organoléptica de los frutos es considerada como una de las causas del descenso del consumo de fruta fresca (Bruhn, 1995; Crisosto, 2002). Sin embargo, en los programas de mejora la mayoría de los esfuerzos se han dedicado, hasta el momento, a la apariencia externa (color, forma y tamaño del fruto) más que a otros aspectos de la calidad organoléptica y nutricional, como la obtención de frutas ricas en compuestos bioactivos (polifenoles, vitaminas u otros antioxidantes) (Infante et al., 2008a). Actualmente, se considera que el éxito de cualquier nuevo cultivar de fruta u hortaliza depende directamente de si responde a las necesidades del consumidor y del grado de satisfacción que el producto le proporcione (Wismer et al., 2005).

1.5.1.1. Mejora de la calidad organoléptica y nutricional

Hasta hoy, la calidad organoléptica y nutricional ha recibido un papel secundario en los programas de mejora. Sin embargo, actualmente, la mejora genética de frutales incluye con prioridad la mejora de estos atributos de calidad (Badenes et al., 2006; Byrne, 2003; Infante et al., 2008a; Liverani, 2002; Wismer et al., 2005). La mejora de la calidad organoléptica de la fruta resulta más compleja que la mejora de la producción o de la calidad nutricional, ya que se trata en muchos casos de caracteres extremadamente complejos, con heredabilidades generalmente bajas, difíciles de identificar y medir, e influenciados por el ambiente (Cubero, 1999, 2000). Por otra parte, la mejora de características organolépticas está muy ligada a aspectos culturales, lo que dificulta su determinación.

Los caracteres organolépticos como los aromas, los sabores o las texturas presentan una elevada variabilidad genética, pero también están sujetos a fuertes influencias ambientales (Brooks et al., 1993; Dirlewanger et al., 1999; Ruiz y Egea, 2008; Sweeney et al., 1970). Habitualmente, estos parámetros presentan variabilidad continua y sus heredabilidades estimadas son habitualmente inferiores a 0,50, mientras que para caracteres como dulzor, sólidos solubles o contenido de azúcares, las heredabilidades son superiores a 0,50, y por tanto más fácilmente de abordar en programas de mejora (Cheng et al., 2004; Oraguzie et al., 2001; Quilot et al., 2005; Rumpunen y Dviklys, 2003; Sánchez-Pérez et al., 2007b; Valentini et al., 2004).

En la última década, se han identificado QTLs (*Quantitative Trait Loci*) y desarrollado marcadores moleculares (SSRs: *Single Sequence Repeat*; AFLPs: *Amplified Fragment Length Polimorphism*) asociados al control de diferentes caracteres organolépticos de la fruta, como el peso del fruto y sus dimensiones, el nivel de SSC, la acidez valorable (TA), la composición de azúcares y ácidos individuales, la coloración roja de la piel y de la pulpa, la jugosidad y/o el dulzor (Abbott et al., 1998; Dirlewanger et al., 1999; Etienne et al., 2002b; Joobeur et al., 1998; Quarta et al., 2000; Quilot et al., 2004c; Sánchez-Pérez et al., 2007a; Sosinski et al., 2000; Testolin, 2003; Verde et al., 2002; Yamamoto et al., 2001), que podrían aplicarse en los programas de mejora.

Hasta el momento, los avances conseguidos en la mejora de caracteres organolépticos se deben principalmente al establecimiento de relaciones entre la composición química y las propiedades físicas de los frutos, con sus atributos sensoriales, y a la identificación y selección de cultivares de calidad organoléptica superior y posterior corrección de deficiencias agronómicas (Colaric et al., 2005; Dosba, 2003; Esti et al., 1997). La identificación de las moléculas responsables de las percepciones sensoriales, la localización de las secuencias génicas que las codifican y su

posterior modificación, permitirán llevar a cabo con mayor eficacia la mejora de la calidad organoléptica (Badenes et al., 2006).

Por otro lado, el valor nutricional de la fruta es un factor con importancia creciente en los programas de mejora de frutales. Los objetivos son aumentar el valor añadido de la fruta e incrementar su consumo, y la obtención de principios activos para la industria farmacológica (Byrne, 2003). Una importante línea de mejora genética actual es la dirigida a la obtención de fruta funcional, con un alto contenido en principios bioactivos beneficiosos para la salud o el bienestar humano, como polifenoles y vitaminas. Sus efectos beneficiosos en los procesos de envejecimiento y en la prevención de distintos tipos de cáncer (Kaur y Kapoor, 2001; Prior y Cao, 2000; Wargovich, 2000), hacen que la obtención de frutas enriquecidas en estos compuestos sea un objetivo en los actuales programas de mejora de melocotón, nectarina y ciruela (Byrne et al., 2004; Cevallos-Casals et al., 2006; Chang et al., 2000; Gil et al., 2002; Rupasinghe et al., 2006; Tavarini et al., 2008; Vizzotto et al., 2007), albaricoque (Ruiz et al., 2005) o fresa (Tulipani et al., 2008).

1.5.2. Control genético de parámetros asociados a la calidad del melocotón

El melocotonero es la especie más estudiada a nivel genético dentro de la familia de las Rosáceas, debido sobre todo al extenso número de programas de mejora existentes y a la antigüedad de muchos de ellos. Por otro lado, es una especie diploide (2n = 16) con un tamaño de genoma relativamente pequeño (580 Mb), una alta auto-compatibilidad y un tiempo de generación relativamente corto (2-3 años) en comparación con otros frutales. Todas estas razones, junto con los numerosos recursos fitogenéticos disponibles, han llevado a que se haya propuesto como la especie modelo para estudios de genómica estructural y funcional dentro del género *Prunus* (Abbott et al., 2002). Hasta el momento existen 13 mapas de ligamiento de distintas especies de *Prunus* conectados entre sí mediante marcadores genéticos (Genome Database for Rosacea, www.bioinfo.wsu.edu/gdr) que incluyen más de 2000 marcadores. El esqueleto de todos ellos es el mapa de referencia (TxE), construido a partir de una F2 proveniente de un cruce interespecífico entre el cultivar de almendro 'Texas' y el cultivar de melocotonero 'Early Gold' (Joobeur et al., 1998). Este mapa, cuenta actualmente con 827 marcadores y una densidad media de 0,63 cM/marcador (Shulaev et al., 2008). Partiendo de los marcadores RFLPs (*Restriction Fragment Length Polimorphism*) del mapa TxE y de dos genotecas tipo BAC (*Bacterial Artificial Chromosome*) se está construyendo un mapa físico de melocotonero (Horn et al., 2005). Las secuencias ESTs (*Expressed Sequence Tags*) obtenidas como parte del Consorcio Internacional de Genómica de Rosáceas, a

partir de melocotonero, almendro y albaricoquero, permitieron la identificación de varios genes, de los cuales, al menos a 28 de ellos se les ha asignado su posición en el mapa TxE (Fig. 1.19). Algunos de estos genes son importantes para la mejora de cultivares y patrones *Prunus*, y están siendo utilizados para la selección asistida por marcadores (MAS) (Arús et al., 2003; Dirlewanger et al., 2004).

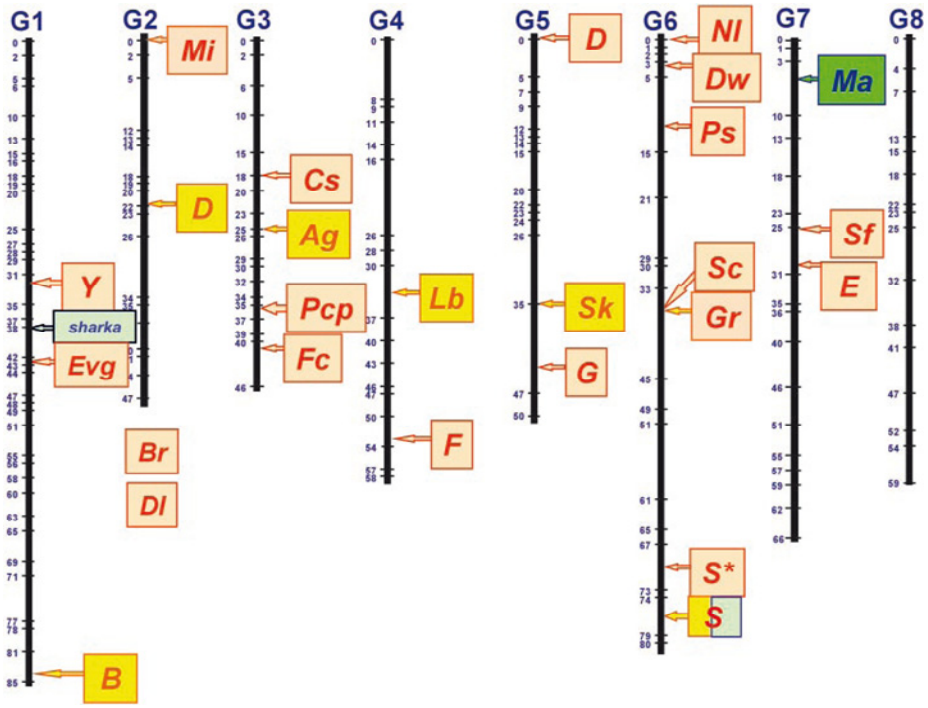

Figura 1.19. Posición aproximada de 28 genes mayores identificados en diferentes poblaciones de albaricoquero (en azul), melocotonero (en naranja), almendro o almendro x melocotonero (en amarillo) y ciruelo (en verde), en el marco del mapa de referencia TxE de *Prunus* (Joobeur et al., 1998). Los nombres abreviados de los genes corresponden a: Y, color de la pulpa; B, color del pétalo en almendro/melocotonero; sharka, resistencia al *Plum pox* virus; Mi, resistencia a nematodos; D, dureza de la cáscara en almendra y fruto no ácido en melocotonero; Br, hábito arbustivo; Dl, doble flor; Cs, coloración alrededor del hueso; Ag, color anteras; Pcp, policarpelo; Fc, color de la flor; Lb, época floración; F, adherencia del hueso; Sk, almendra amarga; G, pubescencia de la piel; NI, forma de la hoja; Dw, planta enana; Ps, esterilidad del polen; Sc, color de la piel del fruto; Gf, color de la hoja; S*, forma del fruto; S, auto incompatibilidad (almendro y albaricoque); Ma, resistencia a nematodos en Mirobolán; E, forma de las glándulas de la hoja; Sf, resistencia a la podredumbre. Los genes Dl y Br se localizan en posiciones desconocidas del G2 (Dirlewanger et al., 2004).

Recientemente, Dirlewanger et al. (2006) describieron por primera vez el carácter fruta abortiva (*Af*) en la var. *platycarpa*. Estos frutos no tienen un crecimiento normal, presentan un rajado característico en la zona pistilar (Fig. 1.20), incluso en estados tempranos de maduración, y caen del árbol unos dos meses después de la floración. Además, estos autores observaron que las flores de estos árboles presentaban pistilos anormales, siendo más cortos y gruesos que los presentes en árboles con frutos no abortivos. Este carácter recesivo fué cartografiado en el grupo de ligamiento 6 (GL6), en el mismo lugar que el gen *S* que controla la forma del fruto (Fig. 1.19). Los resultados de ese trabajo sugieren que ambos caracteres están controlados por un único gen, y que la proteína codificada por dicho gen modula el desarrollo del fruto. Sin embargo, no se descarta la hipótesis de que se trate de dos genes fuertemente ligados que segreguen conjuntamente.

Figura 1.20. Rajado en la zona pistilar, característico de los frutos abortivos.

Algunos de los genes mayores (Fig. 1.19) controlan importantes caracteres de la calidad del fruto mediante herencia mendeliana simple. Sin embargo, la mayoría de los caracteres relacionados con la calidad del fruto son de naturaleza cuantitativa y su control está determinado por la expresión de varios genes o QTLs. El estudio de estos caracteres cuantitativos requiere de familias con un número elevado de individuos, costosas de obtener y de mantener. Se han identificado QTLs que explican algunos de estos caracteres cuantitativos, como el tamaño y diámetro del fruto (Abbott et al., 1998; Hansche et al., 1972; Quilot et al., 2004c), la coloración roja de piel y pulpa (Quilot et al., 2004c; Verde et al., 2002), el contenido de azúcares y ácidos orgánicos (Dirlewanger et al., 1999; Etienne et al., 2002b; Quilot et al., 2004c; Verde et al., 2002), la jugosidad y dulzor (Quilot et al., 2004c), y los síntomas de daños por frío que afectan a la calidad poscosecha del melocotón (2007; Peace et al., 2005a; Peace et al., 2006).

Algunos de los QTLs para características de calidad del fruto, como los que controlan el peso del fruto y su diámetro, el SSC, la acidez valorable, el pH, o las concentraciones de distintos azúcares y ácidos orgánicos, han sido cartografiados en el

mapa consenso TxE de *Prunus* (Arús et al., 2003). La mayoría de estos QTLs tienden a agruparse en regiones específicas de los grupos de ligamiento 4 (G4), 5 (G5) y 6 (G6), coincidiendo con algunos genes mayores para caracteres de calidad (Tabla 1.1).

Tabla 1.1. QTLs descritos para distintos caracteres de calidad del fruto del melocotonero y su localización en el mapa de referencia TxE de *Prunus* (Joobeur et al., 1998).

Carácter	GL*	Poblaciones	Referencias
Azúcares totales	G1	(*P. davidiana* x 'Summergrand') BC2	Quilot et al., 2004
SSC	G2, G4, G6	'Ferjalou Jalusia®' x 'Fantasia'	Etienne et al., 2002
Color de la piel	G2, G6	(*P. ferganensis* x 'IF310828') BC1	Verde et al., 2002
Diámetro del fruto	G3	'Suncrest' x 'Bailey'	Abbot et al., 1998
Peso del fruto	G3	'Suncrest' x 'Bailey'	Abbot et al., 1998
Fructosa	G4	'Ferjalou Jalusia®' x 'Fantasia'	Etienne et al., 2002
Glucosa	G4	'Ferjalou Jalusia®' x 'Fantasia'	Etienne et al., 2002
Sorbitol	G4	(*P. davidiana* x 'Summergrand') BC2	Quilot et al., 2004
Ácido málico	G5	'Ferjalou Jalusia®' x 'Fantasia'	Etienne et al., 2002
pH	G5	'Ferjalou Jalusia®' x 'Fantasia'	Etienne et al., 2002
Sacarosa	G5	'Ferjalou Jalusia®' x 'Fantasia'	Etienne et al., 2002
TA	G5	'Ferjalou Jalusia®' x 'Fantasia'	Etienne et al., 2002

GL*: grupo de ligamiento. Sólo se han considerado aquellos QTLs encontrados al menos en dos evaluaciones independientes en las poblaciones indicadas. SSC: contenido en sólidos solubles; TA: acidez valorable.

Otra estrategia que permitirá la mejora asistida por marcadores es la de los genes candidatos. El mapeo de genes candidatos es una estrategia complementaria al análisis de QTLs que permite la asociación entre genes secuenciados de función conocida y QTLs o genes mayores localizados en el mapa (Arús et al., 2003; Dirlewanger et al., 2004). Esta estrategia fué utilizada por Etienne et al. (2002b) para determinar que uno de los 12 genes candidatos implicados en el metabolismo de azúcares y ácidos de melocotón *(PRUpe-Vp2)*, que correspondía a una pirofosfatasa vacuolar, posiblemente implicada en la acumulación de sacarosa en la vacuola, se localizaba en la misma región del G6 en la que se había descrito un QTL mayor para azúcares y SSC. También mediante el análisis de genes candidatos, Peace et al. (2005b) identificaron que el gen que codifica para la endo-PG, cosegrega con el gen que controla la adherencia del hueso a la pulpa y la firmeza del fruto (F). Además, desarrollaron cebadores a partir del gen de la endo-PG para la selección del carácter firmeza en melocotonero. Esta estrategia, junto con la identificación de genes a partir de genotecas EST desarrolladas a partir de frutos, aportará en un futuro próximo marcadores para su aplicación en la mejora asistida para la calidad del fruto.

Para avanzar en la comprensión de los mecanismos genéticos que controlan el desarrollo de los daños por frío en melocotón y nectarina, se ha construido un mapa de ligamiento (Pop-DG) con una población segregante que procede del cruzamiento entre los cultivares 'Dr. Davis' y 'Georgia Belle' (Ogundiwin et al., 2007; Peace et al., 2005a; Peace et al., 2006). Este mapa ha permitido la identificación de genes y marcadores moleculares (SSRs; RAFs: *Randomly Amplified* DNA *Fingerprint* y SRAPs: *Sequence-Related Amplified Polymorhism*) asociados al control de los daños por frío (Ogundiwin et al., 2007; Peace et al., 2005a). Además, se ha localizado un QTL mayor en el G5 y dos QTLs menores en el G2, para los síntomas de harinosidad, pardeamiento y enrojecimiento de la pulpa (Fig. 1.21) (Ogundiwin et al., 2009a; Ogundiwin et al., 2007; Peace et al., 2005a; 2006). También se ha observado que el gen que codifica para la endo-PG, colocaliza con el QTL mayor que controla la harinosidad y el enrojecimiento de la pulpa en esa población (Peace et al., 2005a; 2005b), lo que sugiere la implicación de esta enzima en el desarrollo de los síntomas de daños por frío. Por otro lado, se ha identificado también un gen de la ruta de biosíntesis de las antocianinas (*leucoanthocyanidin dioxygenase*, PpLDOX), y se ha localizado en la misma región genómica del G5 que el QTL mayor para el pardeamiento de la pulpa (Ogundiwin et al., 2008b). Esta colocalización indica la implicación de las antocianinas del fruto en su predisposición a desarrollar pardeamiento de la pulpa, tras el almacenamiento a bajas temperaturas.

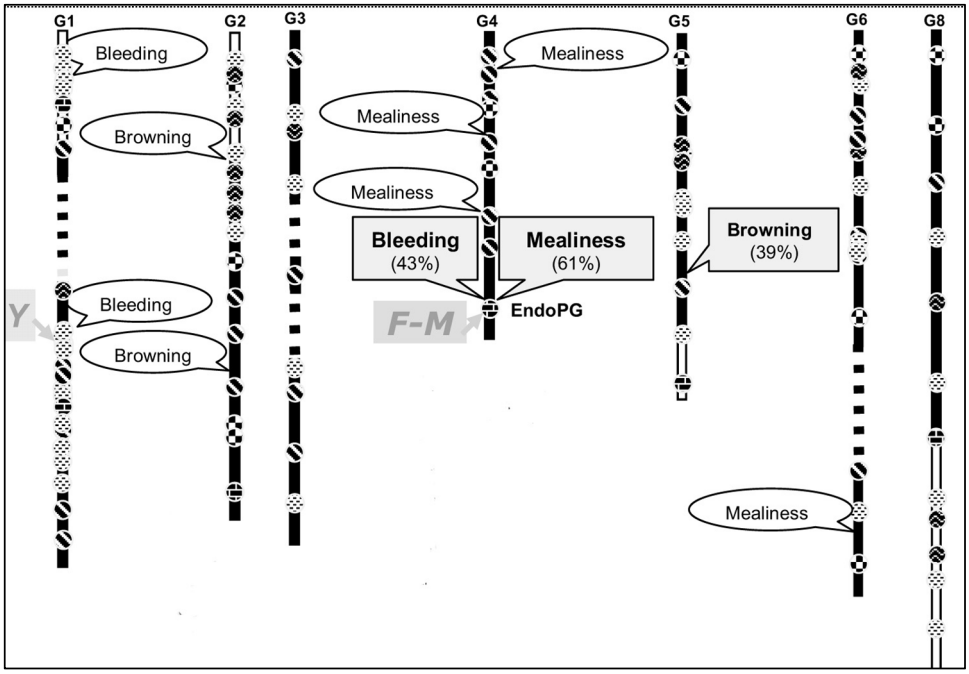

Figura 1.21. QTLs para los distintos síntomas de daños por frío en el mapa de ligamiento Pop-DG (Ogundiwin et al., 2007).

Por último, a partir de dos descendencias procedentes de la población PopDG, se ha desarrollado una base de datos (ChillPeach) que contiene 7.862 ESTs, para la identificación de genes que controlan los daños por frío (Ogundiwin et al., 2008a). Paralelamente a estos estudios, Gónzalez-Agüero et al. (2008) han publicado el desarrollo de un microchip de nylon que contiene 847 ESTs procedentes de una genoteca cDNA construida a partir de pulpa madura de melocotón, para su uso en la comprensión del mecanismo genético que controla la harinosidad. Los resultados de este estudio indican que los cambios moleculares que ocurren durante el desarrollo de este síntoma se deben a cambios en la expresión de 106 genes asociados con el metabolismo de la pared celular y el tráfico de membrana, lo que concuerda con los resultados obtenidos por Peace et al. (2005a; 2005b). La actividad de la mayoría de estos genes (93%) se vio reprimida en los frutos con síntomas de harinosidad.

El reciente desarrollo de las técnicas de mapeo por asociación o análisis del desequilibrio de ligamiento (LD) en los últimos años, augura un gran avance en el análisis de relaciones entre caracteres fenotípicos de interés con marcadores genéticos y la consiguiente selección asistida por marcadores (MAS) (Gupta et al., 2005). Aunque esta técnica ha sido ya utilizada para el mapeo de genes responsables de distintas enfermedades en humanos, su utilización en plantas es más reciente, y por ahora sólo se ha aplicado en algunos cultivos como arroz, maíz, trigo, sorgo o soja (Gupta et al., 2005; Sorkheh et al., 2008). Esta técnica, no sólo permite identificar y cartografíar QTLs, sino que permite también identificar el polimorfismo de un gen responsable de la diferencia entre dos fenotipos alternativos (Gupta et al., 2005). En el mapeo por asociación se utilizan colecciones de cultivares o germoplasma, a diferencia de los cruces biparentales utilizados en el análisis de ligamiento, lo que permite una mayor precisión en la localización de genes o QTLs 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 frutales, un primera aproximación a la utilización de esta técnica se ha llevado a cabo utilizando marcadores tipo SSRs en 225 cultivares de melocotonero (Aranzana et al., 2008; Aranzana et al., 2007). Los resultados han mostrado un alto LD (10-20 cM) para los distintos grupos de cultivares estudiados, que permitirán, junto a los datos fenotípicos disponibles, la selección de grupos apropiados de genotipos para futuros estudios de mapeo por asociación.

Todas estas herramientas y estrategias son y serán claves para profundizar en la comprensión de los mecanismos genéticos que determinan la calidad del fruto, y para la aplicación de la mejora asistida por marcadores en los programas de mejora.

Capítulo 2

Objetivos

El objetivo general de esta Tesis Doctoral es la selección de nuevos cultivares de melocotonero con una alta calidad de fruto adaptados a las condiciones de cultivo del área mediterránea. Los objetivos específicos son:

1. Evaluación de las características agronómicas y parámetros de calidad organoléptica del fruto de los genotipos que conforman las poblaciones de estudio, así como de la variabilidad fenotípica encontrada entre cruzamientos y genotipos con distinto tipo de fruto. Estudio de las relaciones entre parámetros agronómicos y de calidad de fruto (Capítulo 3).
2. Análisis del perfil de azúcares del fruto en una muestra representativa de los genotipos que conforman las poblaciones de estudio. Evaluación de la variabilidad fenotípica y de las diferencias en el perfil de azúcares entre distintos cruzamientos y genotipos con distinto tipo de fruto. Estudio de las relaciones entre la composición de azúcares y otros parámetros de calidad del fruto (Capítulo 4).
3. Análisis del contenido de compuestos bioactivos del fruto en una muestra representativa de los genotipos que conforman las poblaciones de estudio: determinación del contenido en fenoles totales, flavonoides, antocianinas totales, vitamina C y capacidad antioxidante. Evaluación de la variabilidad fenotípica y de las diferencias entre cruzamientos y genotipos con distinto tipo de fruto. Estudio de las relaciones existentes entre dichos compuestos y otros parámetros de calidad del fruto (Capítulo 5).
4. Evaluación de la calidad poscosecha de los frutos en la población de nectarinas ‘Venus’ x ‘Big Top’ mediante la cuantificación de la expresión y la susceptibilidad a los daños por frío. Estudio del efecto del año y la duración del periodo de almacenamiento sobre el desarrollo y la severidad de los síntomas. Evaluación de los mecanismos genéticos que controlan los daños por frío en esta población mediante mapeo del grupo de ligamiento 4 y búsqueda de QTLs implicados en el desarrollo de este desorden fisiológico (Capítulo 6).

Capítulo 3

Phenotypic diversity and relationships of agronomic and fruit quality traits

3.1. ABSTRACT

Agronomic and fruit quality traits were evaluated and compared for three consecutive years on 1111 seedlings coming from fifteen peach and nectarine breeding crosses, grown under a Mediterranean climate. Significant differences among and within the different progenies were found for most of the traits analyzed. The breeding population segregated for several Mendelian characters such as peach or nectarine fruit, round or flat fruit, yellow or white flesh and freestone or clingstone. In addition, aborting fruit flat trees were found in our progeny, and our data seem to support that this trait is controlled by a multi-allelic gene controlling both flat shape and aborting fruit. The variation within the progenies of some traits such as blooming and harvesting date, yield, fruit weight and SSC was continuous, suggesting a polygenic inheritance. Relationships between qualitative pomological traits and these agronomic and fruit quality parameters were also found. Valuable correlations among agronomic and fruit quality parameters were found, although coefficients variation depending on the progeny should be considered. In addition, principal component analysis (PCA) revealed several relationships among quality traits in the evaluated progenies. Based on this evaluation, 26 outstanding genotypes were pre-selected from the initial breeding population for further studies.

3.2. INTRODUCTION

Peach and nectarine [*Prunus persica* (L.) Bastch] are the second most important fruit crop in the European Union (EU) (approximately 4.3 million tons) after apple (FAOSTAT, 2007), and the most important within the genus *Prunus*. Spain is the second producer in Europe and the third in the world with a production of more than one million tons (FAOSTAT, 2007). Among temperate fruit crops, the peach breeding industry is one of the most dynamic and new cultivars are released every year (Byrne, 2002; Fideghelli et al., 1998; Sansavini et al., 2006).

The creation of cultivars through controlled cross pollinitation in peach was first done by Thomas A. Knight in 1806 (Brown, 1975). At present, the most common method for producing new cultivars is via cross of chosen parents. The resulting full-sib families are planted in trials from which the best genotypes, that share the most appropriate combination of traits after evaluation, are selected (Martínez-Calvo et al., 2006; Nicotra et al., 2002; Scorza and Sherman, 1996). The selected seedlings are then budded for clonal testing (Brown, 1975). This is the method used in the present work that deals with fifteen progenies derived from crosses between commercial and/or pre-selected peach cultivars, reaching up to one thousand seedlings. We search for superior peach and nectarine cultivars for the Spanish industry with good adaptation to Mediterranean conditions when grown in the Ebro Valley, one of the biggest production areas in Europe. Most crosses were aimed at developing improved yellow, melting flesh peaches with emphasis on red skin color, good size and firmness and enhance flavor. A few crosses in the program were made for development of improved yellow nectarines, white flesh peaches and flat shape fruits (Moreno, 2005). Besides lowering the production costs and improving pest and disease resistance, breeding objectives of this program include extension of the harvest calendar, new fruit types for mild-winter climate areas, and improvement of fruit quality (shape, flesh and skin color, firmness, flavor, etc.). Like other temperate fruits, peach has chilling and heat requirements for flowering. Early flowering is a desirable character in many breeding programs in Mediterranean areas to obtain earliest yield although spring frosts may reduce production in some years. Extension of the harvest period with very early, as well as late-maturing peach genotypes, is of considerable interest for the peach industry in this area, in order to supply the market for a longer period of time (Badenes et al., 2006; Byrne, 2003; Carusso and Sottile, 1999; Martínez-Calvo et al., 2006).

On the other hand, breeders have traditionally selected primarily for external fruit quality (fruit size and appearance), being organoleptic and nutritional traits a secondary goal (Byrne, 2002; Hilaire, 2003). However, nowadays fruit quality is fundamental for the acceptance of peach and nectarine cultivars by consumers, due to the high competition in

the market with numerous new released cultivars and other fruit species (Crisosto and Crisosto, 2005; Crisosto et al., 2006b; Iglesias and Echeverría, 2009). Quality was defined by Kramer and Twigg (1966) as being composed of those chemical and physical characteristics that give a product consumer appeal and acceptability. Skin appearance (color and freedom from defects), texture, flavor and sugar and acid content are key factors that determine high-quality fresh peaches and nectarines. Also, the shape and proportions of the fruit are aspects of interest to the consumers (Badenes et al., 2006). All these parameters may not be independent from each other, and therefore, should be studied as a whole and should be considered in breeding programs dealing with fruit quality.

Different studies have investigated the phenotypic diversity and relationships of fruit quality traits in peach and nectarine and other fruits germplasm such as apricot (Brooks et al., 1993; Byrne et al., 1991; Esti et al., 1997; Génard and Bruchou, 1992; Ruiz and Egea, 2008). However, there is limited information on the global evaluation of fruit quality in breeding progenies and their relationships with pomological traits. In this study, we investigated different agronomic and fruit quality parameters in fifteen peach and nectarine breeding populations over three consecutive years. The aims of this work were to evaluate the existing phenotypic diversity among and within the breeding progenies, and to study the relationships among agronomic and fruit quality parameters, and with qualitative pomological traits linked to the fruit quality. In addition, principal component analysis was carried out to study correlations among variables and to establish relationships among breeding crosses regarding fruit quality attributes. The materials evaluated are representative of the germplasm available for peach breeding in the Mediterranean area. The high number of genotypes evaluated for many traits, with large genetic variability, will improve the knowledge of the genetic studies on this crop and this will constitute a helpful tool in the future to be applied in peach breeding programs.

3.3. MATERIAL AND METHODS

3.3.1. Plant material

Fifteen controlled biparental crosses between nineteen peach and nectarine commercial and/or pre-selected cultivars (Table 3.1) were made during 2000 and 2001. The resulting seedlings were budded on the same rootstock (GF-677) and established (one tree per genotype) in an experimental orchard at the Experimental Station of Aula Dei-CSIC (Northern Spain, Zaragoza) in 2002. Trees were trained to the standard open vase system and planted at a spacing of 4 m x 2.5 m. Hand thinning was carried out to

reduce fruit load when required. Trees were grown under standard conditions of irrigation, fertilization and pest and disease control. Vegetative and fruit quality traits have been evaluated in a total of 1111 genotypes over three consecutive years (2005-2007). All traits were measured or scored for each seedling tree separately over the three-year period and means of three years were calculated. Finally, superior genotypes were selected by independent culling of the most important agronomic (harvest date and production) and fruit quality traits (fruit weight, soluble solid content, acidity, skin blush, endocarp staining and firmness) evaluated.

3.3.2. Agronomic and quality traits

During the years 2005, 2006 and 2007, agronomic and fruit quality traits were measured individually in each seedling tree. Blooming date was recorded for each progeny according to Fleckinger (1945), i.e., the average date for bloom beginning (E stage), full bloom (F stage) and bloom end (G stage) was scored in each progeny. The mean harvesting date was also calculated for each progeny. Fruits were considered ripe in the tree when their growth had stopped, they began softening, exhibited yellow or orange ground color (which is also representative for each cultivar) and were easily detached. Harvesting date ranged from late-May to mid-September, depending on the genotypes.

Table 3.1. Peach and nectarine commercial and experimental (VAC-) cultivars used as progenitors in the fifteen controlled crosses. Fruit type (round or flat, peach or nectarine), flesh color (yellow or white) and stone adherence (free or cling) for each progenitor is shown.

Cultivar	Fruit type	Flesh colour	Stone
Andross	peach	yellow	cling
Babygold-9	peach	yellow	cling
Big Top	nectarine	yellow	cling
Calante	peach	yellow	cling
Crown Princess	peach	yellow	cling
O´Henry	peach	yellow	free
Orion	peach	yellow	free
Red Top	peach	yellow	free
Rich Lady	peach	white	free
VAC-9510	peach	yellow	cling
VAC-9511	peach	yellow	free
VAC-9512	peach	yellow	free
VAC-9513	nectarine	yellow	free
VAC-9514	nectarine	white	free
VAC-9515	nectarine	yellow	free
VAC-9516	peach	white	free
VAC-9517	flat peach	white	free
VAC-9520	peach	yellow	free
Venus	nectarine	yellow	free

Yield (kg/tree) was determined for each seedling tree recording also the total number of fruits. From these measurements the total average fruit weight was calculated. For the evaluation of fruit quality parameters a representative sample of 30 fruits per tree was selected. The agronomic characters segregating as simple characters were recorded, i.e. peach or nectarine, yellow or white flesh, round or flat fruit, aborting or non-aborting fruit, and freestone or clingstone. Some other pomological traits such as skin blush, stone adhesion, endocarp staining, or fruit shape (diameters), were scored using the rating scales appropriated for each of them. Skin blush was scored as the percentage of skin surface with red color. Stone adhesion and endocarp staining (redness around stone) were scored in an increasing arbitrary scale from 1 to 10. We also measured the three dimensions of the whole fruit: height (H), suture diameter (SD) and cheek diameter (CD). From these parameters, esfericity was calculated as H/SD and H/CD. The suture deformation index (SDI) was estimated as SD/CD (Wert et al., 2007). When the ratios are 1, the shape is considered as globally round. When they are different from 1, the shape is oval, flatted or with protruding sutures. The soluble solids content (SSC) of the juice was

measured with a temperature compensated refractometer (model ATC-1, Atago Co., Tokyo, Japan); and data are given as ºBrix. The titratable acidity (TA) was determined by titration with NaOH 0.1 N to pH 8.1 (AOAC, 1984). Data are given as g malic acid per 100 g fresh weight (FW), since this is the dominant organic acid in peach (Sweeney et al., 1970; Wills et al., 1983). Flesh firmness was determined on opposite sides of the equator of each fruit with a penetrometer fitted with an 8-mm diameter probe on 5 fruit from each tree. The two readings were averaged for each fruit, and data are given as Newtons (N).

3.3.3. Data analysis

All statistical analyses were performed using SPSS 17.0 for Windows (Chicago, IL). To obtain basic statistics for the entire plant material studied, number of observed seedlings, maximum and minimum value, mean, mean standard error and standard deviation for each trait were calculated. Results were analyzed by considering cross and year as fixed factors, and seedling within crosses plus the interaction of seedling with year, as the residual term. Differences between crosses for each trait were analyzed by Duncan's multiple range test ($P \leq 0.05$). When comparing between different fruit types (peach or nectarine, round or flat, yellow or white flesh) *t* test ($P \leq 0.05$) was used. Correlation between traits to reveal possible associations was calculated with raw data based on single plant estimates over the three years, using Pearson correlation coefficient at $P \leq 0.05$. Principal components analysis (PCA) was performed with family means to determinate the relationships among progenies and to obtain an overview of correlation among fruit quality traits.

3.4. RESULTS AND DISCUSSION

3.4.1. Blooming and harvesting dates

Blooming and harvesting dates for the fifteen breeding progenies averaged over the three years of study are shown in Fig. 3.1. Early flowering is a desirable character in Mediterranean areas to obtain earliest yields (Carusso and Sottile, 1999; George and Nissen, 1992) even though spring frosts may reduce production in some years. Although no big differences were found among progenies for the bloom beginning, larger differences were observed for the full bloom and larger bloom end, due to the existing differences on the blooming period length for the different progenies. Blooming date is considered as a quantitative trait in peach and other *Prunus* species (Dirlewanger et al., 1999; Vargas and Romero, 2001). Thus, the differences for the blooming date found

among the seedlings within any progeny from the fifteen studied were somehow expectable.

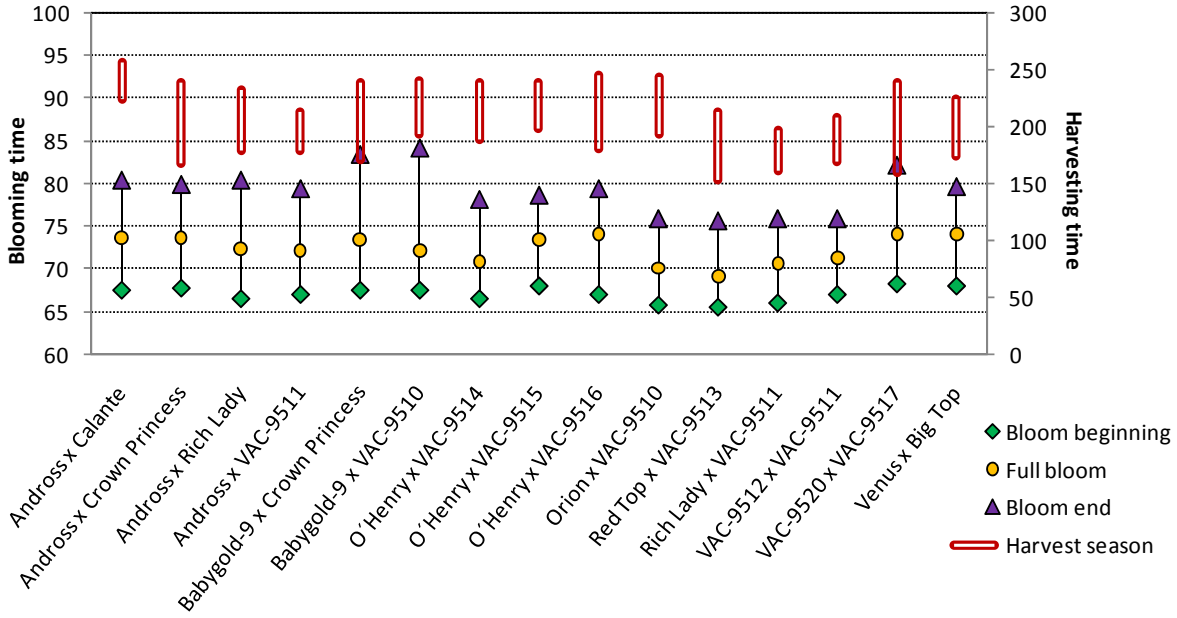

Figure 3.1. Blooming and harvesting time (in Julian days) for the fifteen peach and nectarine breeding progenies. Bloom beginning (E stage), full bloom (F stage) and bloom end (G stage) dates, determined according to Fleckinger (1945). Data are means of 3 consecutive years for each peach and nectarine breeding progeny subjected to assessment.

Regarding harvesting time (Fig. 3.1), large variations were found in the harvest season among the tested genotypes ranging from late-May to mid-September. The earliest seedlings to be harvested (late-May) belonged to the 'Red Top' x 'VAC-9513' progeny. The latest seedlings were those from the 'Andross' x 'Calante' progeny, which were harvested from mid-August to mid-September. The harvesting time showed a normal distribution within each progeny for all the crosses, reflecting a quantitative genetic control. This trait has been established as characteristic of each cultivar, and quantitatively inherited (Dirlewanger et al., 1999; Vargas and Romero, 2001). This variability allows selecting the most interesting harvesting date among the genotypes in order to cover market demands (Byrne, 2003).

Although blooming and harvesting time may change every year depending on the environmental conditions, especially temperature (Mounzer et al., 2008; Ruiz and Egea, 2008; Sánchez-Pérez et al., 2007b), the fruit development period (number of days from full bloom to maturity) remained more or less stable for each seedling over the three years of study. The peach fruit development period is highly dependent on cultivar (Cunha et al.,

2007; Cheng, 2008; Jackson and Sherman, 1980; Mounzer et al., 2008; Muñoz et al., 1986), however, previous research has shown an influence of spring temperatures on the harvest date of peach cultivars (López and Dejong, 2007). Very early-maturing, as well as very late-maturing peach genotypes, are of considerable interest for the peach industry in the Mediterranean area (Carusso and Sottile, 1999), and the main difference between these genotypes is the length of their fruit development period (Mounzer et al., 2008). In the present work, the fruit development period ranged from 80 to 130 days for all the progenies, except for 'Andross' x 'Calante' which showed the longest period (approximately 150 days). Consequently, this was the latest progeny to be harvested (Fig. 3.1). Shortest fruit development period, and earliest harvest season, was found in 'Andross' x 'Crown Princess', 'Red Top' x 'VAC-9513', and 'VAC-9520' x 'VAC-9517' progenies. This interesting trait, among others, was valued in the selection of eleven genotypes from these three progenies.

3.4.2. Cross influence on agronomic and fruit quality traits

Cumulative and annual yield showed a large range of variation among the breeding seedlings (from 0.0 to 137.7 kg for cumulative yield and from 0.0 to 80.8 kg for annual yield). Both of them were significantly different among the fifteen studied progenies (Table 3.2). 'Andross' x 'VAC-9511' and 'Babygold-9' x 'VAC-9510' showed the significant highest average cumulative yield (54.4 and 55.6 kg, respectively) and annual yield (18.3 and 18.9 kg, respectively) among the progenies. On the other hand, 'Andross' x 'Calante' showed the lowest cumulative and annual yield (13.5 and 5.8 kg, respectively) without being significantly different from 'Rich Lady' x 'VAC-9511', 'VAC-9512' x 'VAC-9511' and 'Venus' x 'Big Top' progenies. The combination of 'Andross' with 'Crown Princess', 'Rich Lady', and 'VAC-9511' resulted in higher productive progenies whereas yields were dramatically decreased when it was crossed with 'Calante' cultivar. 'O'Henry' performed similarly in terms of yield for any of the three studied crosses, inducing intermediate yields in their seedlings. Significant differences among seedlings within the progenies were also found. This variability supports the quantitative genetic control reported in peach for yield (Dirlewanger et al., 2004; Dirlewanger et al., 1999).

Fruit weight is a major quantitative inherited factor determining yield, fruit quality and consumer acceptability (Dirlewanger et al., 1999). There was more than 10-fold range (28.9 to 370.3 g) in mean fruit weight among the breeding seedlings, due to the influence of genotype, cultivar and type of fruit (flat and round peach). This result agrees with previous works where high variability in this parameter has been described among peach (Iglesias and Echeverría, 2009; Quilot et al., 2004b) and apricot genotypes (Ruiz and

Egea, 2008). The highest mean fruit weight was found in 'Orion' x 'VAC-9510' progeny (Table 3.2), in spite of being one of the most productive crosses. 'Babygold-9' seemed to induce big fruits in its offspring, although the two other progenitors, involved in crosses with this cultivar, showed also big fruits in different cross combinations. A tendency of having higher mean fruit weight was found for the latest harvesting crosses, such as 'Orion' x 'VAC-9510', 'Babygold-9' x 'VAC-9510', and 'Andross' x 'Calante' which agrees with previous works in peach and apricot, where a positive correlation between harvesting date and fruit weight has been reported (Dirlewanger et al., 1999; López and Dejong, 2007; Ruiz and Egea, 2008). Conversely, 'VAC-9520' x 'VAC-9517' showed the lowest fruit weight among the breeding progenies. 'VAC-9517' is a flat peach (Table 3.1) that segregates for the S gene, which is a dominant gene controlling fruit shape (*S-,* flat or *ss,* round) (Lesley, 1940). The lower mean fruit weight showed by its offspring agrees with the previously described control for fresh weight and productivity detected by Lesley (1940) and the QTL detected by Dirlewanger et al. (1999) near the S gene. This result is corroborated by the significant difference found when comparing yield and fruit weight between round and flat fruits in our study (Table 3.4).

Table 3.2. Agronomic and fruit quality traits for each peach and nectarine breeding progenies subjected to assessment. The number of observed seedlings (n) is shown for each progeny.

Progeny	n	Cumulative yield (kg)	Annual yield (kg)	Fruit weight (g)	Blush (%)	Endocarp staining[a]	SSC (ºBrix)	pH	TA (g 100 g^{-1} FW)	RI	Firmness (N)
1 Andross x Calante	118	13.5 h	5.8 g	185.4 cd	24.8 j	1.7 g	12.6 bc	3.63 a	0.55 hi	23.4 ab	27.2 bc
2 Andross x Crown Princess	25	46.3 b	15.4 b	168.8 ef	57.5 fg	2.9 f	10.8 efg	3.59 ab	0.50 i	21.8 bcd	27.1 bc
3 Andross x Rich Lady	47	40.6 bc	14.5 b	167.6 ef	65.6 de	3.5 def	10.6 fg	3.53 bcd	0.74 c	14.7 ij	23.8 de
4 Andross x VAC-9511	25	54.4 a	18.3 a	173.8 de	69.6 d	2.6 fg	11.3 def	3.46 def	0.61 fgh	18.6 fg	23.6 de
5 Babygold-9 x Crown Princess	102	31.4 d	11.1 cd	187.6 bcd	37.6 i	3.3 ef	10.2 g	3.58 abc	0.52 i	20.0 def	24.0 d
6 Babygold-9 x VAC-9510	130	55.6 a	18.9 a	203.0 b	51.6 h	4.9 bc	11.1 defg	3.63 a	0.59 gh	19.4 ef	28.7 b
7 O´Henry x VAC-9514	159	24.0 efg	9.2 de	187.0 bcd	71.2 cd	6.3 a	14.2 a	3.53 bcd	0.67 cdef	22.2 bc	27.3 bc
8 O´Henry x VAC-9515	75	26.3 def	10.4 d	195.9 bc	70.7 d	6.6 a	13.9 a	3.48 def	0.69 cde	21.0 cde	27.6 bc
9 O´Henry x VAC-9516	99	38.7 c	13.1 bc	154.8 fg	54.8 gh	4.2 cde	11.8 cd	3.48 def	0.63 efg	19.1 efg	21.2 e
10 Orion x VAC-9510	15	41.8 bc	14.1 b	223.7 a	62.2 ef	5.9 ab	11.3 def	3.42 ef	0.73 cd	15.6 hij	29.3 ab
11 Red Top x VAC-9513	100	22.8 fg	8.6 def	119.7 i	67.8 de	4.9 bc	10.7 efg	3.46 def	0.63 efg	17.5 gh	22.5 de
12 Rich Lady x VAC-9511	25	19.6 gh	7.7 efg	128.1 hi	90.4 a	3.6 def	11.0 defg	3.32 g	0.82 b	13.7 jk	31.6 a
13 VAC-9512 x VAC-9511	40	19.9 fgh	8.8 def	141.0 gh	89.5 a	4.6 cd	11.6 de	3.40 fg	0.96 a	12.3 k	29.3 ab
14 VAC-9520 x VAC-9517	76	30.1 de	10.6 d	97.3 j	76.7 c	3.2 ef	12.9 b	3.34 g	0.83 b	16.0 hi	25.1 cd
15 Venus x Big Top	75	18.1 gh	6.6 fg	190.6 bc	83.3 b	4.3 cde	14.6 a	3.50 cde	0.66 def	25.3 a	31.4 a

Mean separation within columns by Duncan´s test ($P \leq 0.05$). In each column, values with the same letter are not significantly different. *Abbreviations:* SSC: soluble solids content; TA: titratable acidity; RI: ripening index (SSC/TA)
[a] Endocarp staining was scored in an increasing arbitrary scale from 1 to 10

It is noticeable the finding of aborting fruits in the flat seedling trees from 'VAC-9520' x 'VAC-9517' progeny, as seen by Dirlewanger et al. (2006), who reported this character for the first time in other genotypes. These trees had flowers, but fruits started to fall 2 months after blooming. Fruits that fell prematurely displayed a crack on the pistilar side, even when they were very small (Fig. 3.2) as observed by Dirlewanger et al. (2006).

These authors studied this character in a F2 progeny obtained from the self-pollination of a single tree, derived by crossing 'Ferjalou Jalousia®' with 'Fantasia'. They suggested that both characters, flat fruit and aborting fruit, are controlled by a single gene, and the protein encoded by this gene modulates fruit development. Therefore, the *s* recessive allele responsible for the round shape appeared to correspond to a necessary gene for fruit development. However, they did not discard the possibility of two linked genes: the dominant allele of the *S* gene being linked to the recessive allele of the *Af* gene. In contrast, our data do not seem to support this hypothesis. If *Flat fruit* and *Aborting fruit* were separate loci, some round aborting fruit phenotypes would be expected to occur in our progeny as recombinants between the loci. Thus, our morphological data supports the hypothesis by Dirlewanger et al. (2006) that *S* and *Af* are at the same locus, with different alleles. However, under their hypothesis, we would have not found aborting fruits, since we have not homozygous genotypes (SS) for flat locus in our progeny. Therefore, we suggest the introduction of a third null allele (*n*) that has the same effect that the absence of the *s* recessive allele, supposed to be necessary for fruit development. The understanding of this trait is very important since marker assisted selection could be used to identify genotypes which will bear no fruits at maturity. Further investigation is necessary to elucidate the mechanism of this premature fall and its genetic control.

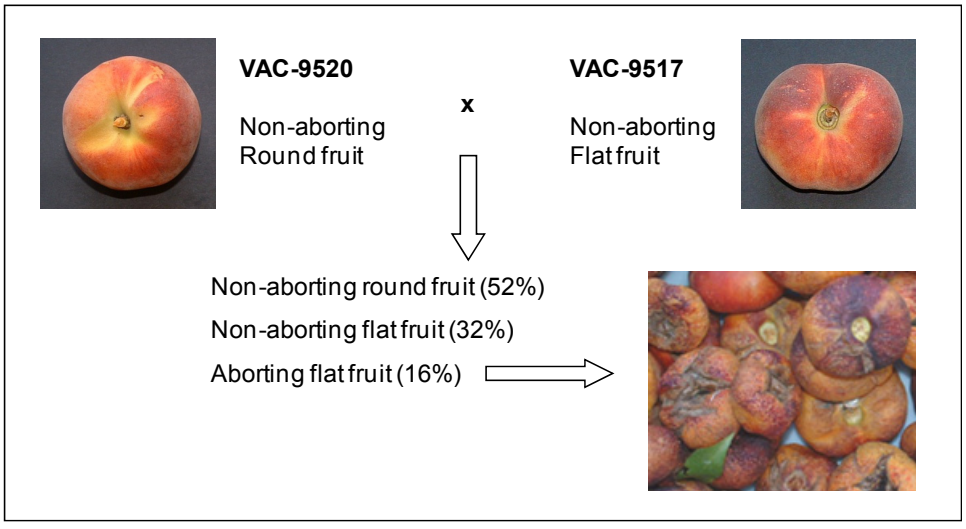

Figure 3.2. Segregation of the 'VAC-9520' x 'VAC-9517' breeding progeny around the fruit shape and aborting fruit traits.

Skin fruit color of peaches and nectarines has a significant impact on consumer acceptance and sales (Liverani, 2002; Scorza and Sherman, 1996). The percentage of skin blush on the fruit surface greatly varied among the breeding seedlings (from 8 to 100%) and significant differences were also found among different progenies (Table 3.2).

'Rich Lady' x 'VAC-9511' and 'VAC-9512' x 'VAC-9511' progenies showed the highest percentage with more than 89% of blush color on the fruit surface, which positively influences their attractiveness. On the other hand, 'Andross' x 'Calante' showed the lowest percentage with less than 25%, having the less attractive fruit among the crosses, since fruit color intensity is nowadays positively related to consumer acceptance for fresh market (Iglesias and Echeverría, 2009). This unfavorable trait, together with low productivity, made that no genotypes were pre-selected from this progeny.

Significant differences were detected among progenies for soluble solids content (SSC) (Table 3.2). Even though SSC values ranged from 7.6 to 24.6 ºBrix among the breeding seedlings, most of them had SSC levels greater than 11 ºBrix. The highest value (24.6 ºBrix) was recorded by a seedling derived from the cross between 'Venus' (an acid nectarine) and 'Big Top' (a non-acid nectarine with high SSC). The minimum SSC established by the EU to market peaches and nectarines is 8 ºBrix [Comission Regulation (EC) No. 1861/2004 of 28 October 2004], although SSC below 11 ºBrix are generally unacceptable to consumer (Crisosto and Crisosto, 2005; Hilaire, 2003; Kader, 1999). However, the relationship between SSC and consumer acceptance is cultivar specific, and there is not a single reliable SSC that assures a given percentage of satisfied consumers (Crisosto and Crisosto, 2005; Hilaire, 2003; Kader, 1999). A tendency of showing the highest SSC values can be observed by the latest harvesting date progenies such as 'Andross' x 'Calante', 'O'Henry' x 'VAC-9514', and 'O'Henry' x 'VAC-9515' (Table 3.2), although no significant correlation was found between harvesting time and SSC. A positive correlation between later harvesting date and SSC has been previously reported in peach and apricot (Dirlewanger et al., 1999; Ruiz and Egea, 2008). A big variability was also found for the SSC among seedlings within the progenies (Cantín et al., 2006), which can be explained by the quantitative regulation of this quality trait (Dirlewanger et al., 1999; Quilot et al., 2004c). This variability allows selecting the most interesting seedlings in terms of sweetness, as it was the case of some pre-selections from 'VAC-9520' x 'VAC-9517' and 'O´Henry' x 'VAC-9514' progenies.

Regarding pH and TA, significant differences were also found among progenies because both are cultivar dependent traits (Table 3.2). There was a five-hold range in TA (from 0.30 to 1.50 g malic acid per 100 g FW) whereas pH ranged from 2.80 to 5.50. Because of different scales, a small change in pH represented a large change in TA. The mean fruit juice pH of different progenies ranged from 3.32 to 3.63. These values are usual for normal acidity fruit. Even in the progeny derived from a cross between the acid nectarine 'Venus' and the non-acid 'Big Top', the average pH value was common for normal acid fruits. All the mean TA values, except for the 'VAC-9512' x 'VAC-9511' progeny, were lower than 0.9%, which is considered the maximum limit for normal acidity

peaches (Hilaire, 2003). Due to the strong correlation between TA and the perception of sourness (Crisosto et al., 2006a), fruits from 'VAC-9512' x 'VAC-9511' would be the tartest among all the studied progenies. TA also plays an important role in consumer acceptance for grapes (Nelson et al., 1973), cherries (Crisosto et al., 2003), and kiwifruit (Marsh et al., 2004). However, the perception of acidity in the mouth depends not only on the acid concentration but also on the type of acid (Pangborn, 1963) and on the concentration and type of sugars (Bassi and Selli, 1990). The high TA, low SSC and consequently low ripening index showed by the 'VAC-9512' x 'VAC-9511' progeny, together with its low yield and fruit weight, resulted in a non interesting progeny for the selection of new high fruit quality genotypes.

Ripening indexes (RI) ranged from 6.5 to 45.7 among the breeding seedlings, depending on their SSC and TA. The sugar-acid ratio is commonly used as a quality index (Bassi and Selli, 1990; Robertson et al., 1989), and higher ratios are usually preferred. Crisosto et al. (1997) observed that RI showed a closer relationship with eating quality than did TA or SSC individually, and Harker et al. (2002a) reported that RI was the best predictor of apple flavor. The RI also plays an important role in consumer acceptance of some peach, nectarine, and plum cultivars (Crisosto and Crisosto, 2005; Crisosto et al., 2004b; Iglesias and Echeverría, 2009). The 'Venus' x 'Big Top' progeny showed the higher RI, being not significantly different from the 'Andross' x 'Calante' progeny (Table 3.2). As expected, higher RI values were usually found in families with the highest SSC. However, some of the progenies with high SSC had low RI because of their high TA, as shown by the two progenies with 'Rich Lady' as a parent and in 'VAC-9512' x 'VAC-9511'. Nevertheless, optimal sugar and acid contents for peaches and nectarines are not universal criteria and they can change with diverse consumer ethnic groups (Crisosto et al., 2006a). High sugar contents and, to a lower extent, high acid contents seem to be favorable to fruit quality as evaluated by consumers (Crisosto and Crisosto, 2005). This equilibrated flavor, combined with other interesting traits, was appreciated in the selection of some genotypes from 'VAC-9520' x 'VAC-9517' and 'Venus' x 'Big Top' progenies. However, in a recent study with nectarines (Iglesias and Echeverría, 2009) the consumer acceptance was always greater for non-acid than for acid cultivars, even at early or advanced stages of fruit maturity. Bassi and Selli (1990) also reported that the high acidity explained the unsatisfactory taste of some acid peach cultivars. The phenotypic variation found in our progenies indicates that there is a genetic potential to develop peaches with optimum sugar and acid contents. Due to their organoleptic relevance, these traits were considered in every pre-selected genotype.

Fruit firmness measured on both cheeks of the fruit was highly variable among all the studied seedlings (from 10.5 to 48.6 N). Maximum levels of fruit firmness for marketing

fresh peaches and nectarines are set by the EU at 63.7 N with an 8 mm diameter probe [Commission Regulation (EC) No. 1861/2004]. Mean firmness values for all the progenies were in the standard commercial firmness range, and more precisely in the range considered as "ready to buy" (18-35 N) (Crisosto et al., 2001a). These authors segregated peaches and nectarines into different classes by using firmness thresholds indicating critical changes during postharvest ripening and susceptibility to bruising damage. The classification of fresh peaches and nectarines into "ready to eat" and "others" was accomplished by using an 18 N threshold. Fruit between 18 and 35 N was considered "ready to buy", and the 35 N threshold was used to define "mature and immature" fruit. Among the breeding progenies, the highest mean fruit firmness was found in 'Rich Lady' x 'VAC-9511' and 'Venus' x 'Big Top' (31.6 and 31.4 N, respectively) (Table 3.2), without being significant different from 'Orion' x 'VAC-9510' and 'VAC-9512' x 'VAC-9511' progenies. It must be noted that the firmest fruit means were found in crosses with nectarines in their progeny, which is corroborated by the significant differences found for firmness between nectarine and peach fruit. Such result has been already observed by other authors (Crisosto et al., 2001a; Valero et al., 2007). On the contrary, lower mean fruit firmness was found in 'O´Henry' x 'VAC-9516' although it was not significantly different from 'Andross' x 'Rich Lady', 'Andross' x 'VAC-9511' and 'Red Top' x 'VAC-9513'. Firmness is an important fruit quality trait to consider in a breeding program, since it is directly related with susceptibility to mechanical damage during postharvest (Crisosto et al., 2001c; Kunze et al., 1975). This trait was highlighted for the pre-selection of some genotypes, such as some seedlings from 'Rich Lady' x 'VAC-9511', 'Venus' x 'Big Top', and 'Babygold-9' x 'VAC-9510' progenies.

Significant differences were also found for fruit shape and size among the studied progenies (Table 3.3). 'Orion' x 'VAC-9510' showed the biggest fruits among the crosses with increased height (H), suture diameter (SD) and cheek diameter (CD), as it was confirmed by its highest mean fruit weight. After 'Orion' x 'VAC-9510' progeny, the highest fruits were found within 'Venus' x 'Big Top', 'Babygold-9' x 'VAC-9510', and 'O'Henry' x 'VAC-9515' progenies. Global shape of fruit (sfericity) was characterized by calculating H/SD and H/CD (Wert et al., 2007). All the populations showed ratios very close to 1 (except 'VAC-9520' x 'VAC-9517' progeny, since it has flat fruits among its offspring), which means that fruits were almost spherical. Fruits from 'Venus' x 'Big Top' and 'Orion' x 'VAC-9510' progenies were significantly more elongated (H/SD and H/CD greater) than the rest of progenies. Fruit shape is an important fruit quality attribute, since it influences consumer's acceptance and postharvest handling. In peach and nectarine, round shapes without protruding tips are preferred by consumer (Badenes et al., 2006). In addition, protruding tips and sutures can be bruised during handling and shipping of fruit and are

therefore undesirable traits for commercial peaches (Kader, 2002). Significant differences in suture deformation among crosses were detected by calculating suture deformation indexes (SDI), although values were always close to 1. SDI ranged from 1.0 for 'Rich Lady' x 'VAC-9511' to 0.95 for 'Andross' x 'Crown Princess' and 'Venus' x 'Big Top' progenies.

Table 3.3. Mean of fruit dimensions for each peach and nectarine breeding progenies subjected to assessment. The number of observed seedlings (n) is shown for each progeny.

Progeny	n	H (mm)	SD (mm)	CD (mm)	H/SD (mm)	H/CD (mm)	SDI (mm)
1 Andross x Calante	118	72.3 cde	73.6 cd	77.0 bc	0.98 cde	0.94 cde	0.96 ef
2 Andross x Crown Princess	25	68.8 f	71.3 de	75.2 c	0.97 cdef	0.92 e	0.95 f
3 Andross x Rich Lady	47	70.7 def	74.2 bc	77.1 bc	0.95 def	0.92 e	0.96 cdef
4 Andross x VAC-9511	25	70.5 ef	73.8 c	77.0 bc	0.96 cdef	0.92 e	0.96 def
5 Babygold-9 x Crown Princess	102	72.5 cde	73.5 cd	75.9 c	0.99 bc	0.96 abcd	0.97 cde
6 Babygold-9 x VAC-9510	130	75.0 bc	76.2 ab	79.2 ab	0.98 cd	0.95 bcde	0.96 def
7 O´Henry x VAC-9514	159	70.2 ef	73.7 cd	75.0 c	0.95 def	0.94 cde	0.98 b
8 O´Henry x VAC-9515	75	73.5 bcd	74.4 bc	76.2 c	0.99 bc	0.97 abc	0.98 bc
9 O´Henry x VAC-9516	99	64.6 g	68.2 f	69.9 d	0.95 ef	0.92 de	0.98 bcd
10 Orion x VAC-9510	15	78.9 a	77.6 a	81.0 a	1.02 ab	0.97 ab	0.96 def
11 Red Top x VAC-9513	100	62.7 g	64.4 g	67.4 e	0.98 cde	0.93 cde	0.96 ef
12 Rich Lady x VAC-9511	25	65.2 g	69.7 ef	69.8 de	0.94 f	0.94 cde	1.00 a
13 VAC-9512 x VAC-9511	40	65.0 g	68.3 f	70.2 d	0.95 def	0.93 de	0.97 bcd
14 VAC-9520 x VAC-9517	76	49.0 h	61.7 h	64.3 f	0.79 g	0.76 f	0.96 def
15 Venus x Big Top	75	75.5 b	73.0 cd	76.9 bc	1.03 a	0.98 a	0.95 f

Mean separation within columns by Duncan´s test ($P \leq 0.05$). In each column, values with the same letter are not significantly different. *Abbreviations*: H, height; SD, suture diameter; CD, cheek diameter; SDI, suture deformation index.

3.4.3. Influence of pomological traits on agronomic and fruit quality traits

Significant differences were found among fruit types for the different agronomic and quality traits evaluated (Table 3.4). As previously reported (Dirlewanger et al., 2006; Iglesias and Echeverría, 2009), significant lower fruit weight was shown by flat fruit trees in relation to the round fruit ones. As mentioned above, this result could be explained by the detection of QTLs for fresh weight and productivity near the gene controlling for fruit shape (Dirlewanger et al., 1999; Lesley, 1940). On the other hand, nectarine genotypes from our progenies showed also lower yield and fruit weight than peach genotypes, whereas no significant differences were found among yellow and white flesh seedlings.

Nectarines had higher percentage of skin blush and endocarp staining than peaches, whereas flat fruit showed higher skin blush than round fruit, but lower endocarp staining (Table 3.4). These differences were probably due to the characteristics of the cultivars involved as progenitors in this breeding program. White flesh fruits showed

higher blush percentage than yellow flesh fruits, which agrees with the higher anthocyanin content found in this type of fruits (see chapter 5).

Nectarines had significantly higher SSC than peaches (Table 3.4) as previously observed by other authors (Crisosto et al., 2006a; Crisosto et al., 2001a; Wu et al., 2005b). This could be explained by the co-localization of a major QTLs for SSC with the morphological marker for peach/nectarine on linkage group 5 (Quilot et al., 2004c). At the same time, flat fruits showed higher SSC than round fruits, in agreement with reports where most flat fruit varieties have shown sweeter taste and higher sugar content (Ma et al., 2003). The interest in flat fruit peach cultivars is increasing to a large extent due to their excellent organoleptic characteristics (Nicotra et al., 2002). On the other hand, white flesh fruits showed significantly higher SSC than yellow flesh fruit, in agreement with Robertson et al. (1990), who reported higher sucrose, glucose, fructose and SSC in white-fleshed than in yellow-fleshed peaches.

Regarding acidity, significantly higher TA (and consequently lower pH) was observed for nectarine as compared to peach (Table 3.4). At the same time, flat fruit showed higher TA than round fruit. However, most flat peach varieties have been reported to have low titratable acidity (Ma et al., 2003). In our work, the higher acidity showed by flat fruit genotypes could be explained by the influence of non-flat progenitors, since this is a continuous trait of quantitative inheritance (Dirlewanger et al., 2006). On the other hand, nectarines showed higher RI than peaches due to their reported higher SSC. Round and yellow flesh fruit showed higher RI than flat and white flesh fruit respectively, due to the higher TA values obtained in the latter.

Firmness was also higher in nectarine than in peach fruits (Table 3.4), as observed by other authors (Crisosto et al., 2001a; Valero et al., 2007). Additionally, softer fruits were showed by white flesh seedlings when comparing with yellow flesh ones, in agreement with Crisosto et al. (2001a).

Table 3.4. Influence of pomological traits on agronomic and fruit quality characteristics of a peach and nectarine breeding population. The number of observed seedlings (n) is shown for each fruit type.

Fruit type	n	Annual yield (kg)	Fruit weight (g)	Blush (%)	Endocarp staining[a]	SSC (ºBrix)	pH	TA (g 100g^{-1} FW)	RI	Firmness (N)
Peach	934	11.8 a	172.5 a	57.4 b	4.2 b	11.6 b	3.54 a	0.61 b	19.6 b	25.6 b
Nectarine	177	6.4 b	156.3 b	75.4 a	4.8 a	15.8 a	3.43 b	0.86 a	20.6 a	30.4 a
Round	1075	11.1 a	172.8 a	59.8 b	7.7 a	12.1 b	3.53 a	0.64 b	19.9 a	26.3 a
Flat	36	8.4 b	84.7 b	69.6 a	5.3 b	14.4 a	3.33 b	0.93 a	16.0 b	27.8 a
Yellow	980	11.0 a	174.3 a	59.5 b	4.3 a	12.1 b	3.54 a	0.64 b	19.9 a	26.6 a
White	131	11.0 a	137.5 b	64.9 a	4.4 a	13.2 a	3.43 b	0.73 a	18.6 b	23.7 b

Mean separation within trait columns by t test ($P \leq 0.05$). In each trait column (Peach and Nectarine; Round and Flat; Yellow and White), values with the same letter are not significantly different. *Abbreviations*: SSC: soluble solids content; TA: titratable acidity; RI: ripening index.
[a] Endocarp staining was scored in an increasing arbitrary scale from 1 to 10

3.4.4. Correlation between traits

Table 3.5 shows the correlations found between the agronomic and fruit quality traits. Most of them appeared significant although no high coefficients were found when all progenies were considered together.

Table 3.5. Correlation coefficients between agronomic and fruit quality traits in fifteen nectarine and peach breeding progenies subjected to assessment. Correlation coefficients were calculated based on single plant estimates.

Trait	Annual yield	Fruit weight	Skin blush	Endocarp staining	SSC	pH	TA	RI	Firmness	Esfericity	SDI
Harvest date[a]	0.060 *	0.091 **	NS	NS	NS	NS	NS	NS	NS	-0.177 **	NS
Annual yield		0.205 **	NS	NS	-0.349 **	NS	-0.148 **	-0.149 **	NS	NS	NS
Fruit weight			-0.218 **	0.255 **	NS	0.330 **	-0.330 **	0.363 **	0.202 **	0.333 **	NS
Skin blush				0.270 **	0.112 **	-0.313 **	0.299 **	-0.164 **	NS	-0.106 **	0.105 **
Endocarp staining[b]					0.260 **	NS	0.127 **	NS	0.255 **	NS	0.168 **
SSC						NS	0.393 **	0.363 **	0.294 **	-0.153 **	0.117 **
pH							-0.436 **	0.444 **	NS	0.127 **	NS
TA								-0.656 **	-0.258 **	-0.293 **	0.176 **
RI									NS	0.180 **	-0.105 **
Firmness										NS	NS
Esfericity											-0.169 **

*, $P \leq 0.05$; **, $P \leq 0.01$; NS, not significant.
Abbreviations: SSC: soluble solids content; TA: titratable acidity; RI: ripening index (SSC/TA); SDI: suture deformation index
[a] Harvest date calculated as Julian days
[b] Endocarp staining was scored in an increasing arbitrary scale from 1 to 10

Harvest date was significantly correlated with fruit weight which means that early harvested seedlings generally had smaller fruits than late ones, as found for different peach and apricot cultivars (Dirlewanger et al., 1999; López and Dejong, 2007; Ruiz and

Egea, 2008). However, correlation coefficients varied depending on the progeny, being higher in specific crosses as 'Rich Lady' x 'VAC-9511' (r = 0.581, $P \leq 0.01$). On the other hand, it has been reported that medium and late season cultivars have a greater capacity to accumulate sugar compared to early season cultivars due to the non-interruption of the growing process (Byrne, 2002; Engel et al., 1988). Although harvest date did not show significant correlation with SSC, a tendency from latest harvesting genotypes, within the same progeny, to have higher SSC were observed, as already mentioned.

Skin blush was, in general, positively correlated with endocarp staining although coefficients were variable depending on the progeny. Significant correlations were found in 'Andross x Calante' (r = 0.377), 'Babygold-9' x 'Crown Princess' (r = 0.271), 'Red Top x VAC-9513' (r = 0.219) and 'Babygold-9' x 'VAC-9510' (r = 0.184). This correlation is expectable since both traits are due to the anthocyanins level of the fruit (Tomás-Barberán et al., 2001). Endocarp staining can be an appreciated trait by specific consumers although a relationship of this trait with postharvest browning has been also reported (Ogundiwin et al., 2009a). In agreement with previous works in peach (Génard et al., 1994) and apricot (Ruiz and Egea, 2008), no relationship between skin color and firmness was found for any cross. In general, fruit weight was positively correlated with annual yield, endocarp staining, pH, RI, firmness and esfericity, and negatively correlated with skin blush and TA. It is worthy to note the significant ($P \leq 0.01$) positive correlation found for annual yield versus fruit weight in some progenies such as 'Red Top' x 'VAC-9513' (r = 0.523), 'VAC-9520' x 'VAC-9517' (r = 0.410), and 'O´Henry' x 'VAC-9514' (r = 0.432), showing that these crosses have a bigger potential to produce higher yields and bigger fruits. However, no significant correlations were found for the highest yielding progenies, such as 'Babygold-9' x 'VAC-9511', 'Andross' x 'Crown Princess' and 'Andross' x 'VAC-9511'. The results suggest that fruit weight increases with annual yield until reaching a value when the tree resources cannot contribute to increasing fruit weight and yield simultaneously. No correlation was found for fruit weight and SSC when all seedlings were considered together. However, a significant positive correlation was observed for some progenies as 'Andross' x 'Rich Lady' (r = 0.508), 'Babygold-9' x 'Crown Princess' (r = 0.481), and 'Red Top' x 'VAC-9513' (r = 0.501) indicating the tendency of bigger fruits to have higher sugar contents. This result is expectable since amount of translocated carbohydrates contributing to SSC, determines fruit growth rate (Mounzer et al., 2008), and at the same time, fruit size increases sink strength to attract sucrose and sorbitol from the plant sources (Lo Bianco and Rieger, 2006). Contrary, yield showed a significant negative correlation versus SSC, showing the sink competition among fruits by the assimilate supply (Mounzer et al., 2008). This effect was variable depending on the progeny, and higher coefficients ($P \leq 0.01$) were found in 'Venus' x 'Big Top' (r = -0.460),

'VAC-9520' x 'VAC-9517' (r = -0.560), 'O´Henry' x 'VAC-9514' (r = -0.566), and 'O´Henry' x 'VAC-9515' (r = -0.405) progenies.

As previously reported for peach (Dirlewanger et al., 1999; Wu et al., 2003), a positive significant correlation was observed for SSC versus TA suggesting a dependent genetic control of both traits. The location of QTLs for nearly all the chemical compounds (sucrose, fructose, sorbitol, malic and citric acid) in the linkage groups 5 and 6 (Dirlewanger et al., 1999; Etienne et al., 2002b) with possible pleiotropic effect, could partly explain this result. Highest coefficients ($P \leq 0.01$) were found in 'Rich Lady' x 'VAC-9511' (r = 0.812), 'VAC-9520' x 'VAC-9517' (r = 0.722), 'O'Henry' x 'VAC-9514' (r = 0.702), and 'O'Henry' x 'VAC-9515' (r = 0.703) progenies. In general, significant correlation was also found between firmness and other traits such as fruit weight, SSC and TA, in agreement with Byrne et al. (1991). A higher correlation between firmness and SSC was found in 'Orion' x 'VAC-9510' (r = 0.634), 'Red Top' x 'VAC-9513' (r = 0.485), 'Andross' x 'Rich Lady' (r = 0.442), and 'O´Henry' x 'VAC-9516' (r = 0.488) progenies, whereas no significant correlation was found in others. 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 of having higher SSC. This correlation is important since selection of high SSC genotypes will aim first at higher firmness, and second at lower susceptibility to mechanical damage during handling and packaging (Crisosto et al., 2001c). The reported genetic correlations are due mainly to pleiotropy, though linkage disequilibrium can be a cause of transient correlation (Falconer and Mackay, 1996). The breeding response for one trait depends on genotypic variations of that trait within the breeding population and on genotypic correlations between traits. Thus, phenotypic correlations are important parameters to take into account in breeding programs.

3.4.5. Principal component analysis and grouping of progenies

Principal component analysis (PCA) model was performed to provide an easy visualization of the complete data set in a reduced dimension plot. PCA has been previously used to establish genetic relationships among cultivars and to study correlations among fruit traits within peach (Brovelli et al., 1999b; Crisosto et al., 2006a; Lavilla et al., 2002) and apricot genotypes (Badenes et al., 1998b; Gurrieri et al., 2001; Ruiz and Egea, 2008).

The PCA carried out in this work showed that more than eighty per cent of the observed variance could be explained by the first five components. PC1, PC2, and PC3, respectively, accounted for 34.3%, 26.2%, and 10.8% of total variability. Table 3.6 shows

the correlation between the original variables and the first 3 components: PC1 represents mainly harvest date, fruit weight, percentage of blush, acidity (pH, TA, and RI), and esfericity; PC2 explains annual and cumulative yield, sugars (SSC), and firmness; PC3 mainly contributes to annual and cumulative yield, together with PC2, and to endocarp staining.

Table 3.6. Component loadings for quality variables and component scores for fifteen peach and nectarine breeding progenies subjected to assessment.

Variable/factor	Component loadings			Progeny	Component scores		
	PC1, λ=34.30 %	PC2, λ=26.20 %	PC3, λ=10.79 %		PC1	PC2	PC3
Harvest date	0.67	-0.59	0.00	Andross x Calante	1.54	-0.34	-2.22
Annual yield	0.17	0.76	0.53	Andross x Crown Princess	0.91	1.43	-0.28
Cumulative yield	0.06	0.71	0.58	Andross x Rich Lady	-0.35	0.11	-0.35
Fruit weight	0.79	-0.22	0.41	Andross x VAC-9511	0.12	1.46	0.84
% Blush	-0.62	-0.46	0.27	Babygold-9 x Crown Princess	1.10	0.71	-0.40
Endocarp staining	-0.17	-0.49	0.56	Babygold-9 x VAC-9510	0.85	0.61	1.74
SSC	0.25	-0.78	0.03	O´Henry x VAC-9514	0.29	-1.28	0.92
pH	0.93	0.12	-0.01	O´Henry x VAC-9515	0.32	-1.37	1.09
TA	-0.81	-0.44	0.17	O´Henry x VAC-9516	0.09	0.83	0.49
RI	0.86	-0.18	-0.04	Orion x VAC-9510	-0.61	-0.50	1.25
Esfericity	0.72	-0.29	0.17	Red Top x VAC-9513	-0.32	0.46	-0.83
SDI	-0.32	-0.37	0.41	Rich Lady x VAC-9511	-1.95	-0.68	-0.37
Firmness	-0.02	-0.64	0.04	VAC-9512 x VAC-9511	-1.12	0.05	-0.40
				VAC-9520 x VAC-9517	-1.79	0.58	-0.90
				Venus x Big Top	0.90	-2.08	-0.59

Abbreviations: PC: principal component; SSC: soluble solids content; TA: titratable acidity; RI: ripening index (SSC/TA); SDI: suture deformation index

Component scores for the 15 studied progenies are shown in Table 3.6. Positive values for PC1 indicate populations with later harvest date, big fruit sizes, low skin blush and low acidity fruits. Progenies such as ‘Andross’ x ‘Calante’, ‘Andross’ x ‘Crown Princess’, both two progenies descendant from ‘Babygold-9’ (‘Babygold-9’ x ‘Crown Princess’ and ‘Babygold-9’ x ‘VAC-9510’), and ‘Venus’ x ‘Big Top’ belong to this group (Fig. 3.4). The lowest values for PC1 indicate early harvest progenies with high acidity and small fruit sizes such as ‘Rich Lady’ x ‘VAC-9511’, ‘VAC-9512’ x ‘VAC-9511’, and ‘VAC-9520’ x ‘VAC-9517’. The highest values for PC2 indicate high yield progenies with low firmness, endocarp staining and SSC fruits. Families such as ‘Andross’ x ‘Crown Princess’ and ‘Andross’ x ‘VAC-9511’ belong to this group. On the contrary, families with lowest values for PC2, such as ‘O´Henry’ x ‘VAC-9514’, ‘O´Henry’ x ‘VAC-9515’, and ‘Venus’ x ‘Big Top’, have low yields but high SSC, firmness, blush, and endocarp staining fruits.

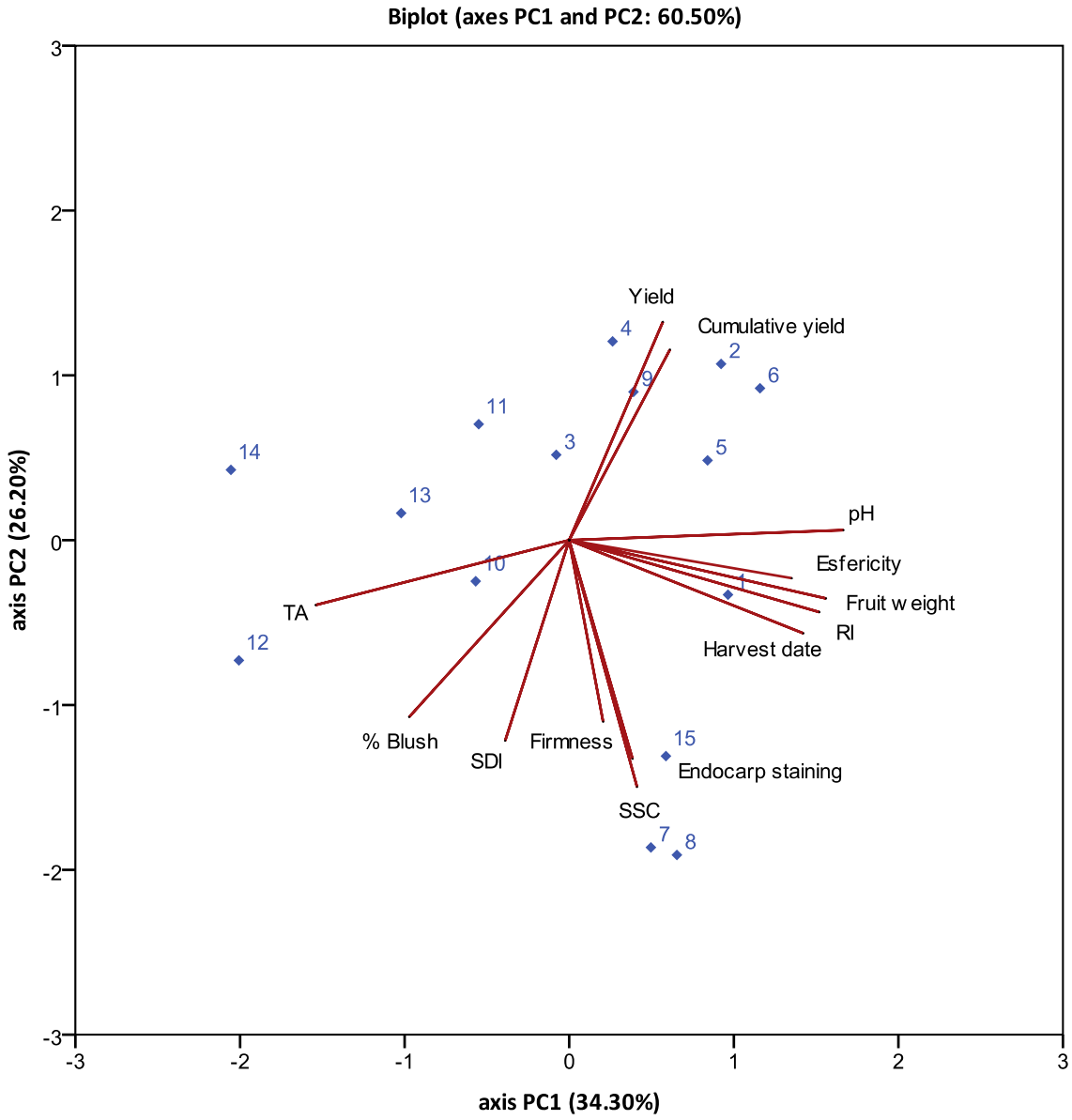

Figure 3.3 Segregation of the fifteen peach and nectarine breeding progenies according to their agronomic and fruit quality characteristics determined by principal component analysis (PCA). Vectors represent the loadings of agronomic and quality traits data along with the principal component scores. Numbers represent peach and nectarine progenies (numbers shown in Tables 2 and 3): 1, 'Andross' x 'Calante'; 2, 'Andross' x 'Crown Princess'; 3, 'Andross' x 'Rich Lady'; 4, 'Andross' x 'VAC-9511'; 5, 'Babygold-9' x 'Crown Princess'; 6, 'Babygold-9' x 'VAC-9510'; 7, 'O´Henry' x 'VAC-9514'; 8, 'O´Henry' x 'VAC-9515'; 9, 'O´Henry' x 'VAC-9516'; 10, 'Orion' x 'VAC-9510'; 11, 'Red Top' x 'VAC-9513'; 12, 'Rich Lady' x 'VAC-9511'; 13, 'VAC-9512' x 'VAC-9511'; 14, 'VAC-9520' x 'VAC-9517'; 15, 'Venus' x 'Big Top'. *Abbreviations*: PC: principal component; RI: ripening index; SDI: suture deformation index; SSC: soluble solids content; TA: titratable acidity.

3.5. CONCLUSIONS

A high variability has been found in the 1111 peach and nectarine genotypes evaluated with regard to the studied pomological traits related to fruit quality, and significant differences among crosses were observed for all quality attributes, which indicates that there is a genetic potential to develop peaches and nectarines with high quality. The high number of evaluated genotypes, coming from very different genetic origins and with a large phenotypic variability, provides valuable information about the peach species, regarding the parameters which influence peach quality. The study shows the existing relationship between different qualitative pomological traits such as peach-nectarine, round-flat shape and yellow-white flesh, and the agronomic and fruit quality attributes. On the other hand, our data support a preliminary hypothesis for the character aborting fruit to be determined by a multi-allelic locus controlling also the flat shape.

The correlations found between some quality attributes such as yield, fruit weight and SSC, SSC versus TA, and SSC versus firmness, could be interesting for quality oriented fruit breeding programs. However, the substantial variation in the correlation coefficients for different cultivars should be taken into account. The study also emphasizes the usefulness of PCA in evaluating the fruit quality of new breeding releases and studying relationships among pomological traits.

Finally, this work enabled the selection of 26 genotypes with the most appropriate combination of agronomic and quality traits within the breeding population. Among the evaluated crosses, ‘Babygold-9’ x ‘Crown Princess’ and ‘Babygold-9’ x ‘VAC-9510’ resulted in the best progenies for selection of new high fruit quality genotypes in the Mediterranean area conditions. ‘O´Henry’ x ‘VAC-9514’, ‘O´Henry’ x ‘VAC-9515’, and ‘Venus’ x ‘Big Top’ crosses showed a good performance regarding fruit quality aspect such as soluble solids content, acidity, fruit weight, firmness, and skin blush although productivity should be improved. On the other hand, crosses with ‘Andross’ as progenitor resulted non-interesting regarding fruit quality, in spite of having higher yields.

Capítulo 4

Analysis of phenotypic
variation of sugar profile

4.1. ABSTRACT

Peaches and nectarines from 205 genotypes coming from 14 different breeding progenies were evaluated by HPLC for specific sugar content. Several qualitative parameters and fruit quality traits were studied in terms of their relationships to sugar concentrations. In addition, the year-to-year effect on sugar profile was also evaluated.

A high contribution of cross to the phenotypic variance of all the evaluated fruit quality traits was found. A relationship of fruit type, flesh color, fruit shape, and stone adhesion with sugar content of fruits was also found. Pre-selected genotypes from the original breeding program showed enhanced SSC, total sugar and sucrose contents. Significant effect of year was found for SSC, sucrose and glucose, whereas no effect was found for fructose and sorbitol content. Individual sugar contents correlated significantly with each other and with other fruit quality traits.

The results showed a significant effect of cross, year and qualitative traits on sugar profile of peaches and nectarines. Moreover, the differences found on sugar traits between the breeding population and the pre-selected genotypes indicated the importance of sugar profile on global quality of peaches and nectarines and the extent of sugar profile modification possible in a peach breeding program.

4.2. INTRODUCTION

Fruit quality is an abstract concept that varies according to the particular perception of consumers. In recent surveys, inconsistent or poor quality has been mentioned as a major limiting factor influencing consumer choice (Crisosto et al., 2001c). Flesh texture and firmness, visual appearance, flavor and aroma are all part of fruit quality as are the levels of sweetness and acidity (Bruhn et al., 1991; Esti et al., 1997; Lockshin and Rhodus, 1991; Sweeney et al., 1970). A lack of sugar or sweetness is among the most common consumer complaints in peaches and apricots (Moreau-Rio and Roty, 1998), since composition of sugars ultimately affect fruit quality and flavor.

Recently, peach breeding programmes have stressed the importance of taste in selection of new cultivars (Crisosto et al., 2006b). In our breeding program, we searched for superior peach and nectarine cultivars for the Spanish industry with good adaptation to Mediterranean conditions when grown in the Ebro Valley (Cantín et al., 2006; Moreno, 2005), one of the biggest production areas in Europe (MARM, 2007). Besides lowering the production costs and improving pest and disease resistance, breeding efforts at this program are directed towards improving both agronomic performance and fruit quality. Soluble sugars are well known for contributing to a range of fruit quality traits such as flavor, texture and healthy properties (Esti et al., 1997). Crisosto and Crisosto (2005) reported that the degree of liking and consumer acceptance were significantly related with SSC, although maximum consumer acceptance was attained at different SSC levels depending on the cultivar. On the other hand, taste is related to water-soluble and non-volatile compounds, while sweetness is mostly attributable to mono- and disaccharides (Colaric et al., 2005).

The collective concentration of sugars present in a given fruit is known as the sugar profile. Sucrose is the predominant sugar in the peach fruit (Albás et al., 2003; Moriguchi et al., 1990a; Robertson et al., 1990). This disaccharide is important as sweetener, energy source and antioxidant of fruit flavors (Huberlant and Anderson, 2003). Other sugars such as glucose, fructose and sorbitol are also present at lower concentrations (Moriguchi et al., 1990a). Fructose is an important monosaccharide in terms of fruit flavor, since it has a higher level of perceived sweetness than sucrose and glucose (Pangborn, 1963). Moreover, fructose has been reported to have beneficial effects on gastrointestinal health, since it favors the growth of bifidobacteria and lactobacilli in the gastrointestinal tract (Muir et al., 2009). In the field of human nutrition, there is an 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 gastrointestinal problems (Ledbetter et al., 2006; Rapaille et al., 2003). Sorbitol can be used as a glucose

substitute for diabetics and as an alternative natural sweetener instead of sucrose (Forni et al., 1992). For these reasons, fruit breeders are also finding specific interest in sorbitol rich fruits (Ledbetter et al., 2006).

On the other hand, qualitative pomological traits such as pubescent or glabrous skin (peach or nectarine), round or flat shape, white or yellow flesh and freestone or clingstone, have been studied from the production and marketing point of view. Few investigations have focused on the relationships of these qualitative traits with sugar profile and therefore with fruit taste, and especially in unselected progenies with such variety of fruit types and genetic backgrounds.

This study was undertaken to: (1) evaluate the sugar content and composition of peach and nectarine seedlings and selections from fourteen breeding progenies, (2) study how sugar profile varies with different pomological qualitative traits, such as peach-nectarine, flat-round, flesh color and/or stone adhesion, (3) determine the year-to-year variations in sugar profile of fruits; and (4) examine relationships among sugar traits and other fruit quality parameters, in order to provide useful information for peach breeding.

4.3. MATERIAL AND METHODS

4.3.1. Plant material

Fourteen controlled biparental crosses between nineteen peach and nectarine cultivars (Table 4.1) were made during 2000 and 2001. The resulting seedlings were budded on the same rootstock (GF-677) and established (one tree per genotype) in an experimental orchard at the Experimental Station of Aula Dei-CSIC (Northern Spain, Zaragoza) in 2002. Trees were trained to the standard open vase system and planted at a spacing of 4 m x 2.5 m. We minimized environmental sources of variations between genotypes and between fruits within genotype by practicing hand thinning when required. Trees were grown under standard conditions of irrigation, fertilization and pest and disease control. Agronomic and fruit quality traits were evaluated in a total of 205 genotypes over three consecutive years (2005-2007). The studied genotypes were selected among the descendants from the 14 crosses for their higher fruit quality or interesting characteristics. All traits were measured or scored for each seedling separately over the 3-year period and means of 3 seasons were calculated. Sugar composition was studied in these 205 genotypes over 2 or 3 years to estimate the seasonal effect on sugar profile. All analyses were carried out on the fruit flesh, since peel is not appreciated by consumers and it is removed prior consumption.

Table 4.1. Peach and nectarine commercial and experimental (VAC-) cultivars used as progenitors in the 14 controlled crosses. Fruit type (round or flat, peach or nectarine), flesh color (yellow or white), and stone adhesion (free or cling) for each progenitor is shown.

Cultivar	Fruit type		Flesh colour	Stone
Andross	round	peach	yellow	cling
Babygold-9	round	peach	yellow	cling
Calante	round	peach	yellow	cling
Crown Princess	round	peach	yellow	cling
O´Henry	round	peach	yellow	free
Orion	round	peach	yellow	free
Red Top	round	peach	yellow	free
Rich Lady	round	peach	white	free
VAC-9510	round	peach	yellow	cling
VAC-9511	round	peach	yellow	free
VAC-9512	round	peach	yellow	free
VAC-9513	round	nectarine	yellow	free
VAC-9514	round	nectarine	white	free
VAC-9515	round	nectarine	yellow	free
VAC-9516	round	peach	white	free
VAC-9517	flat	peach	white	free
VAC-9520	round	peach	yellow	free

4.3.2. Agronomic and fruit quality traits

Agronomic and quality traits were measured in the 205 seedlings over 3 successive years (2005-2007). Fruits were considered ripe in the trees when they no longer grew, softened, exhibited yellow or orange ground color (which is also representative for each cultivar) and were easily detached. They were harvested by a single person to keep consistency of maturity grade. Harvest date ranged from late-May to mid-September, depending on the genotypes. Yield (kg/tree) was determined for each seedling recording also the total number of fruits. From these measurements, the total average fruit weight was calculated. For the evaluation of fruit quality parameters, a representative sample of 30 fruits per tree was selected. The agronomic characters segregating as simple characters were recorded, i.e. peach or nectarine, yellow or white flesh, round or flat fruit, aborting or non-aborting fruit, and freestone or clingstone. Some other pomological traits such as skin blush, stone adhesion, endocarp staining and firmness were scored using the rating scales appropriated for each of them. Skin blush was scored as the percentage of skin surface with red color. Stone adhesion and endocarp staining (redness around stone) were scored in an increasing arbitrary scale

from 1 to 10. The soluble solids content (SSC) of the juice was measured with a temperature compensated refractometer (model ATC-1, Atago Co., Tokyo, Japan); and data are given as ºBrix. The titratable acidity (TA) was determined by titration with NaOH 0.1 N to pH 8.1 (AOAC, 1984) Data are given as g malic acid per 100 g fresh weight (FW), since this is the dominant organic acid in peach (Sweeney et al., 1970; Wills et al., 1983). Flesh firmness was determined on opposite sides of the equator of each fruit with a penetrometer fitted with an 8-mm diameter probe on 5 fruits from each tree. The two readings were averaged per fruit, and data are given in Newtons (N).

4.3.3. Extraction and determination of sugars

Sugar composition was studied in 205 genotypes over 2 or 3 years to estimate the year-to-year variation of sugar profile. Five fruits of each of the 205 seedlings evaluated were peeled with a sharp knife, cut into small pieces, and the flesh was weighted and immediately frozen separately in liquid nitrogen, and stored at -20 ºC until analysis. At the moment of analysis, the frozen fruit material (5 g) was homogenized with a Polytron (2 min on ice) with 10 ml of extraction solution, consisting of ethanol/Milli-Q water (80% v/v). The mixture was centrifuged at 20000 x *g* for 20 min at 4 ºC. The supernatant was recovered and assayed by high-performance liquid chromatography (HPLC) as described below.

For sugar analysis, 250 μl of supernatant was incubated at 80 ºC for 20 min in 200 μl 80% ethanol, and 5 μg/μl of manitol were added as internal standard. At the end of the incubation, samples were filtered through a Waters C18 cartridge to eliminate any interfering apolar residues. The filters were washed twice with 80% ethanol to elute the retained sugars. Samples were then vacuum concentrated to 500 μl of milliQ water. The obtained solutions were consecutively passed through a 0.45 μm and a 0.22 μm filter to eliminate large particles, prior to analysis on a Ca-column (Aminex HPX-87C 300 mm x 7.8 mm column Bio-Rad) with a refractive-index (RI) detector (Waters 2410). The solvent was vacuum de-gassed deionized water at a flow rate of 0.6 ml min^{-1} at 85 ºC, and 20 μl of the sample were injected into the HPLC. Sugars quantification was performed with the Millenium 3.2 software, Waters (Milford, Mass, USA) using standards of analytical grade from Panreac Quimica S.A. (Barcelona, Spain). Sugar concentrations were expressed as mg per g of fresh flesh weight (FW).

4.3.4. Statistical analysis

All statistical analyses were performed using SPSS 17.0 for Windows (Chicago, IL). To obtain basic statistics for the entire plant material studied, the number of observed seedlings, maximum and minimum value, mean, mean standard error and standard deviation for each trait were recorded. The significance of cross, year and cross x year interaction effects on sugar contents was tested on the 205 genotypes by analysis of variance. Data for each seedling over the 2 or 3 years of study were averaged, and mean values were used as estimated genotypic values for future analysis. For every quantitative variable, including ordinal scores, the progenies mean were also estimated to find differences between crosses by using Duncan Multiple Range Test ($P \leq 0.05$). When comparing between different fruit types (peach or nectarine, round or flat, yellow or white flesh) the *t* test ($P \leq 0.05$) was used. The difference on sugar contents between the breeding population and the pre-selected outstanding genotypes was analysed with boxplots drawn. The boxplot can be made to display range, median and distribution density of a variable in a sample size. The median of the data was indicated by the horizontal line in the interior of the box. The height of the box is equal to the interquartile distance, which is the difference between the third quartile of the data and the first quartile. The whiskers extend to a distance (1.5 x inter quartile distance) from the centre. Approximately 99% of the data falls inside the whiskers. The data outside these whiskers are indicated by horizontal lines, and extreme data are indicated by asterisks. Correlations between traits to reveal possible relationships were calculated from raw data of the 3 years, using the Pearson correlation coefficient at $P \leq 0.05$.

4.4. RESULTS AND DISCUSSION

4.4.1. Cross and year effect

The population in this study exhibited considerable phenotypic variation in sugar contents (Table 4.2). The variation ranges extended between those determined at maturity in *P. persica* by other authors (Bassi and Selli, 1990; Colaric et al., 2005; Dirlewanger et al., 1999; Lo Bianco and Rieger, 2002b; Wu et al., 2005b; Yoshida, 1970). In this study, the average content of total sugars (the sum of sucrose, glucose, fructose, and sorbitol contents) in the peeled fruit was 72.1 mg g^{-1} FW. Sucrose, glucose, fructose, and sorbitol contents were analyzed separately as they play an important role in peach flavor quality (Esti et al., 1997). Sucrose was the sugar present at the highest concentration in all genotypes evaluated, ranging from 28.2 to 84.4 mg g^{-1} FW, followed

by fructose, glucose, and sorbitol. The mean levels of glucose and fructose were quite similar (mean glucose/fructose ratio = 0.8) and about 9 times lower than the mean level of sucrose (mean sucrose/glucose ratio = 9). The range found in sucrose, glucose and fructose among genotypes was 5-fold, while in sorbitol the range was 15-fold. A broad range of SSC (from 7.6 to 17.5 ºBrix) was also found among the studied seedlings. On the other hand, a large variation of glucose/fructose ratio (from 0.4 to 2.5) was detected in the studied seedlings. Identifying low glucose/fructose ratio genotypes might be of particular interest, since fructose was rated higher (1.75) than sucrose (1) and glucose (0.75) in terms of sweetness (Pangborn, 1963). The great genotypic variation in this population suggests that there is a genetic potential to develop peaches and nectarines with improved sugar content. Indeed, 12 of the 26 pre-selected outstanding genotypes showed glucose/fructose ratios lower than 0.7. In agreement, Robertson and Meredith (1988) found that 'high quality' contained lower glucose/fructose ratios than 'low quality' peaches.

Table 4.2. Soluble solids content (SSC), individual sugars, total sugar contents, and specific sugar ratios rates for the seedlings from 14 peach and nectarine breeding progenies studied over 3 years. For each trait, number of observed seedlings (n), minimum, maximum, mean value, mean standard error (MSE), and standard deviation (SD) are presented.

Sugar trait	n	Minimum	Maximum	Mean	MSE	SD
SSC (ºBrix)	205	7.6	17.5	11.5	0.12	1.7
Sucrose (mg g^{-1} FW)	205	28.2	84.4	54.3	0.79	11.3
Glucose (mg g^{-1} FW)	205	2.3	14.6	6.5	0.13	1.9
Fructose (mg g^{-1} FW)	205	3.8	16.1	8.6	0.15	2.2
Sorbitol (mg g^{-1} FW)	205	0.9	10.6	3.0	0.14	2.0
Total sugars[a] (mg g^{-1} FW)	205	36.0	109.4	72.1	0.99	14.2
Sucrose/Glucose	205	2.7	18.9	9.0	0.19	2.7
Glucose/Fructose	205	0.4	2.5	0.8	0.02	0.3
% Sorbitol	205	1.2	14.3	4.1	0.17	2.4

Abbreviations: SSC, soluble solids content.
[a] Total sugars: the sum of sucrose, glucose, fructose, and sorbitol for each genotype, analyzed by HPLC

Analysis of variance on the 205 genotypes over 3 years of study (Table 4.3) showed that cross and year significantly affected all evaluated traits, except for fructose and sorbitol content, which were not significantly affected by year. These results suggest that fructose and sorbitol contents appear to be less influenced by the prevailing environmental conditions over the growing season than the other fruit quality traits evaluated. Anyway, contribution of year to the phenotypic variance of sucrose and glucose contents was low (2.2% and 1.7%, respectively), whereas the phenotypic variance of SSC explained by year was higher (17.4%). In peach, a year effect has been

reported for SSC, sucrose, glucose, and sorbitol contents (Brooks et al., 1993; Dirlewanger et al., 1999). The year-to-year variation in sugar profile may be explained by the differences in climate and crop load over the 3 years of study (Brooks et al., 1993; Sweeney et al., 1970). Several studies have previously characterized *Prunus* populations for patterns of variation in sugar contents (Bassi et al., 1996; Dirlewanger et al., 1999; Ledbetter et al., 2006; Quilot et al., 2004a; Wu et al., 2003). These studies agree that environment influences the level of individual sugars (sucrose, glucose, fructose, and sorbitol) although sugar profile seems to be relatively constant across environments. Statistically significant cross x year interactions were only observed for fruit weight and pH. Due to the not significant cross x year interactions on the SSC, TA, and individual sugar contents, the data from different years were analysed together.

Table 4.3. F values and proportion (%) of phenotypic variance obtained in the ANOVA for the studied factors in the seedlings from 14 F1 peach progenies.

Variable	DF	MS	F-value	P	phenotypic variance (%)
Fruit weight					
Cross	13	46769.12	30.85	0.000	52.2
Year	2	23496.62	15.50	0.000	7.8
Cross x Year	22	3003.47	1.98	0.006	10.6
Error	368	1515.94			
TA					
Cross	13	0.44	17.26	0.000	37.9
Year	2	0.13	5.11	0.007	2.7
Cross x Year	22	0.02	0.84	0.673	4.8
Error	368	0.03			
pH					
Cross	13	0.46	12.01	0.000	29.8
Year	2	5.89	153.78	0.000	45.5
Cross x Year	22	0.07	1.92	0.008	10.3
Error	368	0.04			
SSC					
Cross	13	32.22	14.17	0.000	33.4
Year	2	88.08	38.74	0.000	17.4
Cross x Year	22	1.72	0.75	0.781	4.3
Error	368	2.27			
Sucrose					
Cross	13	625.19	4.95	0.000	14.9
Year	2	514.76	4.08	0.018	2.2
Cross x Year	22	90.93	0.72	0.819	4.1
Error	368	126.22			
Glucose					
Cross	13	13.67	3.97	0.000	12.3
Year	2	11.00	3.19	0.042	1.7
Cross x Year	22	1.65	0.48	0.980	2.8
Error	368	3.45			
Fructose					
Cross	13	24.05	5.62	0.000	16.6
Year	2	1.66	0.39	0.679	0.2
Cross x Year	22	2.73	0.64	0.897	3.7
Error	368	4.28			
Sorbitol					
Cross	13	35.10	10.91	0.000	27.8
Year	2	2.04	0.63	0.531	0.3
Cross x Year	22	1.73	0.54	0.958	3.1
Error	368	3.22			

Abbreviations: DF, degrees of freedom; MS, mean squared.

The significant effect of cross found for all the evaluated traits and its high contribution to their phenotypic variance (Table 4.3), agree with previous works where cross effect on peach quality have been reported (Brooks et al., 1993; Usenik et al., 2008). The highest SSC was shown by the 'O´Henry' x 'VAC-9515' progeny (Table 4.4), without being statistically different from the other two progenies descendant from O'Henry, 'Andross' x 'Calante', 'Andross' x 'VAC-9511', 'Rich Lady' x 'VAC-9511', and 'VAC-9520' x 'VAC-9517' progenies. On the contrary, 'Babygold-9' x 'Crown Princess' showed the lowest SSC, without showing significant differences with 'Andross' x 'Crown Princess', 'Andross' x 'Rich Lady', 'Babygold-9' x 'VAC-9510', and 'Red Top' x 'VAC-9513' progenies. SSC is an important quality trait in peaches and nectarines since it has been related with the degree of liking and consumer acceptance (Crisosto and Crisosto, 2005). Average total sugar content in the 'Andross' x 'Calante' progeny was greater than in other progenies in the observed period. However, no significant differences were found with other progenies such as 'Andross' x 'Rich Lady', the three progenies descendant from 'O'Henry' cultivar, 'VAC-9512' x 'VAC-9511', and 'VAC-9520' x 'VAC-9517'. In contrast, the 'Red Top' x 'VAC-9513' progeny showed the lowest mean of total sugars content, being significant different from 'Andross' x 'Calante', 'O'Henry' x 'VAC-9514', 'O'Henry' x 'VAC-9515', and 'VAC-9512' x 'VAC-9511' progenies. Total sugars content is an important quality trait in the fruit breeding programs, since it has been reported to be highly related to aroma and taste of peaches and nectarines (Colaric et al., 2005). Big differences were also found among progenies in the sorbitol content, ranging from 1.4 mg g^{-1} FW in 'Andross' x 'Crown Princess' to 5.3 mg g^{-1} FW in 'O'Henry' x 'VAC-9514'. Consequently, the percentage of sorbitol in the sugar composition was significantly different among progenies, ranging from 2.1% to 7.1%. However, the mean sorbitol content remained lower than 10 mg g^{-1} for all the progenies in agreement with the sorbitol values found in peaches and nectarines (Escobar-Gutiérrez and Gaudillère, 1994; Wu et al., 2005b). Due to the important role of sorbitol in the texture and flavor of peach and nectarine fruits, this interesting trait was valued, among others, in the selection of two genotypes from the 'O'Henry' x 'VAC-9514' progeny. Colaric et al. (2005) reported that sorbitol was the attribute most related to peach aroma and taste among carbohydrates and organic acids. Moreover, it is an interesting polyalcohol in terms of nutrition for special dietary purposes, such as diet control or dental health (Forni et al., 1992; Rapaille et al., 2003). Therefore, genotypes with high sorbitol content are nowadays interesting for fruit breeders (Ledbetter et al., 2006). Glucose/fructose ratios were near one for most progenies, since seasonal changes in glucose and fructose contents have the same pattern (Chapman and Horvat, 1990) and they are usually similar in amount in peach and nectarine fruits (Wu et al., 2003; Wu et al., 2005b). However, significant differences among crosses were found for

this ratio, obtaining values from 0.65 in 'Andross' x 'Calante', and 'Andross' x 'Rich Lady' progenies, to 1.05 in 'O'Henry' x 'VAC-9516' progeny.

Table 4.4. Soluble solids content (SSC, ºBrix), individual sugars, total sugars contents (mg g^{-1} FW), and specific sugar ratios of fruits of 14 breeding progenies averaged for each studied progeny. Values are means of 2 or 3 years of study.

Progeny	n	SSC	Sucrose	Glucose	Fructose	Sorbitol	Total sugars[a]	Sucrose/ Glucose	Glucose/ Fructose	% Sorbitol
Andross x Calante	19	12.2 abc	64.0 a	6.6 ab	10.3 a	5.1 a	85.9 a	10.0 a	0.65 b	5.9 ab
Andross x Crown Princess	9	11.0 cde	51.6 bc	6.4 ab	7.6 ab	1.4 g	67.0 cd	8.5 ab	0.86 ab	2.1 e
Andross x Rich Lady	9	11.1 cde	54.9 abc	5.8 b	9.0 ab	3.6 abcde	73.3 abcd	9.7 ab	0.65 b	4.8 bcd
Andross x VAC-9511	6	11.9 abc	49.6 c	6.7 ab	9.1 ab	1.6 fg	67.0 cd	8.1 ab	0.73 b	2.5 e
Babygold-9 x Crown Princess	19	9.7 e	53.5 abc	5.7 b	7.9 ab	2.4 cdefg	69.4 bcd	9.8 a	0.73 b	3.4 cde
Babygold-9 x VAC-9510	15	11.0 cde	52.5 bc	5.7 b	7.4 ab	2.2 defg	67.7 bcd	9.7 ab	0.79 ab	3.2 de
O´Henry x VAC-9514	14	13.2 ab	54.3 abc	8.0 a	10.1 a	5.3 a	77.7 abc	7.0 b	0.79 ab	7.1 a
O´Henry x VAC-9515	8	13.5 a	60.9 ab	6.8 ab	9.3 ab	3.2 bcdef	80.3 ab	9.3 ab	0.85 ab	4.0 cde
O´Henry x VAC-9516	11	12.5 abc	58.2 abc	6.5 ab	6.9 c	3.9 abc	75.5 abcd	9.8 a	1.05 a	5.1 abcd
Orion x VAC-9510	3	11.6 bcd	47.1 c	7.3 ab	8.9 abc	3.9 abcd	67.2 bcd	7.4 ab	0.82 ab	5.4 abc
Red Top x VAC-9513	37	9.9 de	48.4 c	5.6 b	7.8 ab	2.1 efg	64.0 d	9.6 ab	0.75 b	3.4 cde
Rich Lady x VAC-9511	8	12.3 abc	50.0 bc	6.7 ab	8.4 abc	2.5 bcdefg	67.6 bcd	7.6 ab	0.81 ab	3.6 cde
VAC-9512 x VAC-9511	10	11.6 bcd	57.1 abc	7.0 ab	9.0 bc	4.2 abcd	77.3 abc	8.6 ab	0.79 ab	5.3 abcd
VAC-9520 x VAC-9517	37	12.2 abc	56.5 abc	7.3 ab	8.9 abc	2.8 bcdefg	75.6 abcd	8.5 ab	0.84 ab	3.7 cde

Mean separation in columns by Duncan´s test ($P \leq 0.05$). In each column, values with the same letter are not significantly different. *Abbreviations:* SSC, soluble solids content.

[a] Total sugars: the sum of sucrose, glucose, fructose, and sorbitol for each genotype, analyzed by HPLC

At the end of the agronomic and fruit quality evaluation of the entire breeding population, 26 superior genotypes were selected from the breeding population by independent culling of the most important agronomic (harvest date and yield) and fruit quality traits (fruit weight, soluble solids content, acidity, skin blush, endocarp staining, and firmness) evaluated. A graphical representation showing the median and spread of the distribution of sugar contents of the 205 seedlings of the breeding population (set I) and the 26 pre-selected outstanding genotypes (set II) is given in Figure 4.1.

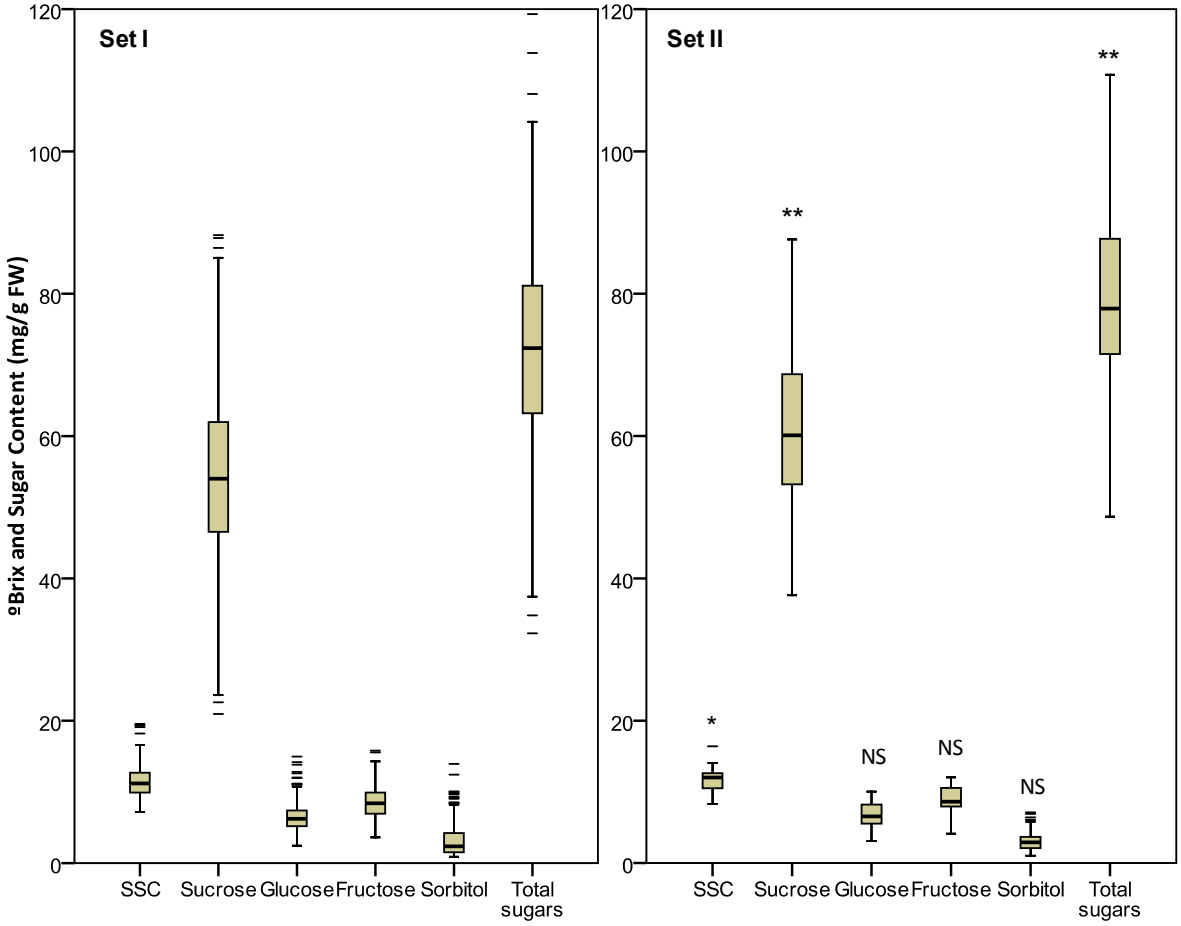

Figure 4.1. Range and distribution of sugars in the 205 seedlings of the breeding population (set I) and in the 26 pre-selected outstanding seedlings (set II). The horizontal lines in the interior of the box are the median values. The height of the box is equal to the interquartile distance, indicating the distribution for 50% of the data. Approximately 99% of the data falls inside the whiskers (the lines extending from the top and bottom of the box). The data outside these whiskers are indicated by horizontal lines. * and ** represent significant differences for each trait between set I and II at $P \leq 0.05$ and $P \leq 0.01$ respectively. NS, not significant differences. *Abbreviations*: SSC, soluble solids content.

Compared with set I, genotypes in set II showed lower variations due to the selection process. Mean sucrose content was significantly higher ($P \leq 0.01$) in set II than in set I. Although not significant differences were found between both sets for the mean contents of glucose, fructose and sorbitol, 12 and 8 outstanding pre-selected genotypes (data not shown) had fructose and sorbitol values over the evaluated population respectively (Table 4.2). Significant higher SSC ($P \leq 0.05$) and total sugar content ($P \leq 0.01$) was also found in set II when comparing with set I, indicating that progress has been

made in our breeding program toward selection of higher sugar content fruits. The significant differences on several sugar traits observed between the pre-selected genotypes and the breeding population confirm the effect of sugar composition on the sensorial quality of the peach fruit (Colaric et al., 2005; Crisosto and Crisosto, 2005), since selection of outstanding seedlings was based on various quality traits which may be influenced by, but are not only restricted to the sugar profile. Harker et al. (2002b) compared instrumental and sensory measurements of apple taste, and reported that SSC was the best predictor of sweet taste. Karakurt et al. (2000) related the lack of flavor of melting peaches with reduced SSC and total sugars. However, the relationship between SSC and consumer acceptance is cultivar specific, and there is not a single reliable SSC that assures consumer satisfaction (Crisosto and Crisosto, 2005). The difference in sucrose content between sets I and II, corroborates the importance of sucrose on the sensorial peach and nectarine quality. Colaric et al. (2005) reported a high contribution of sucrose to aroma and taste of peach and nectarine fruits, indicating that peach fruits with better aroma ratings had also higher sucrose contents. Moreover, sucrose is important as energy source and as a preservative of fruit flavors (Huberlant and Anderson, 2003).

4.4.2. Qualitative traits effect

Different qualitative pomological traits were studied to reveal their possible relationships with the sugar profile of peaches and nectarines (Table 4.5). In the case of pubescent *vs*. glabrous skin, nectarine fruits showed significantly higher SSC, glucose, total sugars, and glucose/fructose ratio than peach fruit, in agreement with other authors (Wu et al., 2005b). Due to their higher glucose content, significant lower sucrose/glucose ratio was found in nectarines than in peaches. Nevertheless, no significant differences between peach and nectarine fruits were observed for sucrose, fructose and sorbitol contents. Similarly to our results, Day et al. (1997) found that 15 peach cultivars averaged approximately 11 ºBrix SSC, whereas 11 nectarine cultivars averaged approximately 14 ºBrix SSC. Moreover, a trained taste panel found that nectarine cultivars were sweeter than peach cultivars. All these results show the advantages of nectarines over peaches in relation to sugar concentrations, although cultivar variations should be also taken into account. The co-localization of major QTLs controlling SSC with the morphological marker for peach vs. nectarine fruit on linkage group 5 (LG5) (Quilot et al., 2005; Quilot et al., 2004c) may partly explain the observed differences.

Significant differences in sugar contents were also found when comparing yellow and white flesh color fruits. White flesh fruits showed significantly higher SSC, individual

and total sugar contents (Table 4.5) than yellow flesh fruits, in agreement with Robertson et al. (1990) who reported higher sucrose, glucose, fructose, and SSC in white-fleshed than in yellow-fleshed peaches. A similar tendency was also found by Wu et al. (2005a) who reported that mean sucrose concentrations in white-fleshed genotypes were about 10% higher than those in yellow-fleshed genotypes. The identification of QTLs controlling fructose content and sweetness (Quilot et al., 2004c) co-localizing with the morphological marker for yellow/white flesh (Bliss et al., 2002) on LG1 might explain the linked segregation of both traits. However, to our knowledge, few studies have focused on the relationship between fruit flesh color and sugar concentrations. It is assumed that white- and yellow-fleshed fruit differ in acidity and sugar composition and this may contribute to the different preferences shown by groups of consumers. In general, Asian consumers prefer white-fleshed cultivars that are considered to have lower acidity and higher sweetness (Wen and Sherman, 2002), while yellow-fleshed peaches are often favoured in Europe and America (Day et al., 1997). However, nowadays and due to the increasing gene exchange between different cultivars occurred on the peach breeding programs, it is also possible to find white-fleshed peaches with lower sweetness and higher acid levels (Wu et al., 2005b).

Although there is only one progeny ('VAC-9520' x 'VAC-9517') segregating for the fruit shape trait (flat *vs*. round), flat fruit genotypes (13.7 ºBrix) showed significant higher SSC than round ones (11.3 ºBrix) (Table 4.5), even when compared to the round fruit within the same progeny (11.5 ºBrix). Higher individual and total sugar contents were also found in flat fruits although differences were not significant. This result agrees with previous works, where flat peach varieties have been reported to have excellent flavor with a sweet taste, low titratable acidity and high sugar content (Ma et al., 2003). This could be explained by the localization of QTLs for SSC (Dirlewanger et al., 1999) and total sugars (Quilot et al., 2005) on LG6, near the dominant gene (S, for 'saucer-shaped') controlling the flat fruit character (Lesley, 1940) originating from China.

In the case of the freestone-clingstone trait, significantly higher SSC, total sugar and individual sugar contents were found in freestone fruits (Table 4.5). These differences may be explained by the identification on LG4 of QTLs involved in SSC, glucose and sucrose contents (Quilot et al., 2004c), near the physical trait controlling flesh adhesion to the stone (F/f) (Dirlewanger et al., 2006; Yamamoto et al., 2001). Also the selection pressure for higher sugar contents on breeding programs for peach freestone cultivars for fresh market may contribute to these results. No differences between both types of fruits were reported in a previous work with a *P. persica* x *P. davidiana* breeding progeny (Wu et al., 2005b). Flesh adhesion to the stone in peach and nectarine is an important quality trait which determines fruit usage (canning, drying, or table fresh fruits). Therefore,

relationships between this trait and sugar concentrations may be useful in breeding strategies. However, until now, studies on the relationship between flesh adhesion and fruit flavor have been scarce. The present study shows a possible functional link between flesh adhesion and sugar profile of peaches and nectarines. Further studies on this aspect would be of interest for peach breeding.

Table 4.5. Soluble solids content (SSC, ºBrix), individual sugars, total sugars contents (mg g^{-1} FW), and specific sugar ratios associated with qualitative traits in peach and nectarine genotypes from 14 breeding progenies. Values are means of 2 or 3 years of study.

Traits	n	SSC	Sucrose	Glucose	Fructose	Sorbitol	Total sugars[a]	Sucrose/ Glucose	Glucose/ Fructose	% Sorbitol
Peach	191	11.3 b	54.8 a	6.4 b	8.6 a	3.1 a	72.6 b	9.1 a	0.78 b	4.2 a
Nectarine	14	14.1 a	57.6 a	7.6 a	8.9 a	3.0 a	77.1 a	7.8 b	0.88 a	3.8 a
Yellow-fleshed	163	11.2 b	53.7 b	6.3 b	8.4 b	2.9 b	71.3 b	9.1 a	0.77 b	4.0 b
White-fleshed	42	12.3 a	56.9 a	7.4 a	9.1 a	3.8 a	77.2 a	8.6 a	0.86 a	4.8 a
Round	190	11.3 b	54.7 a	6.5 a	8.6 a	3.1 a	73.0 a	9.0 a	0.79 a	4.2 a
Flat	15	13.7 a	58.7 a	6.8 a	9.1 a	3.1 a	77.7 a	9.5 a	0.76 a	3.9 a
Freestone	28	12.9 a	59.3 a	7.1 a	9.3 a	4.5 a	80.2 a	8.9 a	0.80 a	5.5 a
Clingstone	177	11.3 b	54.3 b	6.4 b	8.5 b	2.9 b	71.8 b	9.1 a	0.78 a	4.0 b

Mean separation in columns by *t*-test ($P \leq 0.05$). For each trait, in the same column, values with the same letter are not significantly different. *Abbreviations:* SSC, soluble solids content.

[a] Total sugars: the sum of sucrose, glucose, fructose, and sorbitol for each genotype, analyzed by HPLC

4.4.3. Correlations between sugar contents and other fruit quality traits

Table 4.6 shows the correlation coefficients between sugar and other evaluated fruit quality traits. All sugar contents studied were positively correlated between each other and with other fruit composition traits, which could be partly explained by the location of QTLs for nearly all the chemical compounds (sucrose, fructose, sorbitol, malic, and citric acid) in linkage groups 5 and 6 (Dirlewanger et al., 1999). Among individual sugars, the highest correlation was found between glucose and fructose (r = 0.50**), as reported by other authors (Cheng et al., 2004; Dirlewanger et al., 1999; Wu et al., 2003; Wu et al., 2005b). Several authors have shown that seasonal changes in glucose and fructose contents had the same pattern and they had approximately equal amounts during fruit development (Chapman and Horvat, 1990; Ishida et al., 1971). The correlations found in our study were lower than those reported by Esti et al. (1997) and Dirlewanger et al. (1999) because, although most genotypes had higher fructose content than glucose

content, some of them were characterized by a higher content of glucose as shown by Wu et al. (2003) and Moriguchi et al. (1990a). This indicates that independent genetic control of both sugar contents may exist. Sorbitol correlated significantly with the rest of individual sugar contents as previously reported (Wu et al., 2003; Wu et al., 2005b), since even though the absolute sugar contents vary, sugar profile remains generally stable among genotypes (Bassi et al., 1996; Ledbetter et al., 2006), and therefore, higher total sugar content fruits will also have higher glucose, fructose and sorbitol contents.

Table 4.6. Correlation coefficients between traits in the 205 peach and nectarine genotypes of the 14 studied populations over 3 years. Correlation coefficients were calculated with one mean value per genotype.

Trait	SSC	Sucrose	Glucose	Fructose	Sorbitol	Total sugars
Sucrose	0.26 **					
Glucose	0.25 **	0.21 **				
Fructose	0.22 **	0.24 **	0.50 **			
Sorbitol	0.33 **	0.27 **	0.31 **	0.46 **		
Total sugars[a]	0.33 **	0.94 **	0.43 **	0.49 **	0.49 **	
Yield	-0.21 **	NS	NS	NS	-0.16 **	NS
Fruit weight	0.12 **	NS	NS	0.12 *	0.19 **	0.13 **
Blush %	0.14 **	NS	0.13 **	NS	NS	NS
Endocarp staining[b]	0.21 **	NS	NS	0.12 *	0.18 **	0.13 **
pH	NS	-0.11 *	-0.11 *	NS	0.10 *	NS
TA	0.37 **	0.10 *	0.21 **	0.11 *	NS	0.14 **
RI	0.53 **	0.13 **	NS	0.12 *	0.14 **	0.16 **
Firmness	0.27 **	NS	NS	0.13 *	0.17 **	0.12 *

*, $P \leq 0.05$; **, $P \leq 0.01$; NS, not significant.
Abbreviations: SSC, soluble solids content; TA, titratable acidity; RI, ripening index (SSC/TA)
[a] Total sugars: the sum of sucrose, glucose, fructose, and sorbitol for each genotype, analyzed by HPLC
[b] Ten-step scale from no endocarp staining (0) to strongly red staining (10)

Total sugars had a highly significant correlation with sucrose (r = 0.94**), as this is the predominant soluble sugar at maturity (20-90 mg g^{-1} FW) in *P. persica* fruit (Dirlewanger et al., 1999; Gurrieri et al., 2001). Soluble solids content was also positively correlated with sucrose, glucose, fructose and sorbitol as observed in other peach and nectarine cultivars (Wu et al., 2003). Correlation between SSC and total sugar was not very high (r = 0.33**), as reported for citrus (Echeverría and Ismail, 1990) and other peach cultivars (Byrne et al., 1991), probably due to the contribution of other soluble optically active compounds different from sugars such as pectins, salts, and organic acids to SSC value (Jacob, 1944). Indeed, high correlations have been found between SSC and organic acids (Wu et al., 2003). However, correlation coefficients varied depending on the

progeny, being higher in progenies such as 'VAC-9512' x 'VAC-9511' (r = 0.51**) and 'Babygold-9' x 'Crown Princess' (r = 0.52**), indicating a higher contribution of sugars to the SSC of their fruit.

Yield showed a negative significant correlation with SSC, obtaining higher coefficients in progenies such as 'Rich Lady' x 'VAC-9511' (r = -0.50**), 'Andross' x 'Crown Princess' (r = -0.39**), and 'VAC-9520' x 'VAC-9517' (r = -0.53**). Similar results were found when the whole breeding population (1111 genotypes) was analyzed (see chapter 3). This relationship could be explained by the negative effect of high crop loads on the total sugar content of fruits, as shown by Morandi et al. (2008). These authors demonstrated that high crop loads induced lower fruit total sugar content due to sink competition among fruits. On the other hand, fruit weight showed, in general, positive correlations with sugar levels. Slight significant correlation was found between fruit weight and SSC when all the seedlings were evaluated, and higher coefficients were found for certain progenies such as 'Andross' x 'Rich Lady' (r = 0.58**), 'Andross' x 'Crown Princess' (r = 0.47**), and 'O´Henry' x 'VAC-9516' (r = 0.46**). Similarly, a significant loose correlation was found between fruit weight and total sugar content, with higher coefficients for some progenies such as 'Babygold-9' x 'Crown Princess' (r = 0.49**) and 'Red Top' x 'VAC-9513' (r = 0.36**). These results are expectable since amount of translocated carbohydrates determines fruit growth rate as demonstrated by Morandi et al. (2008). Thus, although the fruit growth rate is not only determined by the availability of assimilates, a high carbon supply to the fruit as a sink organ will result into higher growth rates. At the same time, fruit sink size, together with sink activity, determines sink strength to attract sucrose and sorbitol from the leaves (Farrar, 1993; Lo Bianco and Rieger, 2006).

Other positive significant correlations were found between sugar components and other quality traits such as skin blush, endocarp staining, TA, RI, and/or firmness. The positive significant correlation found between some sugar traits with skin blush and endocarp staining, indicates that besides the positive effect of blush and endocarp staining on attractiveness (Iglesias and Echeverría, 2009) and healthy promoting properties of fruits (Tomás-Barberán et al., 2001), these traits could be related to higher SSC and total sugars content, and therefore to better organoleptic quality. This relationship is reasonable since a sufficient accumulation of assimilates, contributing to the SSC, in or near the fruit is essential for phenolic compounds synthesis (Pirie and Mullins, 1976), responsible of the blush and endocarp staining of fruits (Tomás-Barberán and Espín, 2001). Titratable acidity showed a significant positive correlation with sucrose, glucose, fructose, sorbitol, total sugars, and SSC, in agreement with previous works (Cheng et al., 2004; Wu et al., 2003). This result could be due to the co-localization of QTLs involved in fruit weight, SSC, sugars, and acid contents in peach (Dirlewanger et al.,

1999). An expectable high correlation was found between RI and SSC, since RI is the ratio between SSC and TA. This sugar-acid ratio (RI) is commonly used as a quality index (Bassi and Selli, 1990; Robertson et al., 1989), and higher ratios are usually indicative of higher and more acceptable fruit quality (Crisosto and Crisosto, 2005; Ledbetter et al., 2006) since it has been reported that RI is better correlated with perceived sweetness than individual sugars (Colaric et al., 2005). On the other hand, firmness show a significant loose correlation (r = 0.27) with SSC, and other sugar traits. This means that, for the same state of ripening, firmer fruits could have higher SSC and total sugar content, in agreement with what has been reported for sweet cherry (Jiménez et al., 2004).

4.5. CONCLUSIONS

On the basis of this study, the substantial variation among the breeding genotypes suggests that high quality peach and nectarine genotypes based on sugar contents can be developed in the future. The results showed the significant influence of cross and qualitative pomological traits on sugar profile of peaches and nectarines. Nectarine and flat fruits showed a tendency of having higher SSC and total sugars content than peaches and round fruits, respectively. Also, white flesh fruits had in general, higher sugar values than yellow flesh fruits. A slight tendency was also observed for free-stone fruit type to have higher SSC, total sugars and individual sugar contents than cling-stone fruit type. On the other hand, a year effect was found for fruit weight, SSC, TA, sucrose and glucose contents, whereas fructose and sorbitol appear to be more invariable across years. Valuable correlations among agronomic and fruit quality parameters and sugar traits were found, although coefficients variation depending on the progeny should be considered. The results of this work show the importance of the sugar profile as a trait to be considered in a breeding program searching for high quality peaches and nectarines. Further, our results show the extent of sugar profile modification feasible in the peach and nectarine germplasm adapted to the Mediterranean area.

Capítulo 5

Evaluation of the antioxidant capacity, phenolic compounds and vitamin C content

5.1. ABSTRACT

Antioxidant capacity and contents of total phenolics, anthocyanins, flavonoids and vitamin C were evaluated in 218 genotypes from 15 peach and nectarine breeding progenies. Significant differences were found among progenies on the fruit antioxidant profile, corroborated by the high contribution showed by cross to the phenotypic variance of each phytochemical trait analyzed (16-45%). Phytochemical profile varied depending on peach/nectarine and yellow/white flesh color qualitative traits. On the other hand, no significant effect of year was found on the bioactive profile of peaches and nectarines. Antioxidant capacity was linearly correlated to total phenolic content but correlation varied depending on the progeny. No correlation was found for vitamin C versus any other phytochemical trait. The results suggest the importance of genetic background on the antioxidant profile of peaches and nectarines and stress its relevance for the ultimate objective of this work: selecting new peach and nectarine genotypes rich in bioactive compounds to benefit consumer's health.

5.2. INTRODUCTION

The important role of diet in either promoting or preventing diseases has long been recognized, and in recent years, diet and human well-being have received unprecedented attention. Nowadays, there is a growing interest in bioactive compounds of fruits and vegetables due to their putative role in preventing diseases such as diabetes, cancer, stroke, arthritis, and also aging. A clear inverse relationship between the consumption of fruits and vegetables and incidence of cardio- and cerebrovascular, degenerative, and proliferative diseases and mortality has been largely proved by epidemiological studies (Sun et al., 2002). Fruits and vegetables are excellent functional foods as they are high in antioxidant compounds (Tomás-Barberán and Robins, 1997). These naturally occurring substances not only have a role in the visual appearance (pigmentation and browning) and taste (astringency) of fruits and vegetables but also have health-promoting properties, acting as antioxidants by scavenging harmful free radicals, which are implicated in most degenerative diseases (Rice-Evans et al., 1996).

The health benefits of fruits are due to their specific chemical composition, particularly to compounds of nutritional value as phenolic acids, flavonoids, and vitamins (Kaur and Kapoor, 2001). Peaches and nectarines, even though having a lower total antioxidant capacity than other fruits such as strawberry, apple, or orange (Rupasinghe and Clegg, 2007), are nutritionally important because they are one of the most important commodities consumed worldwide. Polyphenols are secondary plant metabolites, and they are the main sources of antioxidant capacity in peaches, although vitamin C and carotenoids also contribute to it (Gil et al., 2002). The basic feature of all polyphenols is the presence of one or more hydroxylated aromatic rings, which seemed to be responsible for their properties as radical scavengers (Fukumoto and Mazza, 2000). The flavonoids are a large class of phenolic compounds, present in cereals, vegetables, and fruits. Evidence is accumulating about their significant contribution to the antioxidant capacity of fruits and vegetables (Prior and Cao, 2000). Anthocyanins are natural colorants and, with flavanols and flavonols, are included in the flavonoid family. They are widely distributed among flowers, fruits, and vegetables and, in addition to their colorful characteristics, they have potent antioxidant properties modulated by their different hydroxylations and glycosylations (Rice-Evans et al., 1996). The main anthocyanins reported in peach are cyanidin-3-glucoside and cyanidin-3-rutinoside (Tomás-Barberán et al., 2001). Besides their relevance in the appearance, taste, and flavor of fruits as well as their health-promoting properties (Tomás-Barberán and Espín, 2001), phenolic compounds have been found to increase the shelf life of food and inhibit the growth of pathogenic microorganisms due to their natural antimicrobial properties (Cevallos-Casals et al.,

2006). Vitamin C is a water soluble antioxidant and is, as are vitamin E and β-carotene, referred to as an antioxidant vitamin. Humans are unable to synthesize vitamin C and are thus entirely dependent upon dietary sources to meet needs. More than 90% of the vitamin C in the human diet is supplied by fruits and vegetables (Davey et al., 2000). These benefits and the increasing consumer interest in functional foods have guided breeders of different crops to consider antioxidant compounds and other nutritional properties as interesting targets in breeding programs (Romandini et al., 2008; Ruiz et al., 2005).

The phytochemical content of fruit is influenced by numerous factors such as genotype, rootstock, climatic conditions, agronomic practices, harvesting time, and postharvest conditions (Cevallos-Casals et al., 2006; Gil et al., 2002; Lee and Kader, 2000; Romandini et al., 2008). Moreover, phenolic compounds are not uniformly distributed within the tissue of fruits, and most of them are concentrated in the epidermal and subepidermal layers of the fruit (Cevallos-Casals et al., 2006). Phenolic distribution is an important aspect of the overall phenolic composition and antioxidant capacity because, due to its characteristics, the peach skin is usually not eaten and therefore it does not contribute to the human diet intake.

The aim of the present work was to screen and compare 218 genotypes from 15 different peach and nectarine breeding progenies by measuring their contents of total phenolics, total flavonoids, total anthocyanins, vitamin C, and relative antioxidant capacity. We also wanted to study the influence of genotype, genetic origin, pomological traits, and year in the bioactive profile of peach and nectarine fruits. The ultimate objective of this study was to select peach genotypes with enhanced antioxidant capacity fruits that will benefit consumers with health-promoting properties.

5.3. MATERIALS AND METHODS

5.3.1. Chemicals

All chemicals were of analytical grade. Folin-Ciocalteu's phenol reagent, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-dipyridyl,1,1-diphenyl-2-picrylhydrazyl (DPPH), 3,4,5-trihydroxybenzoic acid (gallic acid), sodium carbonate (Na_2CO_3), catechin, trichloroacetic acid (TCA), and ascorbic acid (vitamin C) were purchased from Sigma-Aldrich (Steinheim, Germany).

5.3.2. Plant material

Fifteen controlled biparental crosses between 19 peach and nectarine cultivars (Table 5.1) were made during 2000 and 2001 to develop superior peach and nectarine cultivars for the Spanish industry. The resulting seedling trees (one tree per genotype) were grafted on the same rootstock (GF-677) and established in an experimental orchard at the Experimental Station of Aula Dei-CSIC (northern Spain, Zaragoza) in 2002. Trees were trained to the standard open vase system and planted at a spacing of 4 m x 2.5 m. Hand thinning was carried out to reduce fruit load on the heavily loaded trees. They were grown under usual conditions of irrigation, fertilization, and pest control. Vegetative and fruit quality traits were evaluated in a total of 1111 genotypes over three consecutive years (2005-2007). All traits were measured or scored for each seedling separately over the three year period, and means of three years were calculated. Phytochemical composition (total phenolics, total flavonoids, total anthocyanins, and total antioxidant capacity) was studied in 218 genotypes that were common at least for two years to estimate the seasonal effect on phytochemical profile. Vitamin C was also determined in all of the genotypes in the last year of study to corroborate the variability found in other bioactive compounds in the previous years and its contribution to the antioxidant capacity of fruits. The studied genotypes were selected among the descendants from the 15 crosses because of their higher fruit quality. For all analyses, only fruit flesh was used, as it is usually consumed. Fruits were peeled with a sharp knife, and flesh was weighed, immediately frozen separately in liquid nitrogen, and stored at -20 ºC until analysis. Samples for vitamin C determination were kept at -20 ºC in 5% metaphosphoric acid for preservation of ascorbic acid until analysis.

Table 5.1. Peach and nectarine commercial and experimental (VAC-) cultivars used as progenitors in the fifteen controlled crosses. Fruit type (round or flat, peach or nectarine), flesh color (yellow or white), and stone adherence (free or cling) for each progenitor is shown.

Cultivar	Fruit type		Flesh colour	Stone
Andross	round	peach	yellow	cling
Babygold-9	round	peach	yellow	cling
Big Top	round	nectarine	yellow	cling
Calante	round	peach	yellow	cling
Crown Princess	round	peach	yellow	cling
O´Henry	round	peach	yellow	free
Orion	round	peach	yellow	free
Red Top	round	peach	yellow	free
Rich Lady	round	peach	white	free
VAC-9510	round	peach	yellow	cling
VAC-9511	round	peach	yellow	free
VAC-9512	round	peach	yellow	free
VAC-9513	round	nectarine	yellow	free
VAC-9514	round	nectarine	white	free
VAC-9515	round	nectarine	yellow	free
VAC-9516	round	peach	white	free
VAC-9517	flat	peach	white	free
VAC-9520	round	peach	yellow	free
Venus	round	nectarine	yellow	free

5.3.3. Quality parameters

During the years 2005, 2006, and 2007, fruit quality parameters were measured individually in each seedling tree. Fruits were hand-picked at commercial maturity, assessed by peel fruit color and flesh firmness. Yield (kg/tree) was measured, and total number of fruits was counted for each genotype. From these variables, total average fruit weight was calculated. Ten fruits from each plant were randomly selected for the quality evaluations. Some quality traits such as fruit type (peach/nectarine), flesh color (yellow/white), and endocarp staining were scored. Fruit type was scored on a 1-2 scale as peach (1) or nectarine (2). Similarly, flesh color was scored as (1) yellow or (2) white. Endocarp staining (redness around stone) was scored on an increasing scale from no color (1) to high redness (10). The soluble solids content (SSC) of the juice was measured with a temperature-compensated refractometer (model ATC-1, Atago Co., Tokyo, Japan), and data are given as ºBrix. The titratable acidity (TA) was determined by titration with 0.1 N NaOH to pH 8.1 (AOAC, 1984). Data are given as grams malic acid per 100 g of fresh weight (FW), because this is the dominant organic acid in peach (Sweeney et al., 1970; Wills et al., 1983).

5.3.4. Phytochemical analysis

The frozen fruit material (5 g) was homogenized with a Polytron (2 min on ice) with 10 ml of extraction solution, consisting of 0.5 N HCl in methanol/Milli-Q water (80% v/v). The mixture was incubated overnight at 4 ºC and then centrifuged for 20 min at 4 ºC and 20000 *x* g. Supernatant was recovered and the volume measured. This hydroalcoholic extract was used for total phenolics, anthocyanins, flavonoids, and antioxidant capacity assays.

The content of phenolic compounds in methanol extracts was determined according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). The method consisted of mixing 500 μl of the extract diluted in water with 500 μl of Folin-Ciocalteu's reagent. After 3 min of reaction, 1 ml of 1 N sodium carbonate (Na_2CO_3) was added. The tubes were mixed for 15 s and then allowed to stand for 60 min at 20 ºC. Absorbance was measured at 725 nm using a spectrophotometer (Beckman Coulter DU 800). The standard calibration curves were daily prepared using gallic acid (3,4,5-Trihydroxybenzoic acid). The phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per 100 g of FW.

Total flavonoids content was determined using a colorimetric assay based on the method of Zhishen et al. (1999). One ml of the methanolic extract was diluted with water (1:2) and 0.3 ml 5% $NaNO_2$ was added. After five minutes, 0.3 ml 10% $AlCl_3$ were added. After 1 min, 2 ml 1 N NaOH was added and solution was mixed by vortex. Absorbance at 510 nm was measured against a blank with a spectrophotometer (Beckman Coulter DU 800). The results were expressed as milligrams of catechin equivalents (CE) per 100 g FW on the basis of a standard curve using catechin as standard.

Total anthocyanin content of the hydroalcoholic extracts was determined using the method of Fuleki and Francis (1968) adapted to peach tissue. Aliquots of the clear methanol extract were used for spectrophotometric readings at 535 nm by subtracting the absorbance at 700 nm (due to turbidity). The spectrophotometer was zeroed with the anthocyanins extraction solvent as the blank. Anthocyanins were quantified as mg of cyanidin-3-glucoside per kg of FW using a molar extinction coefficient of 25965 cm^{-1} M^{-1} and a molecular weight of 494 (Abdel-Aal and Hucl, 1999).

Vitamin C was determined using the method for the spectrophotometric determination of ascorbic acid (vitamin C) as described by Zaharieva and Abadía (2003). Samples were homogenized with 5% metaphosphoric acid at 4 ºC. Then, they were centrifuged at 20000 *x* g for 15 min at 4 ºC, and the supernatant was immediately used for vitamin C analysis. Absorbance was measured at 525 nm using a spectrophotometer (Beckman Coulter DU 800). The standard calibration curve was daily prepared using

ascorbic acid as standard. Vitamin C was expressed as mg ascorbic acid (AsA) per 100 g of FW.

The antioxidant capacity was measured using the DPPH method adapted from Brand-Williams et al. (1995). Briefly, 100 μl of the methanolic extract was added to 2.9 ml of fresh DPPH radical solution (98.9 μM in methanol) and mixed in the dark by vortex at room temperature. The absorbance of the samples was measured at 515 nm after 10 min. These readings were used for calculation of the relative antiradical capacity (RAC), which indicates the antiradical capacity of the sample compared to Trolox for a specific reaction time (10 min). For each sample, three separate determinations were carried out. The standard calibration curves were prepared daily using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid). Results were expressed in μg of Trolox per gram of FW.

5.3.5. Statistical Analyses

All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL). To obtain basic statistics for the entire plant material studied, maximum and minimum values, mean, mean standard error (MSE), and standard deviation (SD) were calculated for each trait. Data for each genotype in the three years of study were averaged, and mean values were used as estimated genotypic values. The significance of cross, year, and cross x year interaction effects on phytochemical profile was tested on the 218 genotypes by analysis of variance (ANOVA). Duncan´s multiple-range test ($P \leq 0.05$) was used to estimate progeny means and to find differences in phytochemical profile among crosses. A *t* test ($P \leq 0.05$) was run to compare different fruit types. Finally, correlations were calculated with raw data of the three years, according to Pearson´s test at $P \leq 0.01$.

5.4. RESULTS AND DISCUSSION

5.4.1. Genotype and cross effect

Table 5.2 shows the ranges of bioactive compounds and total antioxidant activity in peaches and nectarines. Total phenolics, as determined by the Folin-Ciocalteu assay, varied among genotypes with values in the range of 12.7-71.3 mg of GAE (gallic acid equivalents) per 100 g of FW. Values are within the range reported for peach flesh in the literature, namely, 14-77 mg of GAE per 100 g of FW (Romandini et al., 2008; Tavarini et al., 2008; Tomás-Barberán et al., 2001). Total flavonoids content ranged from 1.8 to 30.9 mg CE (catechin equivalents) per 100 g FW, with an average of 8.8 mg CE per 100 g FW.

Total anthocyanins greatly varied among genotypes [0.1-26.7 mg of cyaniding-3-glucoside equivalents (C3GE) per kg of FW] depending on the red pigmentation of the flesh. Genotypes with red flesh had higher anthocyanins content. Values of flavonoids and anthocyanins in this range have been reported by other authors (Gil et al., 2002; Tomás-Barberán et al., 2001). Higher values of anthocyanins content in peaches and nectarines are found in the literature when skin is included in the sample (Tomás-Barberán et al., 2001) due to unequal distribution of phenolic compounds in the flesh (~30%) and the skin (~70%) of the peach fruit (Asami et al., 2003; Cevallos-Casals et al., 2006). On average, unpeeled fruit contained 1.5-fold higher levels of phenolics than peeled fruit (Asami et al., 2003). However, as already mentioned above, peach skin is not usually appreciated by consumers and, therefore it takes no part in the human diet. The total ascorbic acid (vitamin C) content greatly varied from approximately 1 to 9 mg of AsA/100 g FW, with a mean value of 3.7 mg of AsA/100 g of FW. Genetic background of the genotype is a much more important factor than climatic conditions and cultural practices in producing fruit with high vitamin C content at harvest (Lee and Kader, 2000). Values were in the same range as previously reported for vitamin C contents in peach flesh, namely, 1-14 mg of AsA/100 g of FW (Gil et al., 2002; Proteggente et al., 2002; Tavarini et al., 2008), and lower than values obtained when peach peel was included in the test (Gil et al., 2002). As for phenolic compounds, skin tissues have more vitamin C to protect the fruit from outside stress caused by light and oxidation (Zhishen et al., 1999). The relative antioxidant capacity (RAC) varied among genotypes, with values ranging from 227.3 to 629.9 μg of Trolox/g of FW, with an average of 405 μg of Trolox/g of FW. In recent years, strong attention has been given to this trait as an eligible parameter for fruit quality because many of the biological actions of phytochemicals have been attributed to it. As for anthocyanins, the antioxidant capacity observed in our study was in the range previously reported for peach flesh (100-1000 μg of Trolox/g of FW), but lower than in other studies where peel was included in the test sample (700-6000 μg of Trolox/g of FW) (Cevallos-Casals et al., 2006; Gil et al., 2002). Therefore, the antioxidant capacity of the fruits decreases when they are peeled.

Table 5.2. Basic statistics based on single plant observations for the seedlings from fifteen F1 peach and nectarine progenies studied over three years, for total phenolics, flavonoids, anthocyanins, vitamin C and antioxidant capacity (RAC). For each trait, number of observed seedlings (n), minimum, maximum, mean value, mean standard error (MSE), and standard deviation (SD) are presented.

Trait	n	Minimum	Maximum	Mean	MSE	SD
Total phenolics (mg GAE 100 g^{-1} FW)	218	12.7	71.3	36.4	1.0	15.2
Flavonoids (mg CE 100 g^{-1} FW)	218	1.8	30.9	8.8	0.4	6.0
Anthocyanins (mg C3GE kg^{-1} FW)	218	0.1	26.7	3.0	0.3	4.0
Vitamin C (mg AsA 100 g^{-1} FW)	218	1.2	9.1	3.7	0.1	1.5
RAC (μg Trolox g^{-1} FW)	218	227.3	629.9	405.0	4.9	73.0

Abbreviations: AsA, ascorbic acid; CE, catechin equivalents; GAE, gallic acid equivalents; C3GE, cyanidin-3-glucoside equivalents

Considerable variation was found in the content of antioxidant compounds in the fruits from different progenies (Figure 5.1). The highest total phenolic contents were shown by the three progenies descendant from ‘O´Henry’ cultivar, although no significant differences were found with mean values of ‘Andross’ x ‘Calante’, ‘Andross’ x ‘Crown Princess’, ‘Babygold-9’ x ‘Crown Princess’, and ‘Orion’ x ‘VAC-9510’ progenies. The level of total phenolics in ‘O’Henry’ x ‘VAC-9514’ was more than 2-fold higher than in ‘Rich Lady’ x ‘VAC-9511’, showing the wide variance of total phenolic concentrations in *Prunus persica* already reported in other studies (Gil et al., 2002; Romandini et al., 2008; Tavarini et al., 2008; Tomás-Barberán et al., 2001). Similarly, a high variability in the flavonoids content averaged for the 15 progenies was found in agreement with the variability among *P. persica* cultivars reported by other authors (Tomás-Barberán et al., 2001). A nearly 5-fold difference was measured between the lowest and highest mean values among different progenies. The highest flavonoids content was also shown by ‘O´Henry’ x ‘VAC-9514’ progeny, although no significant difference was found with ‘O´Henry’ x ‘VAC-9515’. The highest values were found in the three ‘O´Henry’ progenies, resulting in the most interesting crosses from which to select peaches and nectarines with higher flavonoid content in the flesh. The ‘O´Henry’ progenies also showed the highest anthocyanins content, although no significant differences were found with ‘Andross’ x ‘VAC-9511’, ‘Babygold-9’ x ‘VAC-9510’, ‘Orion’ x ‘VAC-9510’, ‘VAC-9512’ x ‘VAC-9511’, and ‘VAC-9520’ x ‘VAC-9517’ progenies. Tomás-Barberán et al. (2001) reported higher anthocyanin content in the flesh of ‘O´Henry’ fruits (8.1 mg per kg FW) when compared with other commercial peach cultivars such as ‘September Sun’ (3.7 mg per kg FW), ‘Rich Lady’, and ‘Spring Lady’ (no significant amounts detected in either). In agreement with all of

these results, we could report an influence of ‘O´Henry’ cultivar to induce higher anthocyanins content in its progeny as observed for flavonoids content. On the other hand, the highest vitamin C content was found in the ‘VAC-9520’ x ‘VAC-9517’ progeny, although differences were not significant with ‘Andross’ x ‘VAC-9511’, and ‘VAC-9512’ x ‘VAC-9511’ progenies. The lowest mean value was shown by ‘Venus’ x ‘Big Top’ nectarines progeny without being significantly different from ‘Andross’ x ‘Crown Princess’, both ‘Babygold-9’ progenies, ‘O´Henry’ x ‘VAC-9515’, and ‘Orion’ x ‘VAC-9510’ progenies. A significant effect of cultivar and rootstock on the vitamin C content has been previously reported in different fruits (Lee and Kader, 2000). Tavarini et al. (2008) found a range from 1 to 14 mg AsA per 100 g FW in seven peach cultivars, and Nelson et al. (1972) reported values from 19.3 to 71.5 mg AsA per 100 g FW in six strawberry cultivars. Significant differences among progenies were also found for RAC (Figure 5.1), according to previous results that have shown that antioxidant capacity changes as a function of cultivar and rootstock (Gil et al., 2002; Romandini et al., 2008; Tavarini et al., 2008). As above-mentioned for other bioactive compounds, the highest RAC values were shown by ‘O’Henry’ descendants, without being significantly different from the ‘Andross’ x ‘Calante’ progeny. These results indicate the importance of cultivar and genotype for determining the antioxidant potential and phenolic content of the fruit. The most appropriate combination of phytochemical traits must be considered for the selection of new genotypes with higher nutritional value.

5.4.2. Pomological traits effect

Bioactive compounds and antioxidant content of fruit varied depending peach/nectarine and yellow/white flesh color qualitative traits (Table 5.3). In this work, peaches showed higher phenolic content than nectarines, and phenolic content of white-fleshed fruit was higher than that of yellow-fleshed fruit. This shows a tendency of white-fleshed peaches to have significantly higher antioxidants content than the other genotypes tested (yellow-fleshed peaches and nectarines). No significant differences in flavonoids and anthocyanins content were found between peaches and nectarines; however, they were significantly higher in white-fleshed fruits than in yellow-fleshed fruits as previously found (Tomás-Barberán et al., 2001). This could be explained by the red pigmentation due to anthocyanins in the white-fleshed fruit, especially in the flesh area surrounding the stone, usually found in our studied progenies. This result is different from what occurs in the peel, where yellow-fleshed fruits are reported to produce more anthocyanin pigments than white-fleshed fruits (Tomás-Barberán et al., 2001). No significant differences were found for vitamin C between peach and nectarine fruits, whereas it was higher in white-

fleshed fruit than in yellow-fleshed fruit. Consequently, with all of these results, white-fleshed fruits showed higher antioxidant capacity than the yellow-fleshed ones, as reported in previous works (Gil et al., 2002). In agreement with these results, significant slight positive correlations ($P \leq 0.01$) were found for color flesh fruit versus phenolic compounds, flavonoids, anthocyanins, vitamin C, and RAC ($r = 0.265$, $r = 0.283$, $r = 0.189$, $r = 0.339$ and $r = 0.243$, respectively), indicating different content of these bioactive compounds in white- and yellow-fleshed fruits, as commented above.

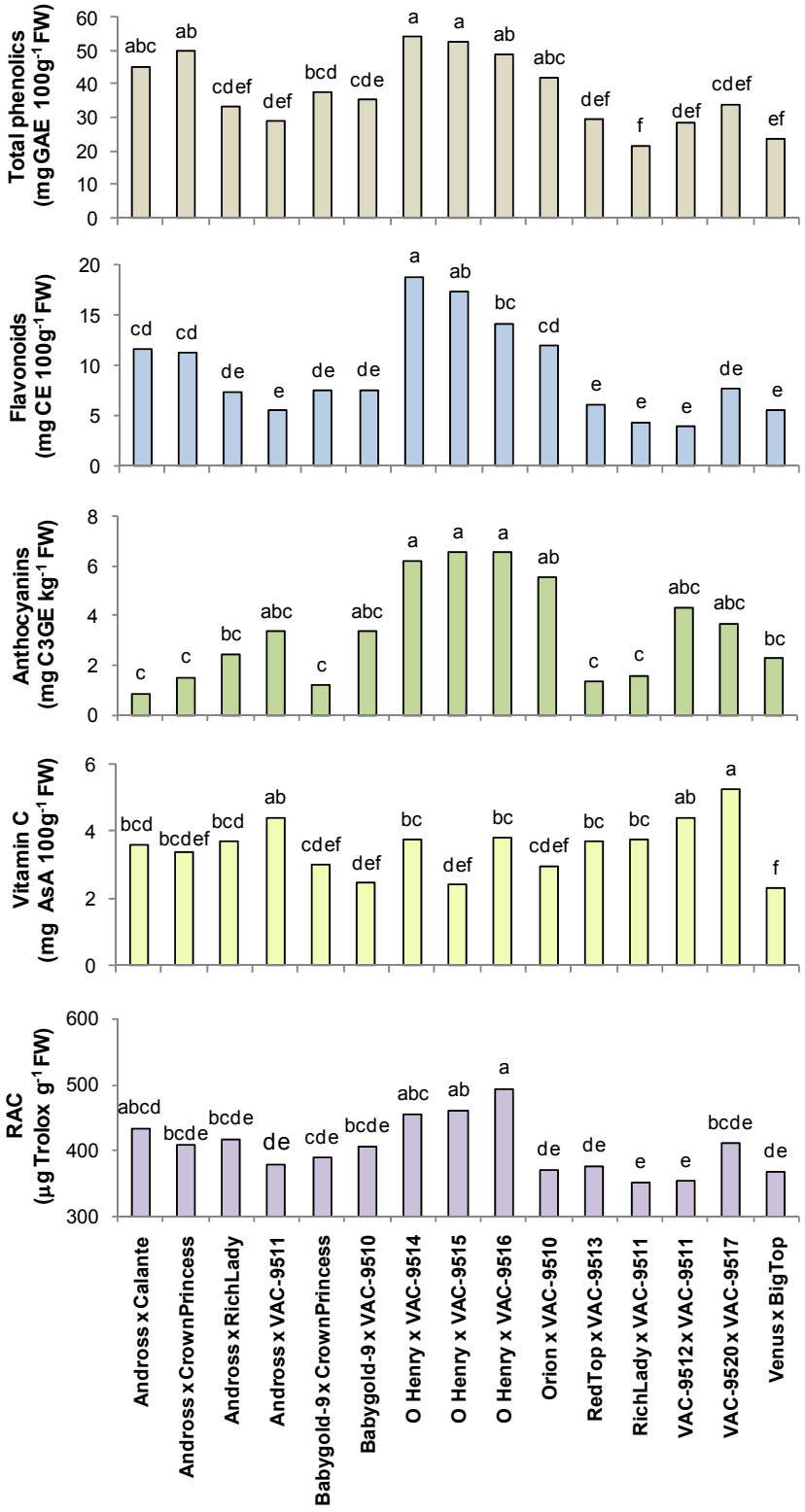

Figure 5.1. Phytochemical profiles of the fifteen peach and nectarine progenies. Data are the means of all the genotypes in each progeny. For each trait, means with the same letter are not significantly different according to Duncan´s test ($P \leq 0.05$). *Abbreviations*: GAE, gallic acid equivalents; CE, catechin equivalents; C3GE, cyanidin-3-glucoside equivalents; AsA, ascorbic acid; RAC, relative antioxidant capacity.

Table 5.3. Total phenolics, flavonoids, anthocyanins, vitamin C, and relative antioxidant capacity (RAC) in different *P. persica* fruit types. For each fruit type, number of observed seedlings (n) is presented. Data are means over the three years of study.

Fruit type	n	Total phenolics (mg GAE 100 g^{-1} FW)	Flavonoids (mg CE 100 g^{-1} FW)	Anthocyanins (mg C3GE kg^{-1} FW)	Vitamin C (mg AsA 100 g^{-1} FW)	RAC (μg Trolox g^{-1} FW)
Peach	192	37.2 a	9.1 a	3.1 a	3.7 a	406.2 a
Nectarine	26	30.5 b	6.9 a	2.2 a	3.9 a	395.7 a
Yellow fleshed	176	34.5 b	8.0 b	2.6 b	3.5 b	396.5 b
White fleshed	42	44.8 a	12.3 a	4.5 a	4.8 a	442.2 a

[a] In each trait column (Peach-Nectarine and Yellow fleshed-White fleshed), means with the same letter are not significantly different according to *t* test ($P \leq 0.05$). *Abbreviations*: GAE, gallic acid equivalents; CE, catechin equivalents; C3GE, cyanidin-3-glucoside equivalents; AsA, ascorbic acid

In agreement with these results, the percentage of phenotypic variance explained by cross (Table 5.4) was high for each bioactive compound analyzed (between 15.7 and 44.6%). Contribution of cross to anthocyanins and antioxidant capacity was lower than to total phenolics, flavonoids, and vitamin C phenotypic variance. These results indicate that cultivar and genotype are decisive in determining the peach fruit antioxidant capacity.

On the other hand, no significant differences were found among the three years of study for total phenolics, flavonoids, anthocyanins and antioxidant capacity (Table 5.4). Despite this result, slight higher flavonoids and anthocyanins contents were observed in the first year of study when compared with the two following years (data not shown), which may be due to differences of climate including temperature, sun irradiation, and/or water stress as mentioned by Tomas-Barberán and Espín (2001). Sun irradiation has demonstrated to increase anthocyanin content of different fruits such as apples and pears, whereas in cherry, grape, and plum, light seems not to be essential for red color formation (Tomás-Barberán and Espín, 2001). Temperature, and in particular the difference between day and night temperatures, has been reported to have a marked effect on anthocyanin accumulation in apples, plums, grapes, and pomegranates (Tomás-Barberán and Espín, 2001).

Table 5.4. Factors affecting phytochemical profile in fifteen peach and nectarine progenies studied over three years. Significant factors ($P \leq 0.01$) and their proportion (%) of phenotypic variance are indicated, as determined by ANOVA.

Variable	F-value	P	phenotypic variance (%)
Total phenolics			
Cross	16.79	0.000	33.6
Year	1.22	0.295	0.5
Cross x Year	0.27	1.000	1.5
Flavonoids			
Cross	26.69	0.000	44.6
Year	0.15	0.865	0.1
Cross x Year	0.42	0.996	2.4
Anthocyanins			
Cross	6.21	0.000	15.7
Year	1.09	0.336	0.5
Cross x Year	0.36	0.999	2.0
Vitamin C			
Cross	8.02	0.000	35.8
Year	-	-	-
Cross x Year	-	-	-
RAC			
Cross	8.08	0.000	19.6
Year	0.98	0.375	0.4
Cross x Year	0.33	1.000	1.9

[a] This proportion of phenotypic variance attributed to Genotype is the broad sense heritability (H); (-) no data available.
Abbreviations: RAC, relative antioxidant capacity

5.4.3. Correlations among phytochemical constituents and other fruit quality traits

A high positive correlation was found between total phenolics and flavonoids content ($r = 0.742$, $P \leq 0.01$), implying that flavonoids are an important group of phenolic compounds in peaches and nectarines (Table 5.5). Moreover, a linear positive relationship (Figure 5.2) was observed between antioxidant capacity and total phenolics for the flesh of the peach and nectarine genotypes, as has been observed for peaches, apricots, and plums (Gil et al., 2002; Rupasinghe and Clegg, 2007). However, higher correlation coefficients ($r > 0.9$) were obtained by Gil et al. (2002) for other peach and nectarine cultivars. This variation could be due to differences in the phytochemical profile of different peach and nectarine cultivars. In addition, the large phenotypic variability within the breeding progenies in our study could induce lower correlation coefficients between those parameters.

Table 5.5. Pearson´s correlation coefficients for phytochemical and quality traits observed over three years in fifteen peach and nectarine progenies.

Trait	Flavonoids	Anthocyanins	Vitamin C	RAC
Total phenolics	0.742 **	0.144 *	NS	0.606 **
Flavonoids		NS	NS	0.553 **
Anthocyanins			NS	NS
Vitamin C				NS

*, $P \leq 0.05$; **, $P \leq 0.01$; NS, not significant.

Total phenolics and flavonoids were the only constituents that correlated significantly ($P \leq 0.01$) with antioxidant capacity ($r = 0.606$ and $r = 0.553$, respectively), indicating that they are important bioactive compounds contributing to antioxidant capacity of peaches and nectarines, in accordance with previous reports on different peach, nectarine and plum cultivars (Cevallos-Casals et al., 2006; Gil et al., 2002). Indeed, correlation coefficients varied depending on the progenies. Higher correlation coefficients ($P \leq 0.01$) were found between total phenolics and RAC in some progenies, such as 'Rich Lady' x 'VAC-9511' ($r = 0.835$), and 'Orion' x 'VAC-9510' ($r = 0.925$) progenies, whereas no significant correlation was found in others ('Venus' x 'Big Top', 'Babygold-9' x 'VAC-9510', 'Andross' x 'VAC-9511', 'O´Henry' x 'VAC-9516', and 'O´Henry' x 'VAC-9514'). Previous works (Remorini et al., 2008; Tavarini et al., 2008) have also shown these differences among peach progenies. Indeed, it is well-known that it is not only the total content of phenols but also their specific structural features, such as the number of available hydroxyl groups, that determine their antioxidant capacity (Rice-Evans et al., 1996).

Proteggente et al. (2002) reported that highest antioxidant capacity is found in fruits such as strawberry, raspberry, and plum due to their high content of anthocyanins. However, no significant correlation was obtained between anthocyanins and RAC in our study (Table 5.5). This fact is probably due to the lower content of anthocyanins in peaches and nectarines compared with contents in strawberries, raspberries, and plums. Vitamin C did not show significant correlation with RAC. All these results suggest that phenolic compounds are mainly responsible for the antioxidant activity on peaches and nectarines, as previously described for stone fruit (Cevallos-Casals et al., 2006; Gil et al., 2002) whereas vitamin C is reported as the main antioxidant compound in oranges, strawberries, raspberries and blueberries (Gardner et al., 2000). The demonstrated beneficial effects of antioxidant compounds on health make the antioxidant capacity of fruits an important trait to be considered in breeding programs. However, due to the lack

of correlation between RAC and other important bioactive compounds such as anthocyanins and vitamin C, we suggest considering and including these traits in a breeding program for the selection of higher fruit quality genotypes.

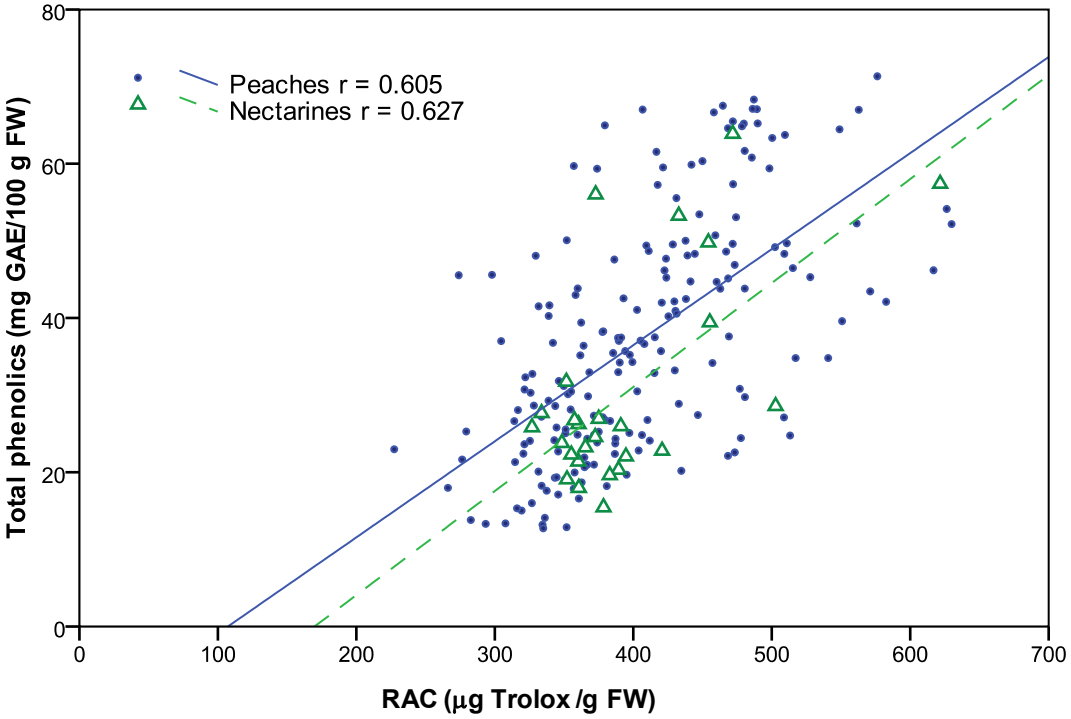

Figure 5.2. Linear regression ($P \leq 0.01$) between relative antioxidant capacity (RAC) and total phenolics (GAE, gallic acid equivalents) in the peach and nectarine genotypes. Each value is the mean over the three years of study for each genotype.

Both total phenolics and flavonoids contents showed a slight significant positive correlation with fruit weight and sugar content (Table 5.6), showing a tendency of bigger and sweeter fruits to have higher levels of these bioactive compounds. This is consistent with the findings reported for most species such as plums (Díaz-Mula et al., 2008), apricots (Bureau et al., 2009a), sweet cherries (Serrano et al., 2005) and apples (Awad and de Jager, 2002). 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). On the other hand, a significant positive correlation was found for total phenolics, flavonoids, and anthocyanins versus endocarp staining (redness around stone), supporting the recognized role of anthocyanin pigments in this quality trait. Higher correlation coefficients between total phenolics and endocarp staining were found in some

progenies such as 'Andross' x 'Calante' (r = 0.522), 'Andross' x 'Rich Lady' (r = 0.730), and 'O´Henry' x 'VAC-9515' (r = 0.691), whereas no correlation was found for others, suggesting that relationships between traits depend on the progeny or cultivar evaluated. A positive correlation between endocarp staining and RAC was also found as a consequence of flavonoid pigments contribution to the antioxidant capacity of fruit (Rice-Evans et al., 1996). This result indicates that higher endocarp-stained fruits have higher antioxidant capacity and, consequently, higher health benefits according to previous papers (Tomás-Barberán et al., 2001). Finally, the positive correlation between vitamin C and TA is due to the contribution of ascorbic acid to the fruit acidity.

Table 5.6. Pearson´s correlation coefficients for phytochemical and quality traits observed over three years in fifteen peach and nectarine progenies.

Trait	Fruit weight	Endocarp staining	SSC	TA
Total phenolics	0.298 **	0.249 **	0.237 **	NS
Flavonoids	0.345 **	0.376 **	0.371 **	NS
Anthocyanins	0.166 *	0.218 **	NS	NS
Vitamin C	-0.450 **	NS	0.154 *	0.308 **
RAC	NS	0.175 **	0.268 **	NS

*, $P \leq 0.05$; **, $P \leq 0.01$; NS, not significant. *Abbreviations*: SSC, soluble solids content; TA, titratable acidity; RAC, relative antioxidant capacity

5.5. CONCLUSIONS

These results confirm the importance of genotype on the availability of bioactive compounds and antioxidant capacity of peach and nectarine fruits and, consequently, on their benefits to health. Therefore, the peach cultivars used as progenitors in the crosses of a breeding program have a vital importance to release new cultivars with high bioactive compounds content. On the other hand, the high number of evaluated genotypes, from different genetic origins and with a large phenotypic variability, constitutes a considerable contribution on peach species and especially on breeding purposes.

Capítulo 6

Chilling injury susceptibility

6.1. ABSTRACT

Chilling injury (CI) is the collective term for various disorders that occur during prolonged cold storage and/or after subsequent ripening of stone fruit. Major symptoms of CI include mealiness, graininess, flesh browning, loss of flavor (off flavor), and red pigmentation (bleeding). These symptoms were evaluated over 2 years in an intra-specific progeny population derived from the cross of cultivars 'Venus' (freestone, melting, yellow-flesh nectarine) and 'Big Top' (clingstone, melting, yellow-flesh nectarine) after storage at 5 ºC (CI inducing conditions) for 2 and 4 weeks. All the traits showed continuous variation which is typical of quantitative or polygenic inheritance. Longer cold storage periods increased the incidence and severity of CI symptoms, except for bleeding and leatheriness. CI symptoms showed high and significant heritability or genotype effect in the studied population, with no significant year effect. Browning, mealiness and graininess were significantly correlated and were the main CI symptoms observed in this population. Mealiness and graininess had negative correlation with stone adhesion which reflects a higher susceptibility of free stone fruit to CI disorders. A genetic linkage map of linkage group 4 was constructed with SSR and candidate genes (CGs). This map was used to validate mealiness and bleeding quantitative trait loci (QTLs) earlier reported on this linkage group from an unrelated progeny population. In addition, QTLs controlling graininess and some agronomic and quality traits were also localized in this linkage group.

6.2. INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is the second most important fruit crop in Europe in terms of production (approximately 4.3 million tons) with a cultivated area of around 260 thousand hectares (FAO, 2007), and it is the most important species of the genus *Prunus*. However, the rapid ripening of fruit results in short shelf-life of the commodity and represents a serious constraint for efficient handling and transportation. Ripening can be slowed by refrigeration, but extended storage of peaches, nectarines and other stone fruit at low temperatures between the freezing point and 10 ºC, or more severely in the range of 2.2-7.6 ºC (killing temperature zone) can negatively affect fruit quality. Under these storage conditions, several physiological disorders, collectively known as chilling injury (CI), are developed (Crisosto et al., 1999; Lill et al., 1989; Manganaris et al., 2006b). These symptoms are of commercial importance because shipping of peaches to distant markets and storage before selling requires low temperature (Campos-Vargas et al., 2006). This disorder, also called internal breakdown, is the major reason for the low consumption level of this fruit when compared to other fresh fruit such as apple and banana (Crisosto, 2006).

Susceptibility to chilling injury is highly influenced by the genetic background of the cultivar (Kader, 1985; Peace et al., 2006; Von Mollendorf, 1987). CI in peaches and nectarines can induce different symptoms, including mealiness or lack of juice, flesh browning and impaired softening which is referred to as leatheriness. The physiological basis of CI symptoms has been studied in detail in peach (reviewed in Lurie and Crisosto, 2005). However, the exact mechanism by which chilling injury affects a commodity is not fully understood. It has been shown to be concerned with loss of membrane integrity and ion leakage from cells and changes in enzyme activity (Brummell et al., 2004; Manganaris et al., 2008a; Zhou et al., 2001a), but exactly why some crops are susceptible and some resistant still remains unclear. Understanding the genetic control of these traits, so that only cultivars free of chilling injury susceptibility are grown, promises to greatly benefit producers, shippers, and consumers.

In the last decade, several linkage maps, obtained by using molecular markers, have been constructed for peach (Abbott et al., 1998; Dirlewanger et al., 2006; Dirlewanger et al., 1998; Dondini, 2007; Etienne et al., 2002a; Lu et al., 1998; Verde et al., 2005; Yamamoto et al., 2001; Yamamoto et al., 2005). As a result of an European project (Arús et al., 1998), a consensus map from an inter-specific almond x peach F_2 population (‘Texas’ × ‘Earlygold’) has been constructed (Howad et al., 2005; Joobeur et al., 1998), and it is considered the reference map of the *Prunus* genus. However, many important peach agronomic characters have not yet been mapped, and very few of which have been

already mapped (such as major genes for disease and pest resistances, fruit quality and self-incompatibility) are currently being used for Marker Assisted Selection (MAS) (Dirlewanger et al., 2004; Shulaev et al., 2008). The genetic control of CI has been already studied and it has been demonstrated that mealiness, browning and bleeding are probably controlled by major genes (Ogundiwin et al., 2007; Peace et al., 2006). Moreover, one major quantitative trait loci (QTL) has been detected for each of these symptoms of CI in linkage groups G4 and G5, using a linkage map constructed from two segregating populations - Pop-DG ('Dr. Davis' × 'Georgia Belle') and Pop-G ('Georgia Belle' selfed) (Ogundiwin et al., 2007; Peace et al., 2005a; Peace et al., 2006). A major QTL for mealiness and bleeding was found at the *F-M* locus at the bottom end of linkage group G4. Other minor QTLs for mealiness were also found on linkage groups 4 and 6. A cell wall-degrading enzyme, endopolygalacturonase (*endoPG*), collocated with the *F-M* locus and the major QTL for mealiness and bleeding (Peace et al., 2005a; Peace et al., 2005b) and an anthocyanin biosynthesis pathway gene, leucoanthocyanidin dioxygenase (*PpLDOX*) co-located with the major QTL for browning on linkage group G5 (Ogundiwin et al., 2008b). An expressed sequence tags (ESTs) database has been developed specifically to study chilling injury (Ogundiwin et al., 2008a). Microarray analysis involving these ESTs has identified several cold-regulated peach genes some of which have been mapped close to CI QTLs on Pop-DG (Ogundiwin et al., 2009b). However, in these populations (Pop-DG and Pop-G), only clingstone non-melting flesh (CNMF) and freestone melting flesh (FMF) progeny were obtained. As CNMF progeny did not get mealy (Ogundiwin et al., 2007; Peace et al., 2006), and FMF and CMF genotypes have the potential to develop this symptom depending on whether they carry further genes for susceptibility, an entirely melting segregating population is of interest for the study of CI susceptibility.

The main objectives of this work were (1) to quantify the expression of different CI symptoms after two different periods of cold storage in an entirely melting nectarine segregating population from 'Venus' × 'Big Top' over a 2-years study; (2) to identify QTLs for quality traits and QTLs mainly involved in the control of the main CI symptoms in the linkage group 4 of this population, and compare these results with the previous results obtained from Pop-DG and Pop-G described above.

6.3. MATERIAL AND METHODS

6.3.1. Plant material

The population assayed was a segregating F_1 population (75 seedlings) obtained from a controlled intra-specific cross made in 2000-2001 between *P. persica* cvs. 'Venus' (female parent) and 'Big Top' (male parent), in collaboration with Agromillora Catalana S.A. 'Venus' is an FMF (freestone melting flesh) nectarine cultivar whereas 'Big Top' is a CMF (clingstone melting flesh) nectarine cultivar. The segregating population is entirely melting flesh, either cling- or freestone. This population is referred to as V×BT throughout the text.

Progeny were established during 2001 in a plot at the Experimental Station of Aula Dei (Zaragoza, Spain). Since 2004 different agronomic and quality traits have been evaluated in this population.

Preliminary SSR marker analysis (see below) identified five selfs of 'Venus'. These selfs contained only alleles present in the 'Venus' parent, and were lacking any 'Big Top' alleles of codominant SSR marker that were inherited by other progeny. Population size was therefore reduced to 70 seedlings. The five selfs remained in the field but were excluded from further molecular genetic analysis.

6.3.2. Agronomic and quality traits evaluation

Quantitative and qualitative traits were recorded over three years (2005, 2006, and 2007). Blooming date according to Fleckinger (1945), harvesting date and annual yield were evaluated in each independent progeny. When the fruit was ripe, yield (kg/tree) was measured and a representative fruit sample (approximately 20 fruit) was taken for the fruit quality evaluations. Some pomological traits such as fruit weight, height, suture diameter (SD), cheek diameter (CD), skin blush, stone type (free or cling), endocarp staining, or flesh firmness were also scored. Flesh firmness measurements were performed by a hand penetrometer with an 8-mm flat probe in two opposite sides of the fruit that had previously been peeled to remove the epidermis. Data were expressed in Newtons. Firmness of fruit was also measured as hardness, with an arbitrary scale from 1 (extremely soft) to 10 (extremely hard). The soluble solid content (SSC) of the juice was measured with a temperature compensated refractometer (model ATC-1, Atago Co., Tokyo, Japan) and the initial pH and TA (titratable acidity) was measured by titration with NaOH 0.1 N to pH 8.1.

6.3.3.Chilling injury symptoms evaluation

Chilling injury (CI) susceptibility was evaluated after cold storage at 5 ºC and 95% RH according to Crisosto et al. (1999). After 2 and 4 weeks of cold storage, a group of 10 fruits from each seedling was ripened at room temperature until firmness reached between 10-18 N. Fruits were then evaluated for different symptoms of CI such as lack of juiciness (flesh mealiness), flesh browning, and flesh bleeding. Observations were made on the mesocarp and the area around the pit immediately after the fruit were cut into two halves through the suture plane. Fruit that had a dry appearance and little or no juice after hand squeezing were considered mealy. Fruit were also informally tasted for a feeling of graininess and/or 'off flavors' to corroborate visual mealiness assessment. Mealiness, graininess and off flavor was scored as the proportion of fruit affected with these symptoms in the sample. Internal browning was visually scored on a scale of 1 (no browning) to 6 (severe browning). Bleeding was visually scored on a scale of 1 (no bleeding) to 3 (more than 50% of the flesh with bleeding). Then the percentage of progenies with each proportion/score was calculated for every CI symptom. Eventually, the degree of CI (CI index) was visually assessed according to the global fruit appearance of each genotype, from healthy fruit with no symptoms (1) to severe CI symptoms (6) when the fruit was extremely injured with mealiness/graininess, browning, and bleeding symptoms.

6.3.4. DNA extraction and molecular analysis

DNA was extracted from leaves of 'Venus', 'Big Top' and each tree of the progeny by using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA) following manufacturer instructions. A total of 38 SSR (simple sequence repeat) and candidate gene (CG) markers were employed to provide marker profiles (Table 6.1). The SSR locus C-0212 (Ogundiwin et al., 2008a) was utilized to identify five selfs in the population.

Table 6.1. SSR and CG markers used in this study. Marker type, reference and specie in what they were developed are also reported.

Marker	Marker type	Reference	Species
ACO SSR*	CG/SSR	Ogundiwin et al., in press	peach
AQP	CG	Ogundiwin et al., in press	peach
ARA	CG	Ogundiwin et al., in press	peach
BPPCT009*	SSR	Dirlewanger et al., 2002	peach
BPPCT010	SSR	Dirlewanger et al., 2002	peach
BPPCT015*	SSR	Dirlewanger et al., 2002	peach
BPPCT017	SSR	Dirlewanger et al., 2002	peach
BPPCT023*	SSR	Dirlewanger et al., 2002	peach
BPPCT025	SSR	Dirlewanger et al., 2002	peach
BPPCT026	SSR	Dirlewanger et al., 2002	peach
BPPCT035*	SSR	Dirlewanger et al., 2002	peach
BPPCT036*	SSR	Dirlewanger et al., 2002	peach
BPPCT040	SSR	Dirlewanger et al., 2002	peach
C0212*	SSR	Ogundiwin et al., in press	peach
C0593*	SSR	Ogundiwin et al., in press	peach
C1077	SSR	Ogundiwin et al., in press	peach
C-PPN18B09*	SSR	Ogundiwin et al., in press	peach
C-PPN70A04*	SSR	Ogundiwin et al., in press	peach
C-PPN52H08	SSR	Ogundiwin et al., in press	peach
CPPCT003*	SSR	Aranzana et al., 2002	peach
CPPCT004	SSR	Aranzana et al., 2002	peach
CPPCT005*	SSR	Aranzana et al., 2002	peach
CPPCT024*	SSR	Aranzana et al., 2002	peach
CPPCT028*	SSR	Aranzana et al., 2002	peach
CPSCT005*	SSR	Mnjja et al., 2004	plum
EndoPG	SSR	Peace et al., 2005	peach
EPPCU0888*	SSR	GDR	almond & peach
EPPCU8503*	SSR	GDR	almond & peach
GPPDE	CG	Ogundiwin et al., in press	peach
pchgms055*	SSR	Sosinski et al., 2000	peach
pchgms2	SSR	Sosinski et al., 2000	peach
pchgms5	SSR	Sosinski et al., 2000	peach
UDA003*	SSR	Testolin et al., 2004	almond
UDA027*	SSR	Testolin et al., 2004	almond
UDP96-003*	SSR	Testolin et al., 2004	almond
UDP97-402	SSR	Testolin et al., 2004	almond
UDP98-024*	SSR	Testolin et al., 2000	peach
UDP98-412	SSR	Testolin et al., 2000	peach

*Polymorphic markers in the 'Venus' × 'Big Top' population.
GDR: Genome Database for Rosacea (http://www.bioinfo.wsu.edu/gdr/)

PCR reactions were carried out according to Etienne et al. (2002a). Twenty nanograms of genomic DNA was amplified in a 10 µl (final volume) PCR reaction containing 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin, 200 µM of each dNTP, 5 pmol each primer, and 0.25 U Taq DNA polymerase (Sigma, St. Louis, Mo.). The PCR conditions were as follows: preliminary denaturation (94 ºC, 3 min), followed by 30 cycles consisting of denaturation (94 ºC, 45 s); annealing (50 or 57 ºC, 45 s, depending on the marker to amplify), and extension (72 ºC, 2 min); and a final extension (72 ºC, 4 min). For CG different PCR conditions were used: preliminary denaturation (95 ºC, 5 min), followed by 30 cycles consisting of denaturation (95 ºC, 30 s); annealing (60 ºC, 45 s) and extension (72 ºC, 90 s); and a final extension (72 ºC, 5 min). The PCR products were then denatured by the addition of one volume of 95 % formamide/dye solution, heated at 94 ºC for 5 min, and chilled on ice. Finally, 4.5 µl of the denatured preparation was loaded on a 4% polyacrilamide sequencing gel containing 7.5 M urea in 1 × TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA). The gels were run at 80 W for 2.5 hours. Following electrophoresis, the gel was silver-stained (Promega Corporation, Madison WI) following the protocol described by Peace et al. (2005a). Fragment sizes were estimated with the 100-bp ladder-DNA sizing markers (Promega Corporation, Madison WI). For initial polymorphism testing of each primer, assays were performed on 'Venus', 'Big Top', and six progenies. Subsequent analyses were performed on all progeny, including selfs, for the primers that were polymorphic.

6.3.5. Mapping and QTL analysis

Genome mapping of linkage group 4 (G4) from the segregating population developed from the cross 'Venus' × 'Big Top' was constructed with JoinMap 4.0 software (Van Ooijen, 2006), using segregation data for morphological, SSR, and CG markers. Linkage analyses involved all linked markers, setting the data type as cross-pollination (CP). QTL analysis was performed with MapQTL 5.0 software (Van Ooijen, 2005). The likelihood value of the presence of a QTL was expressed as a LOD score. Maximum Likelihood-based interval mapping of MapQTL 5.0 software was used for QTL analysis. Permutation (1,000 linkage group-based) was used to determine LOD threshold for quality and other traits while LOD 3 was used as arbitrary threshold for CI symptoms.

6.3.6. Statistical analysis

Data were treated for multiple comparisons by analysis of variance (ANOVA), followed by Tukey's Test with significance level $P \leq 0.05$. ANOVA was performed using the statistical software SPSS 15.0 (SPSS Inc., Chicago, USA).

6.4. RESULTS AND DISCUSSION

6.4.1. Fruit quality traits and CI susceptibility

V×BT population showed variability for the vegetative and fruit quality traits recorded (Table 6.2). All these traits exhibited continuous variation, which is typical of quantitative or polygenic inheritance. Stone adhesion ranged from 1.7 (freestone) to 10.0 (clingstone) showing the segregation of this trait in the population (FMF and CMF progenies). Regarding SSC, all the genotypes showed mean values over 10º Brix, which is considered the minimum value for consumer acceptance for yellow flesh nectarines (Hilaire, 2003; Kader, 1999). The variability found in SSC among the progenies can be explained by the quantitative regulation of this quality trait (Dirlewanger et al., 1999; Quilot et al., 2004c). There was a four-fold range in titratable acidity (TA) whereas pH varied from 3.1 to 4.2. A small change in pH represented a large change in TA because of different scales. Therefore, 'non-acid' and 'acid' fruits were found within the progeny, since fruits with a pH at maturity higher than 4.0 are considered as non-acid (Monet, 1979). 'Venus' is an 'acid' nectarine and 'Big Top' a 'non-acid' nectarine, which explains the segregation of these traits in the progeny. Fruit firmness measured on both cheeks was highly variable among the seedlings (from 10.5 to 50.1 N). Some of the progeny showed firmness values higher than 35 N, that has been defined as the threshold between mature and immature fruit (Crisosto et al., 2001c; Valero et al., 2007) due to the variability of fruit softening within a tree. However, for the CI susceptibility evaluation only mature fruits were selected, because fruit maturity has been reported to affect CI susceptibility (Infante et al., 2008b; Ju et al., 2000; Lill et al., 1989).

The F_1 progeny also showed variability for all evaluated chilling injury symptoms. The distribution of the different traits was calculated using the mean of 2-year data (Fig. 6.1). Continuous distributions were shown for browning, bleeding, mealiness, graininess, off flavor and leatheriness, suggesting polygenic control of these symptoms as was earlier reported in another peach progeny population (Peace et al., 2006). Variation from the Normal distribution was observed for these traits, which indicates that there may be only a few major genes controlling these traits. The progeny distribution for mealiness,

graininess, off flavor and leathery traits was skewed toward lower susceptibility to these symptoms than the parents after 4 weeks of cold storage.

Table 6.2. Basic statistics based on single plant observations in the 'Venus' × 'Big Top' population, for plant and fruit traits, as well as for annual and accumulated yield. For each trait, minimum, maximum, mean value, mean standard error (MSE), and standard deviation (SD) are presented. *Abbreviations*: SSC, soluble solids content; TA, titratable acidity.

Traits	Minimum	Maximum	Mean	MSE	SD
Yield (kg)	0.4	16.0	6.6	0.4	3.3
Cumulative yield (kg)	0.4	48.0	17.9	1.2	10.3
Fruit weight (g)	100.6	270.8	191.2	4.3	37.5
Blush (%)	53.3	100.0	82.7	1.2	10.4
Hardness (1-10)	4.8	8.7	7.9	0.1	0.7
Stone adhesion (1-10)	1.7	10.0	8.3	0.3	2.5
Color around stone (1-10)	1.3	9.5	4.3	0.3	2.8
Height (mm)	51.5	86.7	75.5	0.7	5.8
Suture diameter (mm)	59.5	82.0	73.0	0.6	4.7
Cheek diameter (mm)	57.1	90.6	76.9	0.8	6.4
SSC (%)	10.3	19.7	14.7	0.2	2.1
pH	3.1	4.2	3.5	0.0	0.2
TA (%)	0.3	1.2	0.7	0.0	0.3
Ripening index (RI)	11.2	59.6	25.7	1.3	11.1
Firmness (N)	10.5	50.1	33.0	0.9	7.7

The V×BT population showed lower susceptibility to CI symptoms than previous studied populations - Pop-DG and Pop-G (Ogundiwin et al., 2007; Peace et al., 2005a; Peace et al., 2006). However, similar results were found for bleeding when analyzed only within the FMF progeny and for mealiness when analyzed only within the CNMF progeny of those populations. These authors reported that mealiness was higher in FMF progeny whereas it was almost non-existent in CNMF progeny; in contrast bleeding incidence was higher in CNMF and very low in FMF progeny (Peace et al., 2006). On the other hand, mealiness was lower in V×BT progeny (averaging 27.4 %) than in FMF progeny of Pop-DG (45%) and Pop-G (64%). Differences with other peach populations corroborate the reported genotype influence on the CI susceptibility (Kader, 1985; Peace et al., 2006).

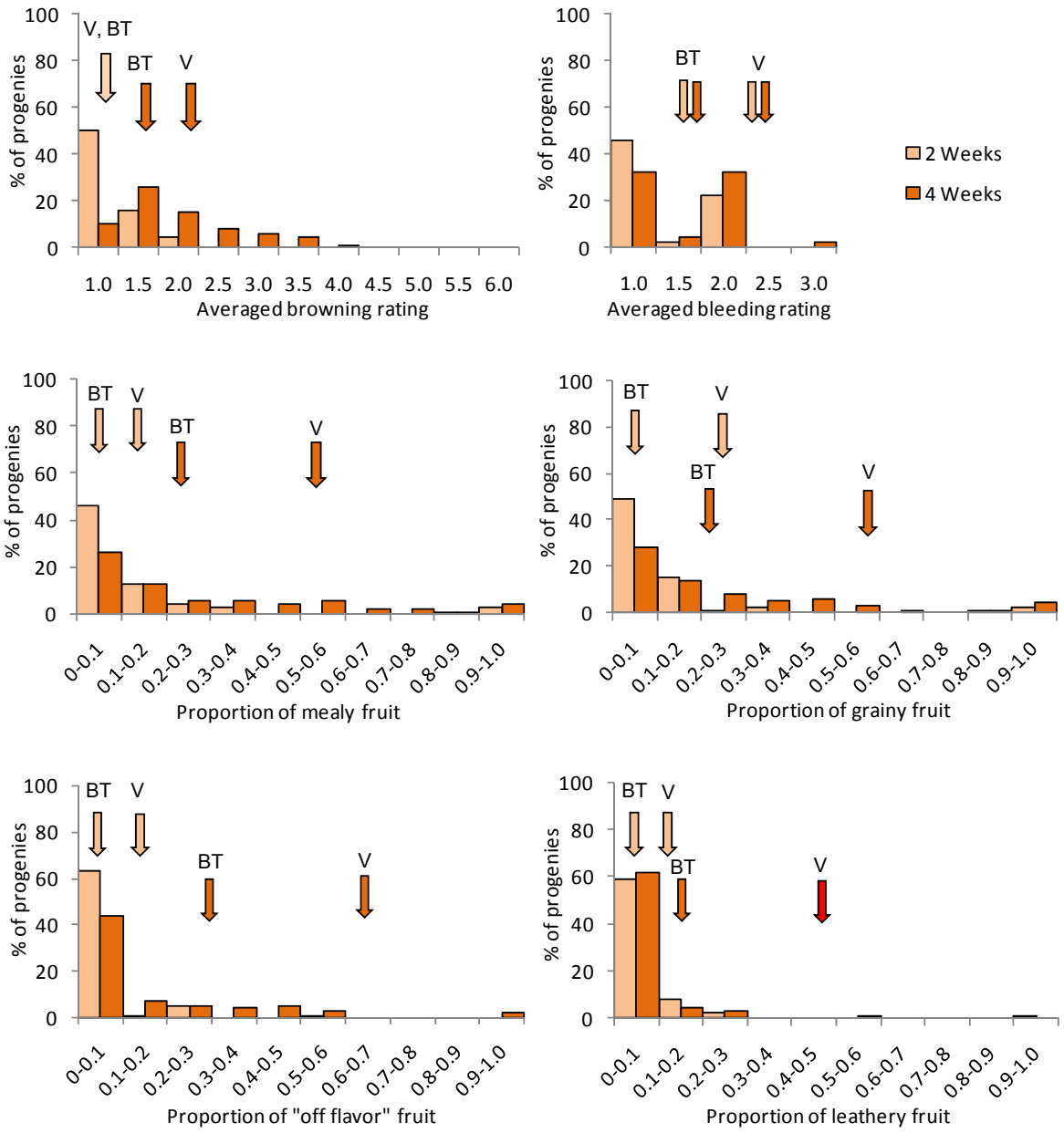

Figure 6.1. Distribution of chilling injury symptoms in the 'Venus' (V) × 'Big Top' (BT) population averaged over two years of study after storage at 5 ºC for 2 and 4 weeks and then ripened at 20 ºC during 2 or 3 days. Intensity of browning was visually scored on a (1-6) scale, and bleeding was scored on a (1-3) scale. Mealiness, graininess, off flavor, and leatheriness were scored according to the percentage of progenies with a determined proportion of injured fruit in the sample.

The duration of the time of storage (2 or 4 weeks at 5 ºC) modified the development and severity of CI symptoms. After 4 weeks of cold storage we found significantly more proportion of fruits affected by CI symptoms (except for bleeding and leatheriness) (Table 6.3), suggesting that these disorders are triggered by the cold storage duration, as has been already reported by other authors (Arana et al., 2007; Ben-Arie and Lavee, 1971; Campos-Vargas et al., 2006; Crisosto and Labavitch, 2002; Lill et al., 1989; Lurie and Crisosto, 2005). However, no significant differences were found for bleeding after 2 and 4 weeks of cold storage. In some cases, flesh bleeding has been associated with fruit senescence and not with CI disorders (Lurie and Crisosto, 2005) which could be an explanation to the low impact of storage duration on this CI symptom. No significant differences were found for leatheriness among both durations of cold storage, maybe due to the low susceptibility of this germplasm to this symptom.

Table 6.3. Comparison of chilling injury symptoms in the 'Venus' × 'Big Top' population after 2 and 4 weeks of cold storage. Averaged proportion of fruit affected by each CI symptom is shown. Data are mean of two years.

Storage time	Browning[a] (1-6)	Bleeding[b] (1-3)	Mealiness	Graininess	Off-Flavour	Leatheriness	CI index[c] (1-10)
2 Weeks	1.2 b	1.4 a	12.0 b	9.1 b	3.7 b	5.5 a	1.5 b
4 Weeks	1.9 a	1.5 a	26.9 a	24.0 a	15.6 a	3.2 a	2.6 a

In each column, values bearing the same letter are not significantly different.
[a]Browning scored on a scale of 1 (no browning) to 6 (severe browning)
[b]Bleeding scored on a scale of 1 (no bleeding) to 3 (more than 50 % of the flesh with bleeding)
[c]CI index scored on a scale of 1 (no CI symptoms) to 6 (severe symptoms)

The main factor contributing to phenotypic variation was genotype for all the CI symptoms measured (Table 6.4), corroborating the significant genetic component on the CI susceptibility (Crisosto et al., 1999; Peace et al., 2006). It is accepted that peach cultivars are more susceptible to CI than nectarine cultivars, and melting flesh cultivars are also more susceptible than the firmer non-melting flesh cultivars (Brovelli et al., 1999a; Lester et al., 1996). Mealiness and graininess showed the higher proportion of phenotypic variance attributed to genotype, reflecting the high genetic control of these symptoms. This is an important result since mealiness is the most important CI symptom affecting peach post-harvest quality. This heritability indicates that there is considerable genetic control that will allow the identification of QTLs in this population and the development of MAS for these CI symptoms. Similar heritability estimates for mealiness, browning, and bleeding have been reported in other mapping populations (Peace et al., 2005b).

Due to the reported variations between years that may occur in CI symptoms (Campos-Vargas et al., 2006; Crisosto and Labavitch, 2002; Peace et al., 2005b) it is important to evaluate the CI susceptibility for several years. In our work, year did not show any significant effect on the CI symptoms (except to CI index). CI symptoms were quite consistent over the two years at different storage durations. This result reflected the high heritability values obtained for all CI symptoms (Table 6.4).

Table 6.4. Factors affecting chilling injury symptoms, observed for two years in the 'Venus' × 'Big Top' population. Significant factors ($P \leq 0.01$) and their proportion (%) of phenotypic variance are indicated, as determined by ANOVA.

CI symptoms	Genotype[a]	Year	Storage duration
Browning	29.5	NS	26.7
Bleeding	46.2	NS	NS
Mealiness	63.5	NS	18.8
Graininess	64.9	NS	21.7
Off flavour	47.8	NS	19.8
Leatheriness	31.8	NS	NS
CI index	44.8	2.4	39.1

[a]This proportion of phenotypic variance attributed to Genotype is the broad sense heritability (H_b). NS, not significant

6.4.2. Correlations between CI symptoms

All pairs of the CI symptoms were positively and significantly correlated except for leatheriness which was only positively correlated with the general CI index and not with any other symptom (Table 6.5). Mealiness and graininess were highly correlated (r = 0.90), probably because graininess is the sensorial feeling of visual mealiness. CI index is a global estimation of CI severity in the fruit, therefore, a significant positive correlation was observed for all the symptoms evaluated. However, it is worthy to note that browning, mealiness, and graininess were highly correlated and contributed the most to the general CI index, corroborating that these symptoms are the main CI disorders affecting peach quality (Brummell et al., 2004; Crisosto et al., 1999; Lurie and Crisosto, 2005). Mealiness and graininess were negatively correlated with stone adhesion (r = -0.25 and r = -0.26, respectively), which reflects a higher susceptibility of free stone fruit to CI disorders. The genetic locus for freestone appears to contain a cluster of *endo-PG* genes controlling these traits (Callahan et al., 2004; Peace et al., 2005b), which can explain the correlation found between both traits. Off flavor was highly positively correlated with mealiness and

graininess (r = 0.63 and 0.68, respectively) which confirms that these CI symptoms negatively affect fruit taste (Lurie and Crisosto, 2005). Moreover, mealiness and graininess were negatively correlated with flowering date (r = -0.31 and -0.32, respectively). These results suggest a tendency from earlier flowering genotypes to be more susceptible to suffer CI symptoms. Different results were shown by Peace et al. (2006) who reported a positive correlation between flowering date and both mealiness and bleeding. On the other hand, bleeding was negatively correlated with harvesting date (r = -0.46). The phenotypic correlations found between traits can be due to shared or linked controlling genes.

Table 6.5. Phenotypic correlations (Spearman R-values) between chilling injury symptoms, observed for two years in the 'Venus' × 'Big Top' population.

CI symptoms	Bleeding	Mealiness	Graininess	Off flavour	Leatheriness	CI index
Browning	NS	0.31 **	0.31 **	0.27 **	NS	0.62 **
Bleeding		0.20 **	0.18 **	0.20 **	NS	0.29 **
Mealiness			0.90 **	0.63 **	NS	0.67 **
Graininess				0.68 **	NS	0.67 **
Off flavour					NS	0.57 **
Leatheriness						0.16 **

**, $P \leq 0.01$; NS, not significant.

6.4.3. Linkage mapping and QTL analysis

Linkage mapping and QTL analysis were used to determine the location, number and effect of genomic sites contributing to the phenotypic variation in the V×BT population for the CI symptoms. The selected markers (Table 6.1) were known to be linked to important regions (G4) involved in the control of the main chilling injury symptoms (Ogundiwin et al., 2008a; Ogundiwin et al., 2007; Peace et al., 2005a). From them, 24 of the 39 markers analyzed were polymorphic in the V×BT population, and 15 (19 loci in total) were anchored to the linkage group G4 (Fig. 6.2). Co-linearity with the *Prunus* consensus (T×E) map (Howad et al., 2005) was found, although distances between markers varied slightly probably due to differences in the rate of recombination in the two sets of parents. The genetic diversity of the individuals involved in the crosses may explain this phenomenon. It is also noticeable that different software was used to elaborate the two maps (JoinMap for V×BT and MAPMAKER for T×E) and differences in the genetic distances have been reported depending on which one is used (Van Ooijen et

al., 1994; Verde et al., 2005). Nevertheless, the order of the common markers within the LG4 was the same, with the exception of an inversion of two adjacent loci (UDP98-024B and CPPCT028). This result confirms the substantial co-linearity and the transferability of the markers among the *Prunus* species observed in previous works (Dirlewanger et al., 2004; Dondini, 2007; Sánchez-Pérez et al., 2007b; Sosinski et al., 2000).

QTLs for several agronomic and quality traits and for CI symptoms were detected on linkage group G4 of the V×BT map by interval mapping (Fig. 6.2) and accounted between the 23% and the 86% of the observed variation (Table 6.6). Significant QTLs for harvesting date, fruit weight, endocarp staining, height, SD, CD, SSC, and firmness were detected on the G4 (Fig. 6.3). Significant QTLs were also found on G4 for mealiness, bleeding, and graininess (Fig. 6.4). Most of the QTLs were consistent through the two years study showing that the expression of the genes could be independent of the environmental conditions as the phenotypic analysis showed (Table 6.4). QTLs for several traits were detected in the same region, what may correspond to linked QTLs or to one QTL with pleiotropic effect. A high contribution QTL (86.5%) for harvesting date was detected near the C-PPN18B09 marker (Table 6.6). Dirlewanger et al. (1999) and Etienne et al. (2002b) also identified QTLs for harvesting date at the top of linkage group 4. The marker linked with the QTL for fruit weight (BPPCT023), was also related to QTLs for fruit dimensions (height, SD and CD). These results agreed with the location of major genes or QTLs on the G4 controlling fruit dimensions described previously using different *Prunus* maps and different molecular markers (Quilot et al., 2004c; Sánchez-Pérez et al., 2007b). With respect to SSC, a QTL explaining 27.5% of the variation observed was found near the *F-M* locus (Fig. 6.4). QTLs for SSC and other sugar components have been previously mapped on G4 by other authors (Dirlewanger et al., 1999; Etienne et al., 2002b; Quilot et al., 2004c).

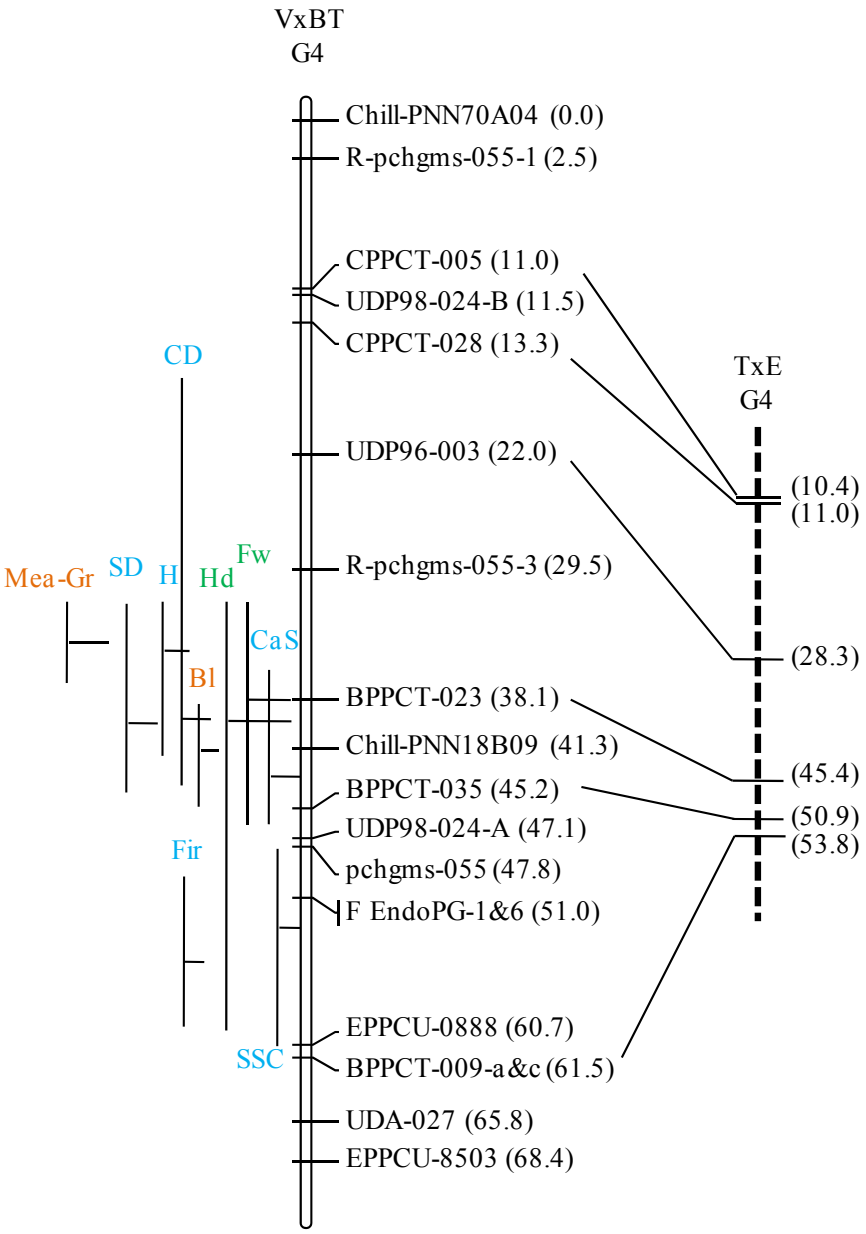

Figure 6.2. Linkage group 4 (G4) of 'Venus' × 'Big Top' (V×BT) F_1 progeny showing in the right the position of DNA markers and on the left QTL peaks for quality and chilling injury traits including harvesting date (*Hd*), fruit weight (*Fw*), endocarp staining (*CaS*), height of the fruit (*H*), suture diameter (*SD*), cheek diameter (*CD*), soluble solids content (*SSC*), firmness (*Fir*), bleeding (*Bl*), mealiness (*Mea*), and graininess (*Gr*). Names and map distances (cM) of the markers are listed on the right. The most likely position for a QTL is indicated by a horizontal line proportional to the percentage of variance explained. The both sides-LOD support confidence interval is indicated by a vertical line. A section of G4 of T×E *Prunus* reference map (Dirlewanger et al., 2004; Howad et al., 2005) is represented showing the position of common SSR markers connected with solid lines to G4 of V×BT.

QTLs for bleeding (Bl), mealiness, and graininess (Mea-Gr) were found on this linkage group. It should be noted that the marker linked with the QTL for bleeding (red pigmentation), was also related with a QTL controlling endocarp staining (Table 6.6). QTLs for mealiness and graininess co-localized probably because graininess is the sensorial manifestation of visual mealiness. Variation explained by these QTLs was 23.8% for bleeding and 53.3% and 53.9% for mealiness and graininess, respectively (Table 6.6). QTLs for browning symptom were not found on linkage group G4, in agreement with other authors (Ogundiwin et al., 2007; Peace et al., 2006). These authors reported QTLs for browning on G5 (one major QTL) and G2 (two minor QTLs). Some of the QTLs controlling quality traits co-localized with CI symptoms QTLs, being a possible explanation for the phenotypic correlations found between them.

Table 6.6. Nearest marker, peak position, maximum LOD score and percentage variance explained for QTLs identified on the linkage group G4 by interval mapping in the F1 progeny population of 'Venus' × 'Big Top'.

Trait	Nearest marker	LOD peak position (cM)	Max. LOD score	% Variance explained
Harvesting date	C-PPN18B09	39.1	23.4	86.5
Fruit weight	BPPCT023	38.1	11.5	59.0
Endocarp staining	C-PPN18B09	42.3	6.7	39.9
Height	BPPCT023	35.5	4.7	34.1
SD	BPPCT023	39.1	6.1	39.7
CD	BPPCT023	39.1	6.2	40.3
SSC	F/EndoPG	53.0	13.5	27.5
Firmness	C-PPN18B09	41.1	4.4	26.4
Bleeding	C-PPN18B09	41.3	3.9	23.8
Mealiness	pchgms0553	33.5	3.2	53.3
Graininess	pchgms0553	33.5	3.3	53.9

Abbreviations: SD= suture diameter; CD= cheek diameter; SSC= soluble solids content

6.5. CONCLUSIONS

The results found in this work confirm the high influence of genetic background on the susceptibility of peaches and nectarines to chilling injury. Moreover, these results supported and validated other QTLs controlling CI susceptibility found in another unrelated peach progeny populations and contributed to a better understanding of the genetic control of this important disorder affecting peach and nectarine fruit.

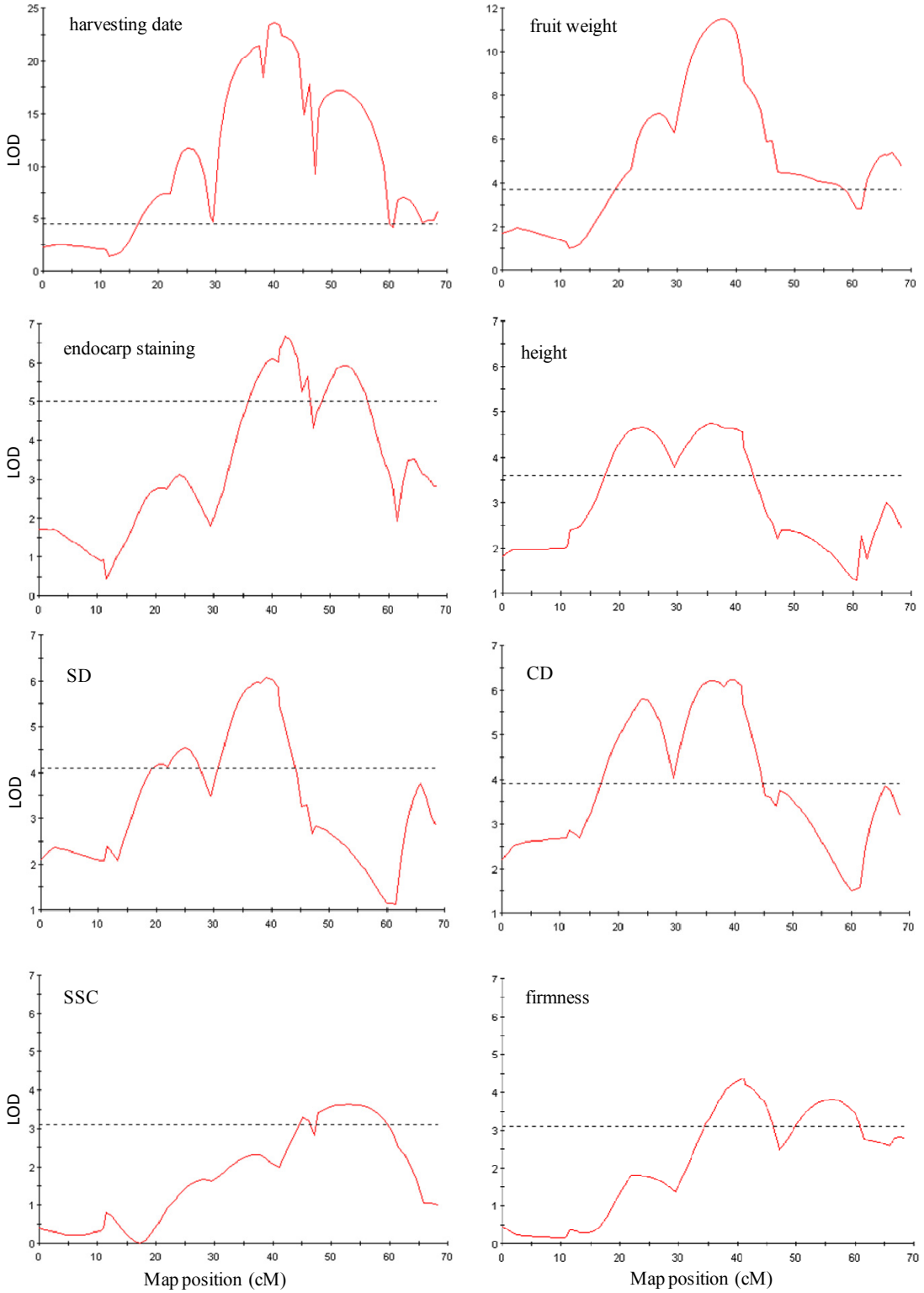

Figure 6.3. LOD plots for several agronomic and fruit quality traits resulting from interval mapping of the 'Venus' × 'Big Top' data averaged for two years on linkage group G4. The dashed line indicates the significance LOD thresholds obtained with 1,000 linkage group-based permutations at $P \leq 0.01$. *Abbreviations:* SD= suture diameter; CD= cheek diameter; SSC= soluble solids content.

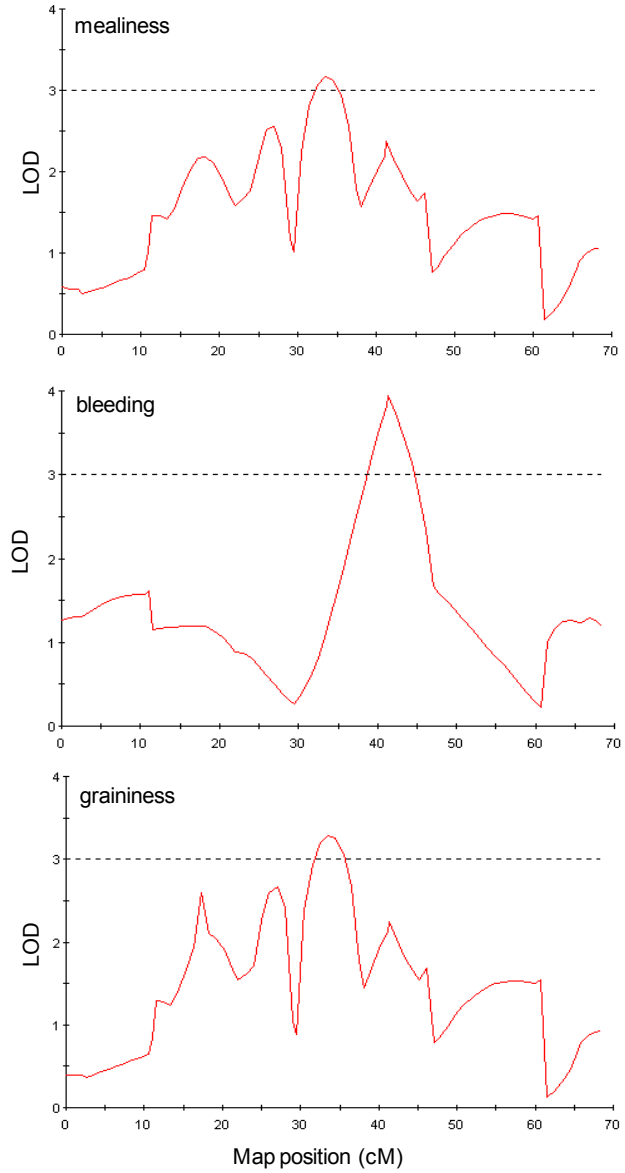

Figure 6.4. LOD plots for three CI symptoms resulting from interval mapping of the 'Venus' × 'Big Top' data averaged for two years on linkage group G4.

Capítulo 7

Discusión general

La variabilidad fenotípica encontrada en todos los parámetros de la población de mejora evaluada en la Estación Experimental de Aula Dei del CSIC (Zaragoza), en las condiciones de cultivo del Valle Medio del Ebro, reveló el alto potencial genético de la misma para la selección de genotipos de melocotonero con un buen comportamiento agronómico y buena calidad de fruto.

Los resultados indicaron que tanto el genotipo como la naturaleza del cruzamiento tuvieron una gran influencia sobre la varianza fenotípica de los caracteres agronómicos y de la calidad organoléptica, nutricional y poscosecha del fruto del melocotonero, como se ha observado en otros estudios (Brooks et al., 1993; Byrne et al., 1991; Crisosto et al., 1999; Chang et al., 2000; Esti et al., 1997; Iglesias y Echeverría, 2009; Peace et al., 2006; Quilot et al., 2004a; Remorini et al., 2008; Tavarini et al., 2008; Tomás-Barberán et al., 2001).

El análisis de componentes principales (PCA), previamente aplicado para el estudio de otras poblaciones de melocotonero (Brovelli et al., 1999b; Crisosto et al., 2006a; Lavilla et al., 2002) y albaricoquero (Badenes et al., 1998b; Gurrieri et al., 2001; Ruiz y Egea, 2008), permitió la simplificación de las variables estudiadas en el presente trabajo, agrupando los distintos cruzamientos de acuerdo a sus características agronómicas y de calidad de fruto.

Dentro de los programas de mejora del área Mediterránea, uno de los objetivos es la obtención de genotipos con fechas de floración tempranas (Carusso y Sottile, 1999; George y Nissen, 1992), junto con la extensión del periodo de cosecha, con el fin de abastecer el mercado durante un mayor periodo de tiempo (Badenes et al., 2006; Byrne, 2003; Carusso y Sottile, 1999; Martínez-Calvo et al., 2006). El estudio de las fechas de floración en nuestra población, mostró una mayor variabilidad en las fechas de plena y final de floración, que en las fechas de inicio de la misma, debido a las diferencias entre progenies en la duración de este periodo. En cuanto a las fechas de cosecha, se observaron grandes diferencias entre las progenies, dado que tanto las fechas de floración como las de cosecha dependen principalmente del cultivar (Crisosto et al., 2001a; Esti et al., 1997; Iglesias y Echeverría, 2009; Mounzer et al., 2008). La variabilidad encontrada tanto para las fechas de floración como para las de cosecha, entre los genotipos de una misma progenie, concuerda con la herencia cuantitativa descrita para estos caracteres agronómicos (Carusso y Sottile, 1999; Dirlewanger et al., 1999; Vargas y Romero, 2001). Esta variabilidad ha permitido la preselección de genotipos con las fechas de maduración más apropiadas para cubrir las demandas del mercado respecto al consumo de melocotones y nectarinas.

La variabilidad fenotípica observada en el perfil de azúcares de los genotipos evaluados, ofrece la posibilidad de la selección de melocotones y nectarinas con mayor contenido de azúcar. Los contenidos de azúcares individuales (sacarosa, glucosa, fructosa y sorbitol) en la población de estudio, concuerdan con los referidos por otros autores en frutos maduros de *P. persica* (Brooks et al., 1993; Colaric et al., 2005; Lo Bianco y Rieger, 2002b; Quilot et al., 2004a; Wu et al., 2005b). El efecto significativo del cruzamiento en todos los parámetros relacionados con el perfil de azúcares, y su alta contribución a la varianza fenotípica de todos ellos, coinciden con resultados previos que muestran además el efecto del cultivar en el perfil de azúcares del fruto del melocotonero (Albás et al., 2004; Brooks et al., 1993; Usenik et al., 2008). Las diferencias en la composición de azúcares entre los genotipos preseleccionados y la población de estudio de la que proceden, demostró la influencia del perfil de azúcares en la calidad global del fruto y la importancia de considerar este parámetro en los programas de mejora. Estos resultados coinciden con trabajos anteriores, en los que la composición de azúcares se ha relacionado directamente con el aroma y el sabor del fruto (Colaric et al., 2005; Crisosto y Crisosto, 2005; Harker et al., 2002b; Karakurt et al., 2000).

Las sustancias con efectos beneficiosos adicionales en la salud humana como polifenoles, vitaminas y otros antioxidantes, tienen una relevancia cada vez más importante en la definición de calidad en la fruta (Cevallos-Casals et al., 2006; Dalla Valle et al., 2007; Di Vaio et al., 2008; Kaur y Kapoor, 2001). Los contenidos de los compuestos antioxidantes (compuestos fenólicos totales, flavonoides, antocianinas y vitamina C) obtenidos en este estudio se encontraron dentro de los rangos descritos para melocotonero (Asami et al., 2003; Gil et al., 2002; Proteggente et al., 2002; Tavarini et al., 2008; Tomás-Barberán et al., 2001), siendo menores que los obtenidos cuando se incluye la piel del fruto en el análisis (Chang et al., 2000; Gil et al., 2002; Remorini et al., 2008; Tomás-Barberán et al., 2001; Vizzotto et al., 2007), debido a la distribución desigual de estos compuestos en la piel y la pulpa de los frutos (Asami et al., 2003; Cevallos-Casals et al., 2006). No obstante, los valores obtenidos en pulpa muestran la gran variabilidad en el contenido de compuestos funcionales y de capacidad antioxidante entre los genotipos de las poblaciones estudiadas (Gil et al., 2002; Tavarini et al., 2008; Tomás-Barberán et al., 2001; Vizzotto et al., 2007), y sugieren la importancia de considerar estos aspectos como factores claves para la selección de nuevos cultivares con propiedades beneficiosas adicionales para el consumidor (Drogoudi et al., 2008; Prior et al., 1998; Ruiz et al., 2005; Tulipani et al., 2008). Por otro lado, la alta heredabilidad encontrada para todos los componentes bioactivos, demuestra la posibilidad de la mejora de estos caracteres en los programas de selección de melocotonero (Oraguzie et al., 2001; Quilot et al., 2005; Rumpunen y Dviklys, 2003). Además, el conocimiento de los parámetros

genéticos que controlan los caracteres en estudio y su herencia, será una herramienta fundamental para el desarrollo de estrategias de mejora de la calidad más eficientes.

Durante la evaluación agronómica llevada a cabo en este trabajo, se encontraron frutos abortivos en algunos de los genotipos var. *platycarpa* de una de las progenies ('VAC-9520' x 'VAC-9517'). El carácter *frutos abortivos* (Af) fue descrito por primera vez por Dirlewanger et al. (2006). Estos autores propusieron que el control genético de este carácter se debía a la participación de distintos alelos del gen que controla la forma achatada del fruto (S), siendo necesaria la presencia del alelo recesivo para el correcto desarrollo del fruto. El análisis de la segregación de este carácter en la progenie de estudio, coincide con la hipótesis del control genético de ambos caracteres bajo el mismo locus. Sin embargo, nuestros resultados contrastan con esta hipótesis ya que, según ésta, no deberían encontrarse frutos abortivos en nuestra población, al no existir genotipos homozigotos dominantes para este carácter. La total comprensión del mecanismo de caída prematura de los frutos y su control genético tiene una gran relevancia, dado que permitiría el desarrollo de marcadores para la identificación temprana de los genotipos susceptibles de producir frutos abortivos.

Por otro lado, también se observó la relación de distintos caracteres pomológicos cualitativos como el tipo y forma del fruto, el color de la pulpa y su adherencia al hueso, con algunos de los parámetros agronómicos y de la calidad organoléptica y nutricional del fruto, sugiriendo la importancia del estudio de dichas relaciones en los programas de mejora. Los genotipos var. *platycarpa* mostraron una producción y tamaño de fruto significativamente menores que los genotipos de fruto redondo. Estos resultados concuerdan con los de otros autores (Dirlewanger et al., 2006; Iglesias y Echeverría, 2009). Este hecho podría deberse a la localización de QTLs para el peso del fruto y la producción, junto al gen que controla la forma achatada del fruto en el grupo de ligamiento 6 (LG6) (Dirlewanger et al., 1999; Lesley, 1940). Por otro lado, los genotipos con fruto de nectarina resultaron ser más firmes que los de melocotón, mientras que los de carne blanca mostraron menor firmeza que los de pulpa amarilla, de acuerdo con otros trabajos (Crisosto et al., 2001a; Valero et al., 2007). Estas diferencias deben considerarse, puesto que la firmeza determina la susceptibilidad de la fruta a los daños mecánicos durante el manejo poscosecha (Crisosto et al., 2001c; Kunze et al., 1975).

Los caracteres cualitativos del fruto resultaron también estar relacionados con la composición de azúcares y de compuestos bioactivos. Estos resultados permitirán establecer distintos perfiles nutricionales para cada una de las tipologías de fruto observadas. Podrían aplicarse a necesidades nutricionales recomendadas (control de peso, dieta para diabéticos, etc.) o a preferencias organolépticas específicas, dependiendo de grupos etnogeográficos o gustos personales. Los genotipos con fruto

tipo nectarina mostraron mayor contenido de sólidos solubles (SSC), glucosa y azúcares totales que los de melocotón, de acuerdo con lo descrito en otros trabajos (Crisosto et al., 2006a; Crisosto et al., 2001a; Day et al., 1997; Wu et al., 2005a). Estas diferencias podrían deberse a la co-localización de un QTL para el contenido en SSC con el gen que controla el carácter fruta glabra o pubescente en el grupo de ligamiento 5 (LG5) (Layne y Bassi, 2008; Quilot et al., 2005; Quilot et al., 2004c). A su vez, los genotipos con fruto achatado o paraguayos mostraron mayor contenido en SSC y en azúcares individuales que los frutos redondos, de acuerdo con distintos trabajos en los que los cultivares var. *platycarpa* se asocian con un sabor más dulce y mayor contenido de SSC (Ma et al., 2003; Nicotra et al., 2002). La localización de QTLs para el contenido de SSC (Dirlewanger et al., 1999) y el de azúcares totales (Quilot et al., 2005) junto al gen para la forma achatada del fruto (Lesley, 1940), podría ser una de las causas que explicase estas diferencias (Layne y Bassi, 2008).

Por otro lado, los genotipos con fruto de carne blanca también mostraron mayor contenido de SSC y de azúcares individuales que los de pulpa amarilla, de acuerdo con lo descrito por Robertson et al. (1990) y Wu et al. (2005b). Además, se encontraron diferencias en el perfil de azúcares entre los genotipos con fruto de hueso libre y los de hueso adherido a la pulpa. Estas diferencias podrían deberse a la localización de QTLs para el contenido de SSC, glucosa y sacarosa en el grupo de ligamiento 4 (LG4) (Quilot et al., 2004c), cerca del gen que controla la adherencia del hueso y la pulpa (Dirlewanger et al., 2006; Layne y Bassi, 2008; Yamamoto et al., 2001). También podría explicarse por una mayor presión de selección hacia mayores contenidos de azúcares, ejercida en los cultivares de hueso libre utilizados como parentales en nuestra población de mejora. En un estudio previo, realizado en una población híbrida obtenida del cruzamiento *P. persica* x *P. davidiana*, no se observaron diferencias en el perfil de azúcares entre frutos de hueso libre y adherente (Wu et al., 2005a). Sin embargo, hasta el momento son muy escasos los trabajos que estudian la relación entre dicho carácter y la calidad organoléptica y nutricional del fruto. En cuanto a los compuestos bioactivos, los genotipos con fruto de pulpa blanca mostraron mayor contenido de compuestos fenólicos, vitamina C y capacidad antioxidante que los de pulpa amarilla, coincidiendo con lo descrito por otros autores (Gil et al., 2002; Tomás-Barberán et al., 2001). Por el contrario, no se observaron diferencias significativas en el contenido de compuestos bioactivos y la capacidad antioxidante entre los genotipos con fruto tipo melocotón y nectarina.

Algunos de los parámetros que influyen en la calidad del fruto, mostraron una variabilidad significativa entre los distintos años de estudio, lo que subraya la importancia del estudio de las poblaciones a lo largo de diferentes años en un programa de mejora. Así, el efecto del año, debido fundamentalmente a diferencias de temperatura (Mounzer

et al., 2008; Ruiz y Egea, 2008; Sánchez-Pérez et al., 2007a), pudo observarse en las fechas de floración y de cosecha. Cabe mencionar que tanto la floración como la cosecha, se produjeron cada año más temprano en el período de los tres años estudiados. A pesar de estas variaciones, el período de desarrollo del fruto permaneció bastante estable a lo largo de los años, confirmando la fuerte dependencia genética de este parámetro (Bassi et al., 1988; Cunha et al., 2007; Cheng, 2008; Mounzer et al., 2008; Muñoz et al., 1986). Estos resultados indican que, conociendo la duración del periodo de desarrollo del fruto, la fecha de plena floración podría utilizarse como una estimación de la fecha de cosecha (Layne y Bassi, 2008). La composición de azúcares, también mostró una variabilidad significativa a lo largo de los años, coincidiendo con lo observado por otros autores (Brooks et al., 1993; Dirlewanger et al., 1999), aunque su perfil se mantuvo estable para cada genotipo (Bassi et al., 1996; Ledbetter et al., 2006; Quilot et al., 2004a; Wu et al., 2003). La variabilidad observada se atribuye principalmente a las oscilaciones en las condiciones climáticas y a la carga de cosecha en los distintos años (Brooks et al., 1993; Sweeney et al., 1970). Sin embargo, los niveles de compuestos bioactivos, mostraron una menor dependencia del efecto del año, aunque también pudieron observarse variaciones anuales en los contenidos de flavonoides y antocianinas, probablemente debido a la diferencia de las condiciones climáticas, especialmente de luz y temperatura, a lo largo de los tres años de evaluación (Kataota et al., 1984; Tomás-Barberán y Espín, 2001). Se ha visto que la intensidad de la radiación solar aumenta el contenido de antocianinas en frutos como la granada, la pera o la manzana (Dussi et al., 1995; Gil et al., 1995; Tomás-Barberán y Espín, 2001), mientras que la mayor oscilación térmica entre el día y la noche ha demostrado un efecto significativo en la acumulación de antocianinas en uva, manzana y ciruela (Tomás-Barberán y Espín, 2001). Por otra parte, la susceptibilidad de los genotipos evaluados a los daños por frío, no varió significativamente entre distintos años de evaluación, lo que contribuyó a la obtención de altas heredabilidades para dichos síntomas, tal y como han descrito otros autores (Peace et al., 2005a; Peace et al., 2006).

Por otro lado, se encontraron correlaciones significativas entre algunos de los caracteres agronómicos y de la calidad organoléptica y nutricional del fruto. La variabilidad de los coeficientes de correlación según la progenie estudiada, demuestra que estas relaciones dependen, en gran medida, de los parentales utilizados. De acuerdo con trabajos anteriores (Byrne, 2002; Dirlewanger et al., 1999; Engel et al., 1988; López y Dejong, 2007; Ruiz y Egea, 2008), la fecha de cosecha mostró una correlación significativa con el tamaño y composición de azúcares del fruto. Esta correlación debe considerarse especialmente en la selección de genotipos tempranos, ya que éstos tienen generalmente frutos más pequeños y con menor contenido de azúcares (Iglesias y

Echeverría, 2009). Por otro lado, las relaciones entre la producción del árbol, el tamaño y la composición nutricional (azúcares y compuestos bioactivos) del fruto, debido a la competencia por los asimilados procedentes de la fotosíntesis (Morandi, 2008), y al efecto de éstos en el tamaño y en la calidad del fruto (Lo Bianco y Rieger, 2006; Mounzer et al., 2008), indican la importancia de buscar un equilibrio óptimo entre dichos parámetros. A su vez, estas relaciones, subrayan la importancia de la comprensión del modelo fuente-sumidero (DeJong, 1999; Farrar, 1993; Lo Bianco y Rieger, 2002a, 2006; Morandi, 2008; Mounzer et al., 2008) en el análisis de los parámetros de calidad que afectan al fruto del melocotonero.

Cabe destacar la relación, ya descrita por otros autores (Dirlewanger et al., 1999; Gurrieri et al., 2001; Wu et al., 2003), entre el contenido en sólidos solubles (SSC) y la acidez valorable, lo que sugiere que ambos parámetros están bajo el mismo control genético. Todos los parámetros relacionados con el perfil de azúcares del fruto se correlacionaron significativamente entre sí y con otros componentes de la calidad del fruto, en mayor o menor medida, debido probablemente a la localización de QTLs para la mayor parte de los compuestos bioquímicos de la composición del fruto en los grupos de ligamiento 5 (LG5) y 6 (LG6) (Arús et al., 2003; Dirlewanger et al., 1999; Etienne et al., 2002b; Quilot et al., 2004c). La mayor correlación entre los azúcares del fruto se observó entre los contenidos de glucosa y fructosa, como habían observado otros autores (Cheng, 2008; Dirlewanger et al., 1999; Esti et al., 1997; Wu et al., 2003; Wu et al., 2005b), lo que se atribuye a la similitud en el patrón de fluctuaciones y concentraciones de ambos monosacáridos a lo largo de todo el desarrollo del fruto (Chapman y Horvat, 1990; Ishida et al., 1971). Sin embargo, aunque la mayoría de los genotipos mostraron un mayor contenido en fructosa que en glucosa, en otros genotipos se encontró lo contrario, tal y como se ha observado en otros trabajos (Moriguchi et al., 1990a; Wu et al., 2003), por lo que el coeficiente de correlación entre dichos azúcares en la población en estudio es más bajo. Entre los azúcares individuales, la sacarosa mostró la correlación más alta con el contenido de sólidos solubles, dado que es el azúcar mayoritario en el fruto maduro de *P. persica* (Gurrieri et al., 2001). La baja correlación observada entre el contenido en SSC y el de azúcares totales, tal y como han descrito otros autores para cítricos (Echeverría y Ismail, 1990) y otros cultivares de melocotonero (Byrne et al., 1991), se atribuye a la contribución de otras sustancias ópticamente activas, diferentes a los carbohidratos, al contenido de sólidos solubles y su medida en ºBrix (Jacob, 1944; Wu et al., 2003).

En cuanto a las relaciones entre compuestos bioactivos, sólo los flavonoides y los compuestos fenólicos totales, mostraron una correlación significativa con la capacidad antioxidante del fruto, indicando que éstos son los principales compuestos antioxidantes de melocotones y nectarinas (Cantín et al., 2009), tal y como ha sido observado en

ciruela, uva y otros cultivares de melocotonero (Cevallos-Casals et al., 2006; Chang et al., 2000; Drogoudi et al., 2008; Gil et al., 2002). La relación lineal encontrada entre el contenido total de compuestos fenólicos y la capacidad antioxidante en la pulpa de los frutos (Cantín et al., 2009), ha sido previamente descrita para ciruelas, albaricoques y otros cultivares de melocotonero (Prior et al., 1998; Rupasinghe y Clegg, 2007; Scalzo et al., 2005). Sin embargo, no se encontró una correlación significativa entre el contenido en vitamina C y la capacidad antioxidante, debido probablemente al bajo contenido en esta vitamina que presentan melocotones y nectarinas, a diferencia de lo que ocurre en cítricos y bayas, en los que dicha vitamina es el principal compuesto antioxidante (Gardner et al., 2000; Pedersen et al., 2000). Por otro lado, la coloración roja de la pulpa, que puede observarse en algunos genotipos de melocotonero, mostró una correlación significativa con la capacidad antioxidante del fruto, lo que apoya el efecto beneficioso que tiene el consumo de frutos con una alta tinción roja (Tomás-Barberán et al., 2001). También se observó una correlación positiva entre el porcentaje de chapa de la piel y la tinción de la pulpa, probablemente debido a que ambos parámetros son derivados del nivel de antocianinas de la fruta (Tomás-Barberán et al., 2001). Por el contrario, no se encontró una correlación significativa entre el color de la piel del fruto y su firmeza, de acuerdo con trabajos previos realizados en melocotón (Génard et al., 1994) y albaricoque (Ruiz y Egea, 2008).

El análisis de la calidad poscosecha en una de las poblaciones de estudio ('Venus' x 'Big Top'), demostró que la susceptibilidad a los daños por frío depende en gran medida del genotipo (Crisosto et al., 1999; Peace et al., 2005a; Peace et al., 2006), obteniéndose una alta heredabilidad para todos los síntomas observados. Todos los síntomas evaluados de los daños por frío (pardeamiento, harinosidad visual y sensorial, enrojecimiento de la pulpa, maduración anormal y problemas de sabor) mostraron una variación continua en la población de estudio, reflejando un control genético cuantitativo o poligénico de este desorden fisiológico. No obstante, el pardeamiento y la harinosidad visual y sensorial fueron los síntomas principales que afectaron a la calidad poscosecha en nuestra población, al igual que se refiere en otros trabajos (Brummell et al., 2004; Crisosto et al., 1999; Lurie y Crisosto, 2005). Es importante destacar que los síntomas de daños por frío se agravaron al aumentar la duración del período de almacenamiento a bajas temperaturas. La importancia de la duración del periodo de almacenamiento en frío en el desarrollo y la severidad de este desorden poscosecha ha sido previamente descrita por otros autores (Arana et al., 2007; Ben-Arie y Lavee, 1971; Crisosto y Labavitch, 2002; Lill et al., 1989; Lurie y Crisosto, 2005). Los genotipos con fruto de hueso libre mostraron una mayor susceptibilidad a los daños por frío que los de hueso adherente, lo que puede ser debido al control genético de este síntoma por el mismo gen

que controla la adherencia al hueso (Callahan et al., 2004; Peace et al., 2005b). En los genotipos evaluados, la harinosidad visual y sensorial se correlacionó negativamente con la fecha de plena floración, sugiriendo una mayor susceptibilidad a estos desórdenes en los genotipos de floración temprana. Este resultado difiere del observado por Peace et al. (2006), que vieron una mayor susceptibilidad en los genotipos de floración más tardía. Estas diferencias son probablemente debidas a las diferentes poblaciones utilizadas en ambos estudios.

Por otra parte, la construcción de un mapa parcial del grupo de ligamiento 4 (LG4) con marcadores tipo SSRs y CGs, permitió la localización de QTLs para los síntomas de harinosidad visual y sensorial, y enrojecimiento de la pulpa (Cantín et al., 2008b). Sin embargo, no se encontró ningún QTL para el pardeamiento del fruto en este grupo de ligamiento, coincidiendo con lo descrito por otros autores (Ogundiwin et al., 2007; Peace et al., 2006). Otros QTLs para la fecha de cosecha, el peso del fruto, la tinción de la pulpa, el contenido en sólidos solubles, la firmeza y las dimensiones del fruto fueron también localizados en dicho grupo de ligamiento (Cantín et al., 2008b), de acuerdo con lo observado en otras poblaciones de melocotonero (Dirlewanger et al., 1999; Etienne et al., 2002b; Quilot et al., 2004c; Sánchez-Pérez et al., 2007a). Varios de los QTLs identificados en el LG4 se localizaron muy próximos en la misma región, lo que puede corresponder a QTLs ligados o a un mismo QTL con efectos pleiotrópicos. Por último, hay que destacar que el orden de los marcadores en el mapa parcial del LG4 de nuestra población, fue el mismo que en el mapa consenso TxE para *Prunus* (Howad et al., 2005), con la excepción de una permutación entre dos loci adyacentes. Este resultado confirma la co-linealidad y transferibilidad de los marcadores moleculares entre distintas especies del género *Prunus* (Dirlewanger et al., 2004; Dondini, 2007; Sánchez-Pérez et al., 2007a; Sosinski et al., 2000).

El estudio agronómico y de la calidad del fruto del melocotonero llevada a cabo en la presente Tesis Doctoral mostró la alta variabilidad genética existente en la población de mejora, lo que ha permitido la preselección de 26 genotipos (ver Capítulo 10) con un buen comportamiento agronómico y una buena calidad de fruto. Los resultados obtenidos en este trabajo destacan la importancia de la evaluación de la calidad organoléptica, nutricional y poscosecha en los programas de mejora para la selección de nuevos cultivares que satisfagan las exigencias del consumidor actual.

Capítulo 8

Conclusiones

1. La amplia variabilidad fenotípica observada entre los genotipos y los cruzamientos de la población de mejora, para todos los parámetros evaluados respecto al comportamiento agronómico, la calidad organoléptica, nutricional y poscosecha del fruto, permitió la preselección de 26 genotipos con la mejor combinación de características agronómicas y de calidad de fruto.

2. La observación del carácter frutos abortivos en algunos genotipos var. *platycarpa* de una de las progenies ('VAC-9520' x 'VAC-9517') y el análisis de su segregación, corroboran la hipótesis previa sobre el control genético de este carácter, mediante distintos alelos del mismo gen que controla la forma achatada del fruto. Sin embargo, la segregación observada en nuestra población no concuerda con dicha hipótesis respecto a la formación alélica de los genotipos con frutos abortivos, y plantea el interés de futuros estudios para comprender el control genético que gobierna este carácter.

3. Se encontraron diferencias significativas en distintos parámetros agronómicos y de la calidad organoléptica y nutricional del fruto, en función de los caracteres cualitativos del tipo y forma de fruto, color de la pulpa y adherencia del hueso. Los genotipos con frutos tipo nectarina y paraguayo mostraron menor producción y tamaño de fruto que los de melocotón y fruta redonda respectivamente. En cuanto a la calidad organoléptica, los genotipos con fruto tipo nectarina mostraron mayor concentración de azúcares y mayor firmeza que los de tipo melocotón. Los genotipos con fruto de pulpa blanca presentaron mayores niveles de azúcares y antioxidantes y menor firmeza que los de pulpa amarilla.

4. Se observó una variación anual en los niveles de azúcares de los frutos, aunque el perfil de los mismos permaneció estable para cada genotipo. El efecto del año fue menor sobre el contenido de los compuestos bioactivos, aunque también se observaron variaciones anuales en la acumulación de flavonoides y antocianinas. La susceptibilidad a los daños por frío no varió significativamente en los años de estudio evaluados, obteniéndose altas heredabilidades para todos los síntomas observados.

5. Los contenidos de azúcares totales, azúcares individuales y sólidos solubles del fruto se correlacionaron significativamente entre sí, y con otros parámetros agronómicos y de la calidad del fruto, como fecha de cosecha, producción, tamaño, acidez y compuestos bioactivos del fruto. La variabilidad observada en el contenido de azúcares individuales permitirá la selección de genotipos con perfiles de azúcares específicos para determinados fines nutricionales o preferencias organolépticas.

6. Los flavonoides y los compuestos fenólicos totales fueron los principales compuestos antioxidantes encontrados en melocotones y nectarinas. La coloración roja de la pulpa, característica del fruto de algunos genotipos, mostró una correlación significativa con la capacidad antioxidante del fruto. La gran variabilidad y las altas heredabilidades encontradas para todos los componentes bioactivos en los genotipos estudiados, muestran la posibilidad de mejorar estos caracteres y sugieren la importancia de su evaluación en la selección de cultivares con frutos que poseen propiedades beneficiosas adicionales para la salud.

7. La evaluación de la calidad poscosecha de la población 'Venus' x 'Big Top' demostró la fuerte influencia del genotipo en la susceptibilidad a los daños por frío, el control cuantitativo de sus síntomas y la importancia del periodo de almacenamiento en el desarrollo y la severidad de este daño poscosecha. En el grupo de ligamiento 4 se localizaron tres QTLs para los síntomas de daños por frío: harinosidad visual y sensorial, y enrojecimiento de la pulpa, así como otros para la fecha de cosecha, el peso y las dimensiones del fruto, la tinción de la pulpa, el contenido en sólidos solubles y la firmeza del fruto. Varios de ellos se localizaron en la misma región del genoma, sugiriendo la existencia de QTLs ligados o efectos pleiotrópicos.

Capítulo 9

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Pa melocotones gordos,
los que cría nuestra tierra,
con seis u a lo más con ocho,
bastan pa una docena

A. Beltrán, 1980

Capítulo 10

Descripción de los genotipos preseleccionados

El objetivo final planteado al inicio de esta Tesis Doctoral fue la selección de aquellos genotipos con un mejor comportamiento agronómico y una mejor calidad organoléptica, nutricional y poscosecha del fruto, en las condiciones de cultivo del Valle Medio del Ebro. Como resultado de los trabajos realizados, se han preseleccionado los mejores 26 genotipos según todos los parámetros evaluados a lo largo de este trabajo. En la siguiente tabla se presenta un resumen de los mismos, y a continuación se adjunta una ficha descriptiva para cada uno de los genotipos con las características más relevantes, medidas durante tres años consecutivos en la Estación Experimental de Aula Dei (CSIC).

Tabla 10.1. Genotipos de melocotonero preseleccionados entre la población de mejora evaluada en este trabajo. Para cada preselección se indica el cruce del que provienen y las características del fruto.

Preselección	Parentales	Tipo fruto	Forma fruto	Color pulpa
D1F1A23	Babygold-9 x VAC-9510	melocotón	redonda	amarilla
D1F1A54	Babygold-9 x VAC-9510	melocotón	redonda	amarilla
D1F2A01	Babygold-9 x VAC-9510	melocotón	redonda	amarilla
D1F3A12	Red Top x VAC-9513	melocotón	redonda	amarilla
D1F3A54	Rich Lady x VAC-9511	melocotón	redonda	amarilla
D1F4A56	Red Top x VAC-9513	melocotón	redonda	amarilla
D1F5A02	Babygold-9 x Crown Princess	melocotón	redonda	amarilla
D1F5A48	Babygold-9 x Crown Princess	melocotón	redonda	amarilla
D1F6A17	Babygold-9 x Crown Princess	melocotón	redonda	amarilla
D1F7A41	Andross x Rich Lady	melocotón	redonda	amarilla
D1F8A07	VAC-9512 x VAC-9511	melocotón	redonda	amarilla
D1F8A39	VAC-9512 x VAC-9511	melocotón	redonda	amarilla
D1F9A01	Orion x VAC-9510	melocotón	redonda	amarilla
D2F1A06	VAC-9520 x VAC-9517	melocotón	paraguayo	blanca
D2F1A08	VAC-9520 x VAC-9517	melocotón	paraguayo	blanca
D2F1A21	VAC-9520 x VAC-9517	melocotón	paraguayo	blanca
D2F1A25	VAC-9520 x VAC-9517	melocotón	paraguayo	blanca
D2F1A43	VAC-9520 x VAC-9517	melocotón	paraguayo	amarilla
D2F1A48	VAC-9520 x VAC-9517	melocotón	paraguayo	amarilla
D2F1A60	VAC-9520 x VAC-9517	melocotón	redonda	blanca
D2F1A72	VAC-9520 x VAC-9517	melocotón	paraguayo	amarilla
D2F2A61	Venus x Big Top	nectarina	redonda	amarilla
D2F2A73	Venus x Big Top	nectarina	redonda	amarilla
D2F4A48	O´Henry x VAC-9514	melocotón	redonda	blanca
D2F5A10	O´Henry x VAC-9514	melocotón	redonda	blanca
D2F7A33	Andross x Crown Princess	melocotón	redonda	amarilla

D1F1A23 Babygold-9 x VAC-9510

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	2ª quincena julio		
	Producción	alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	30		
	Peso fruto (g)	193		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	12,3	Fenoles totales (mg GAE/100 g PF)	23,7
	Acidez (g/L ácido málico)	0,8	Flavonoides (mg CE/100 g PF)	3,7
	Índice madurez	15,5	Antocianinas (mg C3G/kg PF)	0,7
	Azúcares totales (mg/g PF)	64,7	Vitamina C (mg/100 g PF)	1,9
	Sacarosa/Glucosa	11,0	Capacidad antioxidante (μg Trolox/g PF)	387,0
	Glucosa/Fructosa	0,9		
	Sorbitol (%)	3,7		
Calidad organoléptica	Frutos con muy buena presencia Sabor equilibrado			
Calidad postcosecha	Tolerante al almacenamiento en frío de periodos de hasta 15 días A partir de los 15 días, se observa una incidencia moderada de pardeamiento y harinosidad			

D1F1A54 Babygold-9 x VAC-9510

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	finales agosto		
	Producción	alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	24		
	Peso fruto (g)	187		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	13,2	Fenoles totales (mg GAE/100 g PF)	22,6
	Acidez (g/L ácido málico)	0,7	Flavonoides (mg CE/100 g PF)	6,5
	Índice madurez	18,1	Antocianinas (mg C3G/kg PF)	1,2
	Azúcares totales (mg/g PF)	68,4	Vitamina C (mg/100 g PF)	2,3
	Sacarosa/Glucosa	12,3	Capacidad antioxidante (μg Trolox/g PF)	473,3
	Glucosa/Fructosa	0,7		
	Sorbitol (%)	4,0		
Calidad organoléptica	Sabor equilibrado. Fruto firme y de buen calibre Zona pistilar prominente en algunos casos			
Calidad postcosecha	Tolerante al almacenamiento a bajas temperaturas, incluso durante periodos de hasta 30 días			

D1F2A01 Babygold-9 x VAC-9510

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	finales julio		
	Producción	alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	35		
	Peso fruto (g)	179		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,7	Fenoles totales (mg GAE/100 g PF)	27,3
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	8,7
	Índice madurez	16,0	Antocianinas (mg C3G/kg PF)	0,3
	Azúcares totales (mg/g PF)	63,4	Vitamina C (mg/100 g PF)	2,6
	Sacarosa/Glucosa	9,7	Capacidad antioxidante (μg Trolox/g PF)	371,6
	Glucosa/Fructosa	0,9		
	Sorbitol (%)	2,8		
Calidad organoléptica	Fruto de aspecto muy atractivo. Forma redondeada con mucrón. Sutura marcada, con una de las caras más prominente.			
Calidad postcosecha	Sin síntomas de daños por frío tras dos semanas de almacenamiento Alteración de la maduración normal tras periodos de 30 días de almacenamiento			

D1F3A12 Red Top x VAC-9513

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	finales mayo - principios junio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	80		
	Peso fruto (g)	86		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,1	Fenoles totales (mg GAE/100 g PF)	18,2
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	2,3
	Índice madurez	17,5	Antocianinas (mg C3G/kg PF)	1,7
	Azúcares totales (mg/g PF)	49,9	Vitamina C (mg/100 g PF)	3,7
	Sacarosa/Glucosa	6,5	Capacidad antioxidante (μg Trolox/g PF)	380,8
	Glucosa/Fructosa	0,7		
	Sorbitol (%)	1,9		
Calidad organoléptica	Coloración atractiva y sabor equilibrado. Pulpa ligeramente fibrosa. Forma regular, sin mucrón y sutura poco marcada.			
Calidad postcosecha	Susceptibilidad media a los daños por frío. Tras 15 días de almacenamiento en frío, aparecen problemas de textura y sabor.			

D1F3A54 Rich Lady x VAC-9511

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	2ª quincena junio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	90		
	Peso fruto (g)	116		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	9,2	Fenoles totales (mg GAE/100 g PF)	14,1
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	2,8
	Índice madurez	14,1	Antocianinas (mg C3G/kg PF)	0,4
	Azúcares totales (mg/g PF)	58,4	Vitamina C (mg/100 g PF)	3,8
	Sacarosa/Glucosa	6,7	Capacidad antioxidante (μg Trolox/g PF)	336,1
	Glucosa/Fructosa	0,9		
	Sorbitol (%)	2,7		
Calidad organoléptica	Coloración atractiva. Pulpa firme y de textura agradable. Sutura prominente			
Calidad postcosecha	Baja susceptibilidad a los daños por frío. Sin síntomas tras almacenamientos de 15 días Tras 30 días de almacenamiento en frío, aparecen problemas moderados de textura			

D1F4A56 Red Top x VAC-9513

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	finales junio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	85		
	Peso fruto (g)	134		
	Hueso	semi-adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	12,3	Fenoles totales (mg GAE/100 g PF)	53,4
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	10,2
	Índice madurez	21,1	Antocianinas (mg C3G/kg PF)	1,0
	Azúcares totales (mg/g PF)	61,0	Vitamina C (mg/100 g PF)	2,3
	Sacarosa/Glucosa	10,4	Capacidad antioxidante (μg Trolox/g PF)	447,7
	Glucosa/Fructosa	0,7		
	Sorbitol (%)	3,3		
Calidad organoléptica	Coloración y aspecto atractivo. Sabor equilibrado y textura crocante de la pulpa Forma regular, sin mucrón			
Calidad postcosecha	Susceptibilidad alta a los daños por frío. Aparición de problemas de textura y sabor tras almacenamiento en frío durante 15 días			

D1F5A02 Babygold-9 x Crown Princess

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	2ª quincena agosto		
	Producción	media-alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	30		
	Peso fruto (g)	207		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	12,1	Fenoles totales (mg GAE/100 g PF)	34,2
	Acidez (g/L ácido málico)	0,8	Flavonoides (mg CE/100 g PF)	8,7
	Índice madurez	14,8	Antocianinas (mg C3G/kg PF)	0,5
	Azúcares totales (mg/g PF)	98,1	Vitamina C (mg/100 g PF)	4,9
	Sacarosa/Glucosa	13,0	Capacidad antioxidante (μg Trolox/g PF)	390,2
	Glucosa/Fructosa	0,6		
	Sorbitol (%)	5,0		
Calidad organoléptica	Buen sabor. Coloración anaranjada luminosa. Textura algo blanda. Frutos alargados.			
Calidad postcosecha	Baja susceptibilidad a los daños por frío, incluso tras almacenamiento en cámara durante 30 días			

D1F5A48 Babygold-9 x Crown Princess

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	1ª quincena agosto		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	23		
	Peso fruto (g)	197		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,3	Fenoles totales (mg GAE/100 g PF)	49,7
	Acidez (g/L ácido málico)	0,9	Flavonoides (mg CE/100 g PF)	15,1
	Índice madurez	13,1	Antocianinas (mg C3G/kg PF)	1,4
	Azúcares totales (mg/g PF)	86,5	Vitamina C (mg/100 g PF)	2,3
	Sacarosa/Glucosa	7,4	Capacidad antioxidante (μg Trolox/g PF)	510,9
	Glucosa/Fructosa	1,0		
	Sorbitol (%)	5,8		
Calidad organoléptica	Sabor muy bueno. Pulpa firme y crocante. Forma regular. Sin mucrón.			
Calidad postcosecha	Baja susceptibilidad a los daños por frío. Tras almacenamiento en frío durante 30 días, aparición de síntomas leves de pardeamiento y harinosidad.			

D1F6A17 Babygold-9 x Crown Princess

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	mediados agosto		
	Producción	alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	23		
	Peso fruto (g)	181		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,9	Fenoles totales (mg GAE/100 g PF)	64,9
	Acidez (g/L ácido málico)	0,7	Flavonoides (mg CE/100 g PF)	21,4
	Índice madurez	16,5	Antocianinas (mg C3G/kg PF)	1,8
	Azúcares totales (mg/g PF)	59,7	Vitamina C (mg/100 g PF)	2,1
	Sacarosa/Glucosa	10,1	Capacidad antioxidante (μg Trolox/g PF)	478,5
	Glucosa/Fructosa	0,8		
	Sorbitol (%)	3,7		
Calidad organoléptica	Sabor equilibrado. Coloración anaranjada luminosa. Calibre grande. Forma regular y homogénea. Sutura prominente en una de las caras.			
Calidad postcosecha	Baja susceptibilidad a los daños por frío. Tras almacenamiento en frío durante 30 días, aparición de síntomas leves de pardeamiento y harinosidad.			

D1F7A41 Andross x Rich Lady

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	finales junio-principios julio		
	Producción	media-alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	90		
	Peso fruto (g)	118		
	Hueso	semi-adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,5	Fenoles totales (mg GAE/100 g PF)	24,1
	Acidez (g/L ácido málico)	0,8	Flavonoides (mg CE/100 g PF)	2,6
	Índice madurez	14,4	Antocianinas (mg C3G/kg PF)	1,9
	Azúcares totales (mg/g PF)	79,0	Vitamina C (mg/100 g PF)	6,7
	Sacarosa/Glucosa	10,9	Capacidad antioxidante (μg Trolox/g PF)	325,4
	Glucosa/Fructosa	0,6		
	Sorbitol (%)	2,3		
Calidad organoléptica	Fruto jugoso y de sabor equilibrado. Coloración roja atractiva Forma regular, sin mucrón. Sutura prominente en una da las caras.			
Calidad postcosecha	Susceptible a los daños por frío. Aparición de problemas de textura y sabor tras periodos de almacenamiento en frío de 15 días			

D1F8A07 VAC-9512 x VAC-9511

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	2ª quincena junio		
	Producción	media-alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	90		
	Peso fruto (g)	158		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,9	Fenoles totales (mg GAE/100 g PF)	42,6
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	7,0
	Índice madurez	18,8	Antocianinas (mg C3G/kg PF)	0,8
	Azúcares totales (mg/g PF)	72,1	Vitamina C (mg/100 g PF)	4,7
	Sacarosa/Glucosa	7,5	Capacidad antioxidante (μg Trolox/g PF)	393,0
	Glucosa/Fructosa	0,7		
	Sorbitol (%)	2,2		
Calidad organoléptica	Sabor dulce. Pulpa teñida y de textura crocante. Atractiva coloración roja de la piel. Forma regular, con mucrón.			
Calidad postcosecha	Susceptibilidad baja a los daños por frío. Sin síntomas de daños incluso tras periodos de almacenamiento en frío de 30 días			

D1F8A39 VAC-9512 x VAC-9511

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	1ª quincena julio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	90		
	Peso fruto (g)	156		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,3	Fenoles totales (mg GAE/100 g PF)	27,2
	Acidez (g/L ácido málico)	0,9	Flavonoides (mg CE/100 g PF)	3,6
	Índice madurez	20,1	Antocianinas (mg C3G/kg PF)	2,0
	Azúcares totales (mg/g PF)	87,7	Vitamina C (mg/100 g PF)	1,6
	Sacarosa/Glucosa	7,3	Capacidad antioxidante (μg Trolox/g PF)	333,7
	Glucosa/Fructosa	0,8		
	Sorbitol (%)	7,8		
Calidad organoléptica	Fruto jugoso y de buen sabor. Pulpa teñida y de textura crocante. Buen calibre. Forma regular. Sin mucrón.			
Calidad postcosecha	Baja susceptibilidad a los daños por frío. Sin síntomas tras almacenamientos de 15 días. Tras 30 días de almacenamiento en frío, problemas moderados de textura.			

D1F9A01 Orion x VAC-9510

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	finales julio-principios agosto		
	Producción	alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	20		
	Peso fruto (g)	240		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	12,5	Fenoles totales (mg GAE/100 g PF)	33,0
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	5,8
	Índice madurez	20,4	Antocianinas (mg C3G/kg PF)	10,3
	Azúcares totales (mg/g PF)	62,3	Vitamina C (mg/100 g PF)	3,1
	Sacarosa/Glucosa	5,2	Capacidad antioxidante (μg Trolox/g PF)	368,3
	Glucosa/Fructosa	0,9		
	Sorbitol (%)	4,6		
Calidad organoléptica	Fruto aromático y de buen sabor. Textura crocante. Forma regular, con una cara más prominente. Buen calibre.			
Calidad postcosecha	Tolerante al almacenamiento en frío durante períodos de hasta 15 días. Incidencia de problemas graves de pardeamiento, textura y sabor tras almacenamientos más largos.			

D2F1A06 VAC-9520 x VAC-9517

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	mediados junio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	blanca		
	Forma	paraguayo		
	Chapa (%)	60		
	Peso fruto (g)	163		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,6	Fenoles totales (mg GAE/100 g PF)	24,8
	Acidez (g/L ácido málico)	0,9	Flavonoides (mg CE/100 g PF)	5,8
	Índice madurez	12,4	Antocianinas (mg C3G/kg PF)	1,6
	Azúcares totales (mg/g PF)	92,9	Vitamina C (mg/100 g PF)	7,5
	Sacarosa/Glucosa	10,0	Capacidad antioxidante (μg Trolox/g PF)	406,3
	Glucosa/Fructosa	0,8		
	Sorbitol (%)	1,7		
Calidad organoléptica	Sabor dulce y aromático. Pulpa firme. Coloración atractiva. Calibre pequeño. Cavidad pistilar abierta en algunos frutos.			
Calidad postcosecha	Buena tolerancia al almacenamiento en frío. Buen estado de los frutos incluso tras periodos de almacenamiento de 30 días.			

D2F1A08 VAC-9520 x VAC-9517

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	1ª quincena julio		
	Producción	alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	blanca		
	Forma	paraguayo		
	Chapa (%)	90		
	Peso fruto (g)	95		
	Hueso	libre		
Calidad nutricional	Sólidos solubles (ºBrix)	14,4	Fenoles totales (mg GAE/100 g PF)	64,5
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	13,8
	Índice madurez	24,6	Antocianinas (mg C3G/kg PF)	3,4
	Azúcares totales (mg/g PF)	100,8	Vitamina C (mg/100 g PF)	5,3
	Sacarosa/Glucosa	9,6	Capacidad antioxidante (μg Trolox/g PF)	549,0
	Glucosa/Fructosa	0,7		
	Sorbitol (%)	5,5		
Calidad organoléptica	Fruto jugoso, dulce y aromático. Color rosado atractivo. Calibre medio. Zona pistilar abierta en algunos frutos.			
Calidad postcosecha	Susceptible a los daños por frío tras almacenamiento a bajas temperaturas. Problemas de textura tras 15 de almacenamiento.			

D2F1A21 VAC-9520 x VAC-9517

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	mediados junio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	blanca		
	Forma	paraguayo		
	Chapa (%)	80		
	Peso fruto (g)	60		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	12,8	Fenoles totales (mg GAE/100 g PF)	57,4
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	10,6
	Índice madurez	21,7	Antocianinas (mg C3G/kg PF)	1,7
	Azúcares totales (mg/g PF)	36,0	Vitamina C (mg/100 g PF)	6,5
	Sacarosa/Glucosa	12,0	Capacidad antioxidante (μg Trolox/g PF)	472,1
	Glucosa/Fructosa	0,6		
	Sorbitol (%)	4,8		
Calidad organoléptica	Sabor equilibrado y aromático. Calibre medio y aspecto atractivo. Zona pistilar abierta en algunos frutos.			
Calidad postcosecha	Buena tolerancia al almacenamiento en frío. Buen estado de los frutos incluso tras periodos de almacenamiento de 30 días.			

D2F1A25 VAC-9520 x VAC-9517

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	mediados junio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	blanca		
	Forma	paraguayo		
	Chapa (%)	73		
	Peso fruto (g)	79		
	Hueso	semi-adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	12,0	Fenoles totales (mg GAE/100 g PF)	30,8
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	7,1
	Índice madurez	18,5	Antocianinas (mg C3G/kg PF)	2,1
	Azúcares totales (mg/g PF)	90,9	Vitamina C (mg/100 g PF)	5,9
	Sacarosa/Glucosa	12,8	Capacidad antioxidante (μg Trolox/g PF)	476,9
	Glucosa/Fructosa	0,5		
	Sorbitol (%)	2,0		
Calidad organoléptica	Sabor muy aromático y dulce. Calibre medio-alto y presencia muy atractiva. Zona pistilar abierta en algunos frutos.			
Calidad postcosecha	Buena tolerancia al almacenamiento en frío. Buen estado de los frutos incluso tras periodos de almacenamiento de 30 días.			

D2F1A43 VAC-9520 x VAC-9517

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	mediados junio		
	Producción	media-alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	paraguayo		
	Chapa (%)	67		
	Peso fruto (g)	78		
	Hueso	semi-libre		
Calidad nutricional	Sólidos solubles (ºBrix)	12,8	Fenoles totales (mg GAE/100 g PF)	45,3
	Acidez (g/L ácido málico)	0,7	Flavonoides (mg CE/100 g PF)	10,0
	Índice madurez	19,2	Antocianinas (mg C3G/kg PF)	1,9
	Azúcares totales (mg/g PF)	87,9	Vitamina C (mg/100 g PF)	5,6
	Sacarosa/Glucosa	7,4	Capacidad antioxidante (μg Trolox/g PF)	527,8
	Glucosa/Fructosa	1,1		
	Sorbitol (%)	4,1		
Calidad organoléptica	Sabor equilibrado y aromático Calibre medio y buena presencia. Zona pistilar abierta en algunos frutos			
Calidad postcosecha	Baja susceptibilidad a los daños por frío. Sin síntomas tras almacenamientos de 15 días Tras 30 días de almacenamiento en frío aparecen problemas moderados de textura			

D2F1A48 VAC-9520 x VAC-9517

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	mediados junio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	paraguayo		
	Chapa (%)	85		
	Peso fruto (g)	68		
	Hueso	semi-adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	13,1	Fenoles totales (mg GAE/100 g PF)	19,7
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	6,5
	Índice madurez	21,8	Antocianinas (mg C3G/kg PF)	1,1
	Azúcares totales (mg/g PF)	71,5	Vitamina C (mg/100 g PF)	5,5
	Sacarosa/Glucosa	9,1	Capacidad antioxidante (μg Trolox/g PF)	395,2
	Glucosa/Fructosa	1,0		
	Sorbitol (%)	2,2		
Calidad organoléptica	Fruto jugoso y de sabor equilibrado. Calibre medio. Pulpa teñida y algo blanda. Zona pistilar abierta en algunos frutos			
Calidad postcosecha	Susceptibilidad moderada a los daños por frío. Aparición de problemas de textura tras almacenamiento en frío durante 15 días			

D2F1A60 VAC-9520 x VAC-9517

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	mediados junio		
	Producción	alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	blanca*		
	Forma	redonda		
	Chapa (%)	80		
	Peso fruto (g)	97		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	10,7	Fenoles totales (mg GAE/100 g PF)	34,2
	Acidez (g/L ácido málico)	0,7	Flavonoides (mg CE/100 g PF)	8,7
	Índice madurez	15,9	Antocianinas (mg C3G/kg PF)	0,5
	Azúcares totales (mg/g PF)	98,1	Vitamina C (mg/100 g PF)	4,9
	Sacarosa/Glucosa	13,0	Capacidad antioxidante (μg Trolox/g PF)	390,2
	Glucosa/Fructosa	0,6		
	Sorbitol (%)	5,0		
Calidad organoléptica	Coloración roja estriada sobre fondo blanco rosado. Jugoso y aromático *Pulpa muy teñida. Algo blanda			
Calidad postcosecha	Baja susceptibilidad a los daños por frío, incluso tras almacenamiento en cámara durante 30 días			

D2F1A72 VAC-9520 x VAC-9517

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	mediados junio		
	Producción	media-alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	paraguayo		
	Chapa (%)	85		
	Peso fruto (g)	89		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	14,6	Fenoles totales (mg GAE/100 g PF)	21,0
	Acidez (g/L ácido málico)	0,6	Flavonoides (mg CE/100 g PF)	6,1
	Índice madurez	23,5	Antocianinas (mg C3G/kg PF)	1,7
	Azúcares totales (mg/g PF)	74,5	Vitamina C (mg/100 g PF)	4,1
	Sacarosa/Glucosa	10,7	Capacidad antioxidante (μg Trolox/g PF)	371,6
	Glucosa/Fructosa	0,7		
	Sorbitol (%)	4,0		
Calidad organoléptica	Sabor ácido y refrescante. Coloración atractiva y calibre medio Zona pistilar bien cerrada en la mayoría de los frutos			
Calidad postcosecha	Buena tolerancia al almacenamiento en frío. Buen estado de los frutos incluso tras periodos de almacenamiento de 30 días			

D2F2A61 Venus x Big Top

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	finales julio-principios agosto		
	Producción	alta		
Fruto	Tipo fruto	nectarina		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	90		
	Peso fruto (g)	192		
	Hueso	libre		
Calidad nutricional	Sólidos solubles (ºBrix)	14,0	Fenoles totales (mg GAE/100 g PF)	20,3
	Acidez (g/L ácido málico)	0,5	Flavonoides (mg CE/100 g PF)	5,4
	Índice madurez	32,2	Antocianinas (mg C3G/kg PF)	3,2
	Azúcares totales (mg/g PF)	79,7	Vitamina C (mg/100 g PF)	1,8
	Sacarosa/Glucosa	7,9	Capacidad antioxidante (μg Trolox/g PF)	389,4
	Glucosa/Fructosa	1,2		
	Sorbitol (%)	3,1		
Calidad organoléptica	Sabor aromático. Pulpa firme y crocante Buen calibre. Aspecto atractivo			
Calidad postcosecha	Baja susceptibilidad a los daños por frío. Tras almacenamiento en frío durante 30 días, aparición de pardeamiento de la pulpa			

D2F2A73 Venus x Big Top

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	1ª quincena julio		
	Producción	media		
Fruto	Tipo fruto	nectarina		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	90		
	Peso fruto (g)	166		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	13,8	Fenoles totales (mg GAE/100 g PF)	19,6
	Acidez (g/L ácido málico)	0,4	Flavonoides (mg CE/100 g PF)	4,3
	Índice madurez	14,0	Antocianinas (mg C3G/kg PF)	2,5
	Azúcares totales (mg/g PF)	77,0	Vitamina C (mg/100 g PF)	2,4
	Sacarosa/Glucosa	8,9	Capacidad antioxidante (μg Trolox/g PF)	383,0
	Glucosa/Fructosa	1,1		
	Sorbitol (%)	3,7		
Calidad organoléptica	Sabor muy dulce, sin acidez. Tamaño grande. Pulpa firme Forma ligeramente oblonga.			
Calidad postcosecha	Buena tolerancia al almacenamiento en frío. Buen estado de los frutos incluso tras periodos de almacenamiento de 30 días			

D2F4A48 O´Henry x VAC-9514

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	2ª quincena julio		
	Producción	media		
Fruto	Tipo fruto	melocotón		
	Color pulpa	blanca*		
	Forma	redonda		
	Chapa (%)	87		
	Peso fruto (g)	172		
	Hueso	semi-adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	13,2	Fenoles totales (mg GAE/100 g PF)	60,8
	Acidez (g/L ácido málico)	0,8	Flavonoides (mg CE/100 g PF)	12,2
	Índice madurez	16,3	Antocianinas (mg C3G/kg PF)	2,4
	Azúcares totales (mg/g PF)	109,4	Vitamina C (mg/100 g PF)	3,6
	Sacarosa/Glucosa	8,5	Capacidad antioxidante (μg Trolox/g PF)	485,7
	Glucosa/Fructosa	0,8		
	Sorbitol (%)	2,8		
Calidad organoléptica	Muy aromático y buen sabor. Aspecto aterciopelado y atractivo *Pulpa muy teñida. Algo blanda			
Calidad postcosecha	Susceptibilidad alta a los daños por frío. Síntomas de pardeamiento y problemas de textura tras almacenamiento en frío durante 15 días			

D2F5A10 O´Henry x VAC-9514

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	2ª quincena julio		
	Producción	media-alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	blanca*		
	Forma	redonda		
	Chapa (%)	80		
	Peso fruto (g)	182		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	12,2	Fenoles totales (mg GAE/100 g PF)	46,5
	Acidez (g/L ácido málico)	0,9	Flavonoides (mg CE/100 g PF)	23,8
	Índice madurez	13,1	Antocianinas (mg C3G/kg PF)	8,7
	Azúcares totales (mg/g PF)	66,3	Vitamina C (mg/100 g PF)	3,1
	Sacarosa/Glucosa	5,5	Capacidad antioxidante (μg Trolox/g PF)	515,3
	Glucosa/Fructosa	0,7		
	Sorbitol (%)	4,5		
Calidad organoléptica	Muy aromático y buen sabor. Aspecto atractivo. Calibre medio *Pulpa muy teñida			
Calidad postcosecha	Buena tolerancia al almacenamiento en frío durante períodos de hasta 15 días Tras 30 días de almacenamiento, problemas graves de pardeamiento, textura y sabor			

D2F7A33 Andross x Crown Princess

Cosecha	Fecha de plena floración	1ª quincena marzo		
	Fecha de cosecha	finales julio-principios agosto		
	Producción	media-alta		
Fruto	Tipo fruto	melocotón		
	Color pulpa	amarilla		
	Forma	redonda		
	Chapa (%)	67		
	Peso fruto (g)	163		
	Hueso	adherente		
Calidad nutricional	Sólidos solubles (ºBrix)	11,6	Fenoles totales (mg GAE/100 g PF)	46,2
	Acidez (g/L ácido málico)	0,9	Flavonoides (mg CE/100 g PF)	9,8
	Índice madurez	12,4	Antocianinas (mg C3G/kg PF)	2,7
	Azúcares totales (mg/g PF)	92,9	Vitamina C (mg/100 g PF)	2,0
	Sacarosa/Glucosa	10,0	Capacidad antioxidante (μg Trolox/g PF)	422,5
	Glucosa/Fructosa	0,8		
	Sorbitol (%)	1,7		
Calidad organoléptica	Sabor equilibrado. Textura crocante. Apariencia atractiva Sutura prominente, forma achatada			
Calidad postcosecha	Buena tolerancia al almacenamiento en frío. Buen estado de los frutos incluso tras periodos de almacenamiento de 30 días			

Capítulo 11

Anexo

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Evaluation of the Antioxidant Capacity, Phenolic Compounds, and Vitamin C Content of Different Peach and Nectarine [*Prunus persica* (L.) Batsch] Breeding Progenies

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Antioxidant capacity and contents of total phenolics, anthocyanins, flavonoids, and vitamin C were evaluated in 218 genotypes from 15 peach and nectarine breeding progenies. Significant differences were found among progenies on the fruit antioxidant profile, corroborated by the high contribution showed by cross to the phenotypic variance of each phytochemical trait analyzed (16−45%). Phytochemical profile varied depending on peach/nectarine and yellow/white flesh color qualitative traits. On the other hand, no significant effect of year was found on the bioactive profile of peaches and nectarines. Antioxidant capacity was linearly correlated to total phenolic content, but correlation varied depending on the progeny. No correlation was found for vitamin C versus any other phytochemical trait. The results suggest the importance of genetic background on the antioxidant profile of peaches and nectarines and stress its relevance for the ultimate objective of this work: selecting new peach and nectarine genotypes rich in bioactive compounds to benefit consumer's health.

KEYWORDS: ***Prunus persica*; total phenolics; anthocyanins; flavonoids; vitamin C; antioxidant capacity; phytochemical profiling**

INTRODUCTION

The important role of diet in either promoting or preventing diseases has long been recognized, and in recent years, diet and human well-being have received unprecedented attention. Nowadays, there is a growing interest in bioactive compounds of fruits and vegetables due to their putative role in preventing diseases such as diabetes, cancer, stroke, arthritis, and also aging. A clear inverse relationship between the consumption of fruits and vegetables and incidence of cardio- and cerebrovascular, degenerative, and proliferative diseases and mortality has been largely proved by epidemiological studies (*1*). Fruits and vegetables are excellent functional foods as they are high in antioxidant compounds (*2*). These naturally occurring substances not only have a role in the visual appearance (pigmentation and browning) and taste (astringency) of fruits and vegetables but also have health-promoting properties, acting as antioxidants by scavenging harmful free radicals, which are implicated in most degenerative diseases (*3*).

The health benefits of fruits are due to their specific chemical composition, particularly to compounds of nutritional value such as phenolic acids, flavonoids, and vitamins (*4*). Peaches and nectarines, even though having a lower total antioxidant capacity than other fruits such as strawberry, apple, or orange (*5*), are nutritionally important because they are one of the most important commodities consumed worldwide. Polyphenols are secondary plant metabolites, and they are the main sources of antioxidant capacity in peaches, although vitamin C and carotenoids also contribute to it (*6*). The basic feature of all polyphenols is the presence of one or more hydroxylated aromatic rings, which seemed to be responsible for their properties as radical scavengers (*7*). The flavonoids are a large class of phenolic compounds, present in cereals, vegetables, and fruits. Evidence is accumulating about their significant contribution to the antioxidant capacity of fruits and vegetables (*8*). Anthocyanins are natural colorants and, with flavanols and flavonols, are included in the flavonoid family. They are widely distributed among flowers, fruits, and vegetables and, in addition to their colorful characteristics, they have potent antioxidant properties modulated by their different hydroxylations and glycosylations (*3*). The main anthocyanins reported in peach are cyanidin-3-glucoside and cyanidin-3-rutinoside (*9*). Besides their relevance in the appearance, taste, and flavor of fruits as well as their health-promoting properties (*10*), phenolic compounds have been found to increase the shelf life of food and inhibit the growth of pathogenic microorganisms due to their natural antimicrobial properties (*11*). Vitamin C is a water-soluble antioxidant and is, as are vitamin E and β-carotene, referred to as an antioxidant vitamin. Humans are unable to synthesize vitamin C and are thus entirely dependent upon dietary sources to meet needs. More than 90% of the vitamin C in the human diet is supplied by fruits and vegetables (*12*). These benefits and the increasing consumer interest in functional foods have guided breeders of different crops to consider antioxidant compounds and other nutritional properties as interesting targets in breeding programs (*13*, *14*).

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Table 1. Peach and Nectarine Commercial and Experimental (VAC-) Cultivars Used as Progenitors in the 15 Controlled Crosses[a]

cultivar	fruit type		flesh color	stone
Andross	round	peach	yellow	cling
Babygold-9	round	peach	yellow	cling
Big Top	round	nectarine	yellow	cling
Calante	round	peach	yellow	cling
Crown Princess	round	peach	yellow	cling
O'Henry	round	peach	yellow	free
Orion	round	peach	yellow	free
Red Top	round	peach	yellow	free
Rich Lady	round	peach	white	free
VAC-9510	round	peach	yellow	cling
VAC-9511	round	peach	yellow	free
VAC-9512	round	peach	yellow	free
VAC-9513	round	nectarine	yellow	free
VAC-9514	round	nectarine	white	free
VAC-9515	round	nectarine	yellow	free
VAC-9516	round	peach	white	free
VAC-9517	flat	peach	white	free
VAC-9520	round	peach	yellow	free
Venus	round	nectarine	yellow	free

[a] Fruit type (round or flat, peach or nectarine), flesh color (yellow or white), and stone adherence (free or cling) for each progenitor is shown.

The phytochemical content of fruit is influenced by numerous factors such as genotype, rootstock, climatic conditions, agronomic practices, harvesting time, and postharvest conditions (*6*, *11*, *14*, *15*). Moreover, phenolic compounds are not uniformly distributed within the tissue of fruits, and most of them are concentrated in the epidermal and subepidermal layers of the fruit (*11*). Phenolic distribution is an important aspect of the overall phenolic composition and antioxidant capacity because, due to its characteristics, the peach skin is usually not eaten and therefore it does not contribute to the human diet intake.

The aim of the present work was to screen and compare 218 genotypes from 15 different peach and nectarine breeding progenies by measuring their contents of total phenolics, total flavonoids, total anthocyanins, vitamin C, and relative antioxidant capacity. We also wanted to study the influence of genotype, genetic origin, pomological traits, and year in the bioactive profile of peach and nectarine fruits. The ultimate objective of this study was to select peach genotypes with enhanced antioxidant capacity fruits that will benefit consumers with health-promoting properties.

MATERIALS AND METHODS

Chemicals. All chemicals were of analytical grade. Folin−Ciocalteu's phenol reagent, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-dipyridyl-1,1-diphenyl-2-picrylhydrazyl (DPPH), 3,4,5-trihydroxybenzoic acid (gallic acid), sodium carbonate (Na_2CO_3), catechin, trichloroacetic acid (TCA), and ascorbic acid (vitamin C) were purchased from Sigma-Aldrich (Steinheim, Germany).

Plant Material. Fifteen controlled biparental crosses between 19 peach and nectarine cultivars (**Table 1**) were made during 2000 and 2001 to develop superior peach and nectarine cultivars for the Spanish industry. The resulting seedling trees (one tree per genotype) were grafted on the same rootstock (GF-677) and established in an experimental orchard at the Experimental Station of Aula Dei-CSIC (northern Spain, Zaragoza) in 2002. Trees were trained to the standard open vase system and planted at a spacing of 4 m × 2.5 m. Hand thinning was carried out to reduce fruit load on the heavily loaded trees. They were grown under usual conditions of irrigation, fertilization, and pest control. Vegetative and fruit quality traits were evaluated in a total of 1111 genotypes over three consecutive years (2005−2007). All traits were measured or scored for each seedling separately over the three year period, and means of three years were calculated. Phytochemical composition (total phenolics, total flavonoids, total anthocyanins, and total antioxidant capacity) was studied in 218 genotypes that were common at least for two years to estimate the seasonal effect on phytochemical profile. Vitamin C was also determined in all of the genotypes in the last year of study to corroborate the variability found in other bioactive compounds in the previous years and its contribution to the antioxidant capacity of fruits. The studied genotypes were selected among the descendants from the 15 crosses because of their higher fruit quality. For all analyses, only fruit flesh was used, as it is usually consumed. Fruits were peeled with a sharp knife, and flesh was weighed, immediately frozen separately in liquid nitrogen, and stored at −20 °C until analysis. Samples for vitamin C determination were kept at −20 °C in 5% metaphosphoric acid for preservation of ascorbic acid until analysis.

Quality Parameters. During the years 2005, 2006, and 2007, fruit quality parameters were measured individually in each seedling tree. Fruits were hand-picked at commercial maturity, assessed by peel fruit color and flesh firmness. Yield (kg/tree) was measured, and total number of fruits was counted for each genotype. From these variables, total average fruit weight was calculated. Ten fruits from each plant were randomly selected for the quality evaluations. Some quality traits such as fruit type (peach/nectarine), flesh color (yellow/white), and endocarp staining were scored. Fruit type was scored on a 1−2 scale as peach (1) or nectarine (2). Similarly, flesh color was scored as (1) yellow or (2) white. Endocarp staining (redness around stone) was scored on an increasing scale from no color (1) to high redness (10). The soluble solids content (SSC) of the juice was measured with a temperature-compensated refractometer (model ATC-1, Atago Co., Tokyo, Japan), and data are given as °Brix. The titratable acidity (TA) was determined by titration with 0.1 N NaOH to pH 8.1. Data are given as grams of malic acid per 100 g of fresh weight (FW), because this is the dominant organic acid in peach.

Phytochemical Analysis. The frozen fruit material (5 g) was homogenized with a Polytron (2 min on ice) with 10 mL of extraction solution, consisting of 0.5 N HCl in methanol/Milli-Q water (80% v/v). The mixture was incubated overnight at 4 °C and then centrifuged for 20 min at 4 °C and 20000*g*. Supernatant was recovered and the volume measured. This hydroalcoholic extract was used for total phenolics, anthocyanins, flavonoids, and antioxidant capacity assays.

The content of phenolic compounds in methanol extracts was determined according to the Folin−Ciocalteu method (*16*). The method consisted of mixing 500 μL of the extract diluted in water with 500 μL of Folin−Ciocalteu's reagent. After 3 min of reaction, 1 mL of 1 N sodium carbonate (Na_2CO_3) was added. The tubes were mixed for 15 s and then allowed to stand for 60 min at 20 °C. Absorbance was measured at 725 nm using a spectrophotometer (Beckman Coulter DU 800). The standard calibration curves were daily prepared using gallic acid (3,4,5-trihydroxybenzoic acid). The phenolic content was expressed in milligrams of gallic acid equivalents (GAE) per 100 g of FW.

Total flavonoids content was determined using a colorimetric assay based on the method of Zhishen et al. (*17*). One milliliter of the methanolic extract was diluted with water (1:2), and 0.3 mL of 5% $NaNO_2$ was added. After 5 min, 0.3 mL of 10% $AlCl_3$ were added. After 1 min, 2 mL of 1 N NaOH was added, and the solution was mixed by vortex. Absorbance at 510 nm was measured against a blank with a spectrophotometer (Beckman Coulter DU 800). The results were expressed as milligrams of catechin equivalents (CE) per 100 g of FW on the basis of a standard curve using catechin as standard.

Total anthocyanin content of the hydroalcoholic extracts was determined using the method of Fuleki and Francis (*18*) adapted to peach tissue. Aliquots of the clear methanol extract were used for spectrophotometric readings at 535 nm by subtracting the absorbance at 700 nm (due to turbidity). The spectrophotometer was zeroed with the anthocyanins extraction solvent as the blank. Anthocyanins were quantified as milligrams of cyanidin-3-glucoside per kilogram of FW using a molar extinction coefficient of 25965 $cm^{-1}\,M^{-1}$ and a molecular weight of 494.

Vitamin C was determined using the method for the spectrophotometric determination of ascorbic acid (vitamin C) as described by Zaharieva and Abadía (*19*). Samples were homogenized with 5% metaphosphoric acid at 4 °C. Then, they were centrifuged at 20000*g* for 15 min at 4 °C, and the supernatant was immediately used for vitamin C analysis. Absorbance was measured at 525 nm using a spectrophotometer (Beckman Coulter DU 800). The standard calibration curve was daily prepared using ascorbic acid as standard. Vitamin C was expressed as milligrams of ascorbic acid (AsA) per 100 g of FW.

Table 2. Basic Statistics Based on Single Plant Observations for the Seedlings from 15 F1 Peach and Nectarine Progenies Studied over 3 Years, for Total Phenolics, Flavonoids, Anthocyanins, Vitamin C, and Antioxidant Capacity (RAC)[a]

trait	*N*	min	max	mean	MSE	SD
total phenolics (mg of GAE/100 g of FW)	218	12.7	71.3	36.4	1.0	15.2
flavonoids (mg of CE/100 g of FW)	218	1.8	30.9	8.8	0.4	6.0
anthocyanins (mg of C3GE/kg of FW)	218	0.1	26.7	3.0	0.3	4.0
vitamin C (mg of AsA/100 g of FW)	218	1.2	9.1	3.7	0.1	1.5
RAC (μg of Trolox/g of FW)	218	227.3	629.9	405.0	4.9	73.0

[a] For each trait, number of observed seedlings (*N*), minimum, maximum, mean value, mean standard error (MSE), and standard deviation (SD) are presented. Abbreviations: GAE, gallic acid equivalents; CE, catechin equivalents; C3GE, cyanidin-3-glucoside equivalents; AsA, ascorbic acid; RAC, relative antioxidant capacity.

The antioxidant capacity was measured using the DPPH method adapted from Brand-Williams et al. (*20*). Briefly, 100 μL of the methanolic extract was added to 2.9 mL of fresh DPPH radical solution (98.9 μM in methanol) and mixed in the dark by vortex at room temperature. The absorbance of the samples was measured at 515 nm after 10 min. These readings were used for calculation of the relative antiradical capacity (RAC), which indicates the antiradical capacity of the sample compared to Trolox for a specific reaction time (10 min). For each sample, three separate determinations were carried out. The standard calibration curves were prepared daily using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid). Results were expressed in micrograms of Trolox per gram of FW.

Statistical Analyses. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL). To obtain basic statistics for the entire plant material studied, maximum and minimum values, mean, mean standard error (MSE), and standard deviation (SD) were calculated for each trait. Data for each genotype in the three years of study were averaged, and mean values were used as estimated genotypic values. The significance of cross, year, and cross × year interaction effects on phytochemical profile was tested on the 218 genotypes by analysis of variance (ANOVA). Duncan's multiple-range test ($P \leq 0.05$) was used to estimate progeny means and to find differences in phytochemical profile among crosses. A *t* test ($P \leq 0.05$) was run to compare different fruit types. Finally, correlations were calculated with raw data of the three years, according to Pearson's test at $P \leq 0.01$.

RESULTS AND DISCUSSION

Table 2 shows the ranges of bioactive compounds and total antioxidant activity in peaches and nectarines. Total phenolics, as determined by the Folin−Ciocalteu assay, varied among genotypes with values in the range of 12.7−71.3 mg of GAE per 100 g of FW. Values are within the range reported for peach flesh in the literature, namely, 14−77 mg of GAE per 100 g of FW (*9*, *14*, *21*). Total flavonoids content ranged from 1.8 to 30.9 mg of CE per 100 g of FW, with an average of 8.8 mg of CE per 100 g of FW. Total anthocyanins greatly varied among genotypes [0.1−26.7 mg of cyanidin-3-glucoside equivalents (C3GE) per kg of FW] depending on the red pigmentation of the flesh. Genotypes with red flesh had higher anthocyanins content. Values of flavonoids and anthocyanins in this range have been reported by other authors (*6*, *9*). Higher values of anthocyanins content in peaches and nectarines are found in the literature when skin is included in the sample (*9*) due to unequal distribution of phenolic compounds in the flesh (~30%) and the skin (~70%) of the peach fruit (*11*, *22*). On average, unpeeled fruit contained 1.5-fold higher levels of phenolics than peeled fruit (*22*). However, as already mentioned above, peach skin is not usually appreciated by consumers and, therefore it takes no part in the human diet. The total ascorbic acid (vitamin C) content greatly varied from approximately 1 to 9 mg of AsA/100 g of FW, with a mean value of 3.7 mg of AsA/100 g of FW. Genetic background of the genotype is a much more important factor than climatic conditions and cultural practices in producing fruit with high vitamin C content at harvest (*15*). Values were in the same range as previously reported for vitamin C contents in peach flesh, namely, 1−14 mg of AsA/100 g of FW (*6*, *21*, *23*) and lower than values obtained when peach peel was included in the test (*6*). As for phenolic compounds, skin tissues have more vitamin C to protect the fruit from outside stress caused by light and oxidation (*17*). The relative antioxidant capacity (RAC) varied among genotypes, with values ranging from 227.3 to 629.9 μg of Trolox/g of FW, with an average of 405 μg of Trolox/g of FW. In recent years, strong attention has been given to this trait as an eligible parameter for fruit quality because many of the biological actions of phytochemicals have been attributed to it. As for anthocyanins, the antioxidant capacity observed in our study was in the range previously reported for peach flesh (100−1000 μg of Trolox/g of FW), but lower than in other studies in which peel was included in the test sample (700−6000 μg of Trolox/g of FW) (*6*, *11*). Therefore, the antioxidant capacity of the fruits decreases when they are peeled.

Considerable variation was found in the content of antioxidant compounds in the fruits from different progenies (**Figure 1**). The highest total phenolic contents were shown by the three progenies descendant from O'Henry cultivar, although no significant differences were found with mean values of Andross × Calante, Andross × Crown Princess, Babygold-9 × Crown Princess, and Orion × VAC-9510 progenies. The level of total phenolics in O'Henry × VAC-9514 was >2-fold higher than in Rich Lady × VAC-9511, showing the wide variance of total phenolic concentrations in *Prunus persica* already reported in other studies (*6*, *9*, *14*, *21*). Similarly, a high variability in the flavonoids content averaged for the 15 progenies was found in agreement with the variability among *P. persica* cultivars reported by other authors (*9*). A nearly 5-fold difference was measured between the lowest and highest mean values among different progenies. The highest flavonoids content was also shown by O'Henry × VAC-9514 progeny, although no significant difference was found with O'Henry × VAC-9515. The highest values were found in the three O'Henry progenies, resulting in the most interesting crosses from which to select peaches and nectarines with higher flavonoid content in the flesh. The O'Henry progenies also showed the highest anthocyanins content, although no significant differences were found with Andross × VAC-9511, Babygold-9 × VAC-9510, Orion × VAC-9510, VAC-9512 × VAC-9511, and VAC-9520 × VAC-9517 progenies. Tomás-Barberán et al. (*9*) reported higher anthocyanin content in the flesh of O'Henry fruits (8.1 mg/kg of FW) when compared with other commercial peach cultivars such as September Sun (3.7 mg/kg of FW), Rich Lady, and Spring Lady (no significant amounts detected in either). In agreement with all of these results, we could report an influence of O'Henry cultivar to induce higher anthocyanins content in its progeny as observed for flavonoids content. On the other hand, the highest vitamin C content was found in the VAC-9520 × VAC-9517 progeny, although differences were not significant with Andross × VAC-9511 and VAC-9512 × VAC-9511 progenies. The lowest mean value was shown by Venus × Big Top nectarines progeny without being significantly different from Andross × Crown Princess, both Babygold-9 progenies, O'Henry × VAC-9515, and Orion × VAC-9510 progenies. A significant effect of cultivar and rootstock on the vitamin C content has been previously reported in different fruits (*15*). Tavarini et al. (*21*) found a range from 1 to 14 mg of AsA/100 g of FW in seven peach cultivars, and Nelson et al. (*24*) reported values from 19.3 to 71.5 mg of AsA/100 g of FW in six strawberry cultivars. Significant differences among progenies were also found for RAC (**Figure 1**), according

to previous results that have shown that antioxidant capacity changes as a function of cultivar and rootstock (*6*, *14*, *21*). As above-mentioned for other bioactive compounds, the highest RAC values were shown by O'Henry descendants, without being significantly different from the Andross × Calante progeny. These results indicate the importance of cultivar and genotype for determining the antioxidant potential and phenolic content of the fruit. The most appropriate combination of phytochemical traits must be considered for the selection of new genotypes with higher nutritional value.

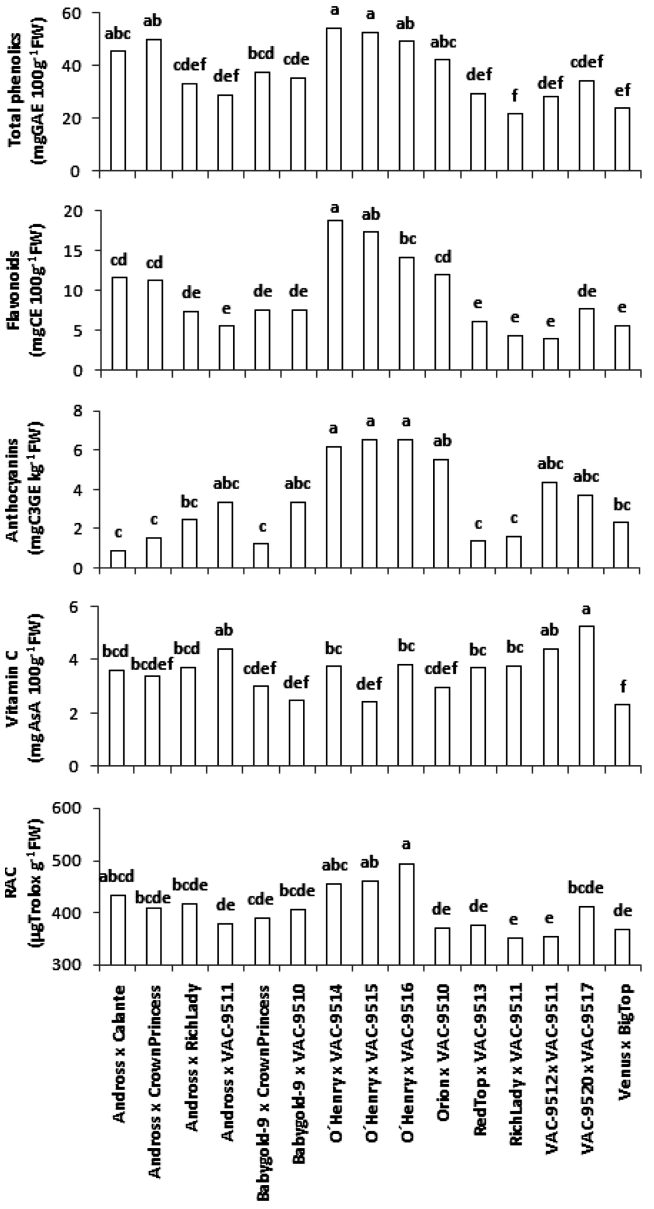

Figure 1. Phytochemical profiles of the 15 peach and nectarine progenies. Data are the means of all of the genotypes in each progeny. For each trait, means with the same letter are not significantly different according to Duncan's test ($P \leq 0.05$). Abbreviations: GAE, gallic acid equivalents; CE, catechin equivalents; C3GE, cyanidin-3-glucoside equivalents; AsA, ascorbic acid; RAC, relative antioxidant capacity.

Bioactive compounds and antioxidant content of fruit varied depending peach/nectarine and yellow/white flesh color qualitative traits (**Table 3**). In this work, peaches showed higher phenolic content than nectarines, and phenolic content of white-fleshed fruit was higher than that of yellow-fleshed fruit. This shows a tendency of white-fleshed peaches to have significantly higher antioxidants content than the other genotypes tested (yellow-fleshed peaches and nectarines). No significant differences in flavonoids and anthocyanins content were found between peaches and nectarines; however, they were significantly higher in white-fleshed fruits than in yellow-fleshed fruits as previously found (*9*). This could be explained by the red pigmentation due to anthocyanins in the white-fleshed fruit, especially in the flesh area surrounding the stone, usually found in our studied progenies. This result is different from what occurs in the peel, where yellow-fleshed fruits are reported to produce more anthocyanin pigments than white-fleshed fruits (*9*). No significant differences were found for vitamin C between peach and nectarine fruits, whereas it was higher in white-fleshed fruit than in yellow-fleshed fruit. Consequently, with all of these results, white-fleshed fruits showed higher antioxidant capacity than the yellow-fleshed ones, as reported in previous works (*6*). In agreement with these results, significant slight positive correlations ($P \leq 0.01$) were found for color flesh fruit versus phenolic compounds, flavonoids, anthocyanins, vitamin C, and RAC ($r = 0.265$, $r = 0.283$, $r = 0.189$, $r = 0.339$, and $r = 0.243$, respectively), indicating different contents of these bioactive compounds in white- and yellow-fleshed fruits, as commented above.

In agreement with these results, the percentage of phenotypic variance explained by cross (**Table 4**) was high for each bioactive compound analyzed (between 15.7 and 44.6%). Contribution of cross to anthocyanins and antioxidant capacity was lower than to total phenolics, flavonoids, and vitamin C phenotypic variance. These results indicate that cultivar and genotype are decisive in determining the peach fruit antioxidant capacity. On the other hand, no significant differences were found among the three years of study for total phenolics, flavonoids, anthocyanins and antioxidant capacity (**Table 4**). Despite this result, slight higher flavonoids and anthocyanins contents were observed in the first year of study when compared with the two following years (data not shown), which may be due to differences of climate including temperature, sun irradiation, and/or water stress as mentioned by Tomas-Barberán and Espín (*10*). Sun irradiation has been demonstrated to increase anthocyanin content of different fruits such as apples and pears, whereas in cherry, grape, and plum,

Table 3. Total Phenolics, Flavonoids, Anthocyanins, Vitamin C, and Relative Antioxidant Capacity (RAC) in Different *P. persica* Fruit Types[a]

fruit type	*N*	total phenolics (mg of GAE/100 g of FW)	flavonoids (mg of CE/100 g of FW)	anthocyanins (mg of C3GE/kg of FW)	vitamin C (mg of AsA/ 100 g of FW)	RAC (μg of Trolox/g of FW)
peach	192	37.2 a	9.1 a	3.1 a	3.7 a	406.2 a
nectarine	26	30.5 b	6.9 a	2.2 a	3.9 a	395.7 a
yellow fleshed	176	34.5 b	8.0 b	2.6 b	3.5 b	396.5 b
white fleshed	42	44.8 a	12.3 a	4.5 a	4.8 a	442.2 a

[a] For each fruit type, number of observed seedlings (*N*) is presented. Data are means over the three years of study. In each trait column (peach, nectarine, yellow fleshed, white fleshed), means with the same letter are not significantly different according to *t* test ($P \leq 0.05$). Abbreviations: GAE, gallic acid equivalents; CE, catechin equivalents; C3GE, cyanidin-3-glucoside equivalents; AsA, ascorbic acid.

light seems not to be essential for red color formation (*10*). Temperature, and in particular the difference between day and night temperatures, has been reported to have a marked effect on anthocyanin accumulation in apples, plums, grapes, and pomegranates (*10*).

Correlations among Phytochemical Constituents and Other Fruit Quality Traits. A high positive correlation was found between total phenolics and flavonoids content ($r = 0.742$, $P \leq 0.01$), implying that flavonoids are an important group of phenolic compounds in peaches and nectarines (**Table 5**). Moreover, a linear positive relationship (**Figure 2**) was observed between antioxidant capacity and total phenolics for the flesh of the peach and nectarine genotypes, as has been observed for peaches, apricots, and plums (*5*, *6*). However, higher correlation coefficients ($r > 0.9$) were obtained by Gil et al. (*6*) for other peach and nectarine cultivars. This variation could be due to differences in the phytochemical profile of different peach and nectarine cultivars. In addition, the large phenotypic variability within the breeding progenies in our study could induce lower correlation coefficients between those parameters. Total phenolics and flavonoids were the only constituents that correlated significantly ($P \leq 0.01$) with antioxidant capacity ($r = 0.606$ and $r = 0.553$, respectively), indicating that they are important bioactive compounds contributing to the antioxidant capacity of peaches and nectarines, in accordance with previous studies on different peach, nectarine, and plum cultivars (*6*, *11*). Indeed, correlation coefficients varied depending on the progenies. Higher correlation coefficients ($P \leq 0.01$) were found between total phenolics and RAC in some progenies, such as Rich Lady × VAC-9511 ($r = 0.835$) and Orion × VAC-9510 ($r = 0.925$) progenies, whereas no significant correlation was found in others (Venus × Big Top, Babygold-9 × VAC-9510, Andross × VAC-9511, O′Henry × VAC-9516, and O′Henry × VAC-9514). Previous works (*21*, *25*) have also shown these differences among peach progenies. Indeed, it is well-known that it is not only the total content of phenols but also their specific structural features, such as the number of available hydroxyl groups, that determine their antioxidant capacity (*3*). Proteggente et al. (*23*) reported that highest antioxidant capacity is found in fruits such as strawberry, raspberry, and plum due to their high content of anthocyanins. However, no significant correlation was obtained between anthocyanins and RAC in our study (**Table 5**). This fact is probably due to the lower content of anthocyanins in peaches and nectarines compared with contents in strawberries, raspberries, and plums. Vitamin C did not show significant correlation with RAC. All of these results suggest that phenolic compounds are mainly responsible for the antioxidant activity of peaches and nectarines, as previously described for stone fruits (*6*, *11*), whereas vitamin C is reported as the main antioxidant compound in oranges, strawberries, raspberries, and blueberries (*26*). The

Table 4. Factors Affecting Phytochemical Profile in 15 Peach and Nectarine Progenies Studied over 3 Years[a]

variable	*F* value	*P*	phenotypic variance (%)
total phenolics			
cross	16.79	0.000	33.6
year	1.22	0.295	0.5
cross × year	0.27	1.000	1.5
flavonoids			
cross	26.69	0.000	44.6
year	0.15	0.865	0.1
cross × year	0.42	0.996	2.4
anthocyanins			
cross	6.21	0.000	15.7
year	1.09	0.336	0.5
cross × year	0.36	0.999	2.0
vitamin C			
cross	8.02	0.000	35.8
year	—	—	—
cross × year	—	—	—
RAC			
cross	8.08	0.000	19.6
year	0.98	0.375	0.4
cross × year	0.33	1.000	1.9

[a] *F* values and proportion (%) of phenotypic variance are indicated as determined by ANOVA. (—) no data available. Abbreviations: RAC, relative antioxidant capacity.

Table 5. Pearson's Correlation Coefficients between Phytochemical Traits Observed over 3 Years in 15 Peach and Nectarine Progenies[a]

trait	flavonoids	anthocyanins	vitamin C	RAC
total phenolics	0.742**	0.144*	ns	0.606**
flavonoids		ns	ns	0.553**
anthocyanins			ns	ns
vitamin C				ns

[a] *, $P \leq 0.05$; **, $P \leq 0.01$; ns, not significant. Abbreviations: RAC, relative antioxidant capacity.

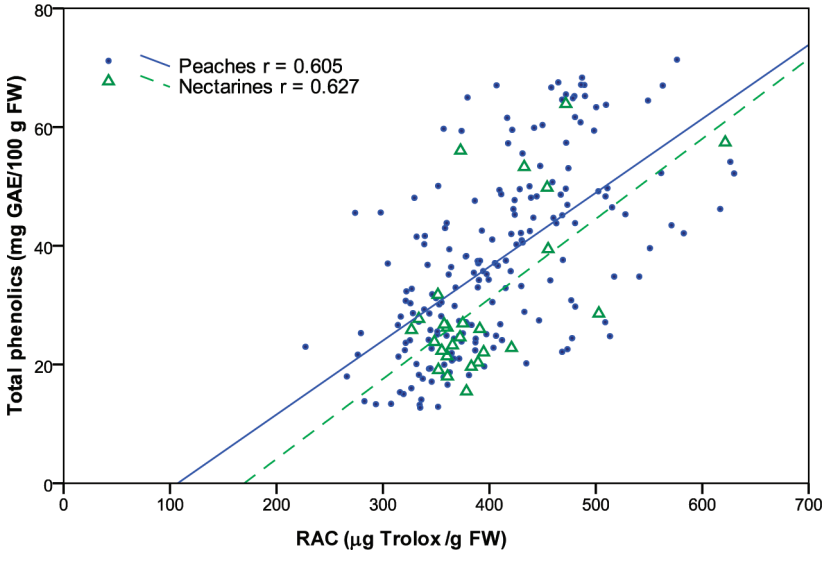

Figure 2. Linear regression ($P \leq 0.01$) between relative antioxidant capacity (RAC) and total phenolics (GAE, gallic acid equivalents) in the peach and nectarine genotypes. Each value is the mean over the three years of study for each genotype.

Table 6. Pearson's Correlation Coefficients for Phytochemical and Quality Traits Observed over 3 Years in 15 Peach and Nectarine Progenies[a]

trait	fruit weight	endocarp staining	SSC	TA
total phenolics	0.298**	0.249**	0.237**	ns
flavonoids	0.345**	0.376**	0.371**	ns
anthocyanins	0.166*	0.218**	ns	ns
vitamin C	−0.450**	ns	0.154*	0.308**
RAC	ns	0.175**	0.268**	ns

[a] *, $P \leq 0.05$; **, $P \leq 0.01$; ns, not significant. Abbreviations: SSC, soluble solid content; TA, titratable acidity; RAC, relative antioxidant capacity.

demonstrated beneficial effects of antioxidant compounds on health make the antioxidant capacity of fruits an important trait to be considered in breeding programs. However, due to the lack of correlation between RAC and other important bioactive compounds such as anthocyanins and vitamin C, we suggest considering and including these traits in a breeding program for the selection of higher fruit quality genotypes.

Both total phenolics and flavonoids contents showed a slight significant positive correlation with fruit weight and sugar content (**Table 6**), showing a tendency of bigger and sweeter fruits to have higher levels of these bioactive compounds. This is consistent with the findings reported for most species such as plums (*27*), apricots (*28*), sweet cherries (*29*), and apples (*30*). 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 (*31*). On the other hand, a significant positive correlation was found for total phenolics, flavonoids, and anthocyanins versus endocarp staining (redness around stone), supporting the recognized role of anthocyanin pigments in this quality trait. Higher correlation coefficients between total phenolics and endocarp staining were found in some progenies such as Andross × Calante ($r = 0.522$), Andross × Rich Lady ($r = 0.730$), and O'Henry × VAC-9515 ($r = 0.691$), whereas no correlation was found for others, suggesting that relationships between traits depend on the progeny or cultivar evaluated. A positive correlation between endocarp staining and RAC was also found as a consequence of flavonoid pigment contribution to the antioxidant capacity of fruit (*3*). This result indicates that higher endocarp-stained fruits have higher antioxidant capacity and, consequently, higher health benefits according to previous papers (*9*). Finally, the positive correlation between vitamin C and TA is due to the contribution of ascorbic acid to the fruit acidity.

These results confirm the importance of genotype on the availability of bioactive compounds and antioxidant capacity of peach and nectarine fruits and, consequently, on their benefits to health. Therefore, the peach cultivars used as progenitors in the crosses of a breeding program have a vital importance to release new cultivars with high bioactive compounds content. On the other hand, the high number of evaluated genotypes, from different genetic origins and with a large phenotypic variability, constitutes a considerable contribution on peach species and especially on breeding purposes.

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