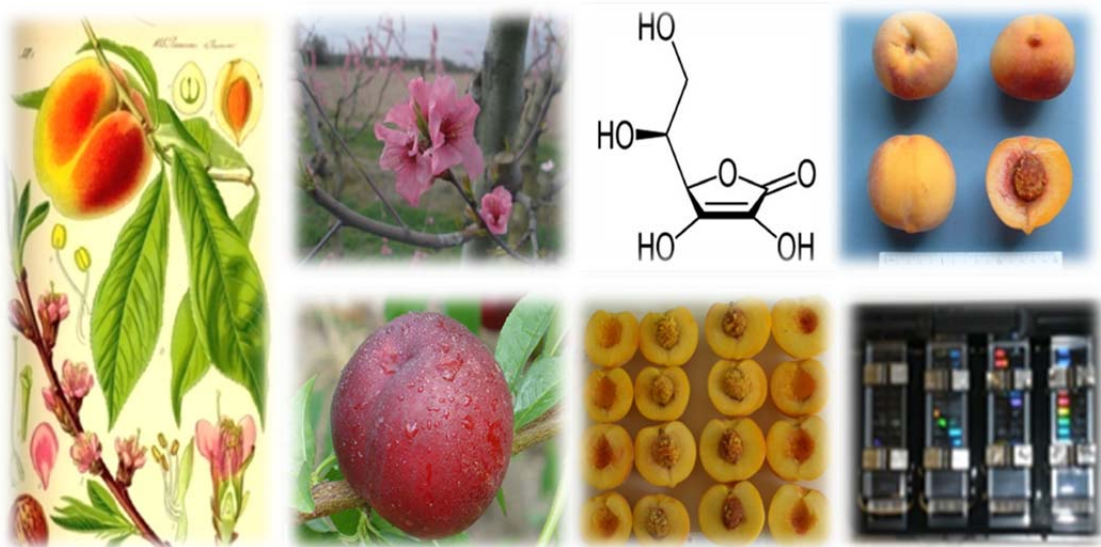Universidad
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Evaluation of agronomical and biochemical traits and mapping QTLs controlling fruit quality traits in peach [*Prunus persica* (L.) Batsch] progenies

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Tesis Doctoral:

Evaluation of agronomical and biochemical traits and mapping QTLs controlling fruit quality traits in peach [*Prunus persica* (L.) Batsch] progenies.

Memoria presentada por D. Walid ABIDI, Ingeniero Agrónomo, para optar al grado de Doctor por la Universidad de Zaragoza.

Zaragoza, Junio 2012

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CERTIFICAN

Que la Tesis Doctoral titulada "**Evaluation of agronomical and biochemical traits and mapping QTLs controlling fruit quality traits in peach [*Prunus persica* (L.) Batsch] progenies**", ha sido realizada por el Ingeniero Agrónomo Walid ABIDI, en el Departamento de Pomología de la Estación Experimental de Aula Dei (EEAD) del Consejo Superior de Investigaciones Científicas (CSIC) bajo su dirección durante los años 2008, 2009, 2010, 2011; y reúne, a su juicio, las condiciones requeridas para optar al Grado de Doctor por la Universidad de Zaragoza.

Y para que conste a los efectos oportunos firman el presente informe en Zaragoza, a 4 de Junio de 2012.

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Zaragoza, Junio de 2012

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Fdo. Mª Yolanda Gogorcena Aoiz   **Fdo. Mª Ángeles Moreno Sánchez**

Agradecimientos

A mis padres y
a mis hermanos

Índice General

Abreviaturas

AFLP= Amplified Fragment Length Polimorphism
ANOVA= Análisis de Varianza
AsA= Ácido Ascórbico
BT= 'Big Top'
C3G eq= Cyanidin-3-Glucoside Equivalents
CE= Catechin Equivalents
CG= Genes Candidatos
CI= Chilling Injury
cM= centiMorgan
CMF= Clingstone Melting Flesh
CNMF= Clingstone Non Melting Flesh
DHA= Dehydroxyascorbic Acid
DHAA= Ácido Dehidroascórbico
dNTP= Desoxiribonucleótidos
DPPH= 2, 2-dipyridyl-1, 1-diphenyl-2-picrylhydrazyl
EDTA= Ácido Etilendiaminotetraacético
EST= Expressed Sequence Tag
FMF= Freestone Melting Flesh
FW= Fresh Weight
GAE= Gallic Acid Equivalents
GC= Cromatografía de Gases
GC-MS= Gas Chromatography-Mass Spectrometry
ha= hectárea
HPLC= Cromatografía Líquida de Alta Resolución
HS-SPME= Head Space Solid Phase Micro-Extraction
HS-SPME-GC-MS= Cromatografía de Gases acoplada a la Espectrometría de Masas
LD=Desequilibrio de Ligamiento
LG= Linkage Group
LOD= Logarithm of the Odds
MAS= Selección Asistida por Marcadores
MSE= Error Estándar de la Media
N= Newton
NIRS= Near Infrared Reflectance Spectroscopy
NS= No Significativo
PCA= Análisis de Componentes Principales
PCR= Reacción en Cadena de la Polimerasa
QTLs= Quantitative Trait Loci
RAC= Relative Antioxidant Capacity
RAPD= Randomly Amplified Polymorphic DNA
RFL= Restriction Fragment Length Polymorphism
RH= Humedad Relativa
RI= Ripening Index (SSC/TA)
ROS= Especies Reactivas de Oxígeno
SD= Desviación Estándar
SRAP= Sequence-Related Amplified Polymorhism
SSC= Soluble Solids Content (ºBrix)
SSR= Simple Sequence Repeats
TA= Titratable Acidity
V= 'Venus'
VOCs= Volatile Compounds Content

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Resumen

La calidad del fruto es un criterio de selección muy amplio en las *rosáceas*, y los caracteres agronómicos y bioquímicos son componentes importantes de la calidad nutricional del fruto. Los polifenoles, entre otros compuestos bioactivos, son micronutrientes abundantes en nuestra dieta y fundamentales en la prevención de enfermedades degenerativas y cardiovasculares. En el melocotón, los compuestos fenólicos son una fuente importante de antioxidantes con influencia en la calidad nutricional de la fruta.

En este estudio, se analizaron caracteres agronómicos y bioquímicos en una población F1 de nectarina (75 genotipos) derivada del cruzamiento 'Venus' × 'Big Top' y en una población F1 de melocotonero (130 genotipos) derivada del cruzamiento 'Babygold 9' x 'VAC-9510'. Los resultados obtenidos mostraron una alta variabilidad entre genotipos para todos los caracteres analizados. Los valores promedio para los diferentes parámetros de calidad evaluados estaban dentro del rango obtenido en los dos parentales y algunos de ellos mostraron una variación continua típica de caracteres cuantitativos.

Por otra parte, se observaron correlaciones positivas entre los sólidos solubles totales (SSC) y la firmeza del fruto, de gran importancia desde el punto de vista comercial para la selección de genotipos con alto contenido en SSC y menos susceptibles a los daños mecánicos. Los fenoles totales y los flavonoides mostraron también correlaciones positivas con la capacidad antioxidante (RAC), demostrando que estos compuestos bioactivos tienen un papel predominante en la RAC de la fruta. El estudio permitió destacar ocho genotipos en la población 'Venus' x 'Big Top' y otros seis genotipos en la población 'Babygold 9' x 'VAC-9510' con mayor contenido en fenoles totales, flavonoides y capacidad antioxidante.

Los compuestos volátiles (COVs) como responsables del aroma desempeñan un papel significativo en la percepción del aroma de la fruta e influyen en la calidad del melocotón. En la población 'Venus' x 'Big Top' se han estudiado las características de los COVs utilizando el análisis de la Cromatografía de Gases acoplada a la Espectrometría de Masas (HS-SPME-GC-MS). Se identificaron setenta y siete compuestos, incluyendo ácidos, aldehídos, alcoholes, compuestos C6, esteres, cetonas, lactonas y terpenos. La composición en volátiles totales fue relativamente consistente en las muestras analizadas, pero el contenido relativo de los mismos varió significativamente entre genotipos. El análisis de componentes principales de los principales volátiles identificó genotipos que destacaron por su alto contenido en los compuestos más representativos del aroma del melocotón (lactonas, esteres y terpenos). La metodología utilizada confirmó la presencia de dos volátiles (4-Methyl-5-penta-1,3-dienyltetrahydrofuran-2-one y 2,4 heptadienal) descritos recientemente en melocotón.

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Uno de los problemas más importantes en el manejo postcosecha en melocotonero, son los daños por frío (DF) porque afectan negativamente a su comercialización. Para identificar genotipos con baja susceptibilidad a los DF, se ha evaluado la población 'Babygold 9' x 'VAC-9510' durante dos años consecutivos. Se determinaron la harinosidad, granulosidad, pardeamiento, pigmentación roja de la pulpa y falta de sabor. Esta población presentó variabilidad para todos los síntomas evaluados, aumentando la severidad de los mismos con la mayor duración del almacenaje. Después de dos semanas en cámara, los principales síntomas observados fueron el enrojecimiento y la harinosidad, pero con menor severidad que en otras progenies de melocotonero. Tras cuatro semanas de almacenaje, el principal síntoma de DF fue el pardeamiento. Las correlaciones observadas entre los compuestos antioxidantes y los síntomas de DF indican la importancia y la influencia de los mismos en el mayor desarrollo de los daños. Estos efectos deben ser tenidos en consideración en los programas de selección de variedades, ya que además de buscar un mayor contenido en compuestos bioactivos, las nuevas variedades deben mostrar baja susceptibilidad a los daños por frío.

Finalmente, con el fin de conocer el control genético de caracteres que regulan el contenido en compuestos bioquímicos y por tanto implicados en la calidad organoléptica del fruto en melocotonero, se realizó un mapa genético con marcadores SNPs y SSRs en la población 'Venus' x 'Big Top'. El análisis de QTLs identificó en el grupo de ligamiento 4 (GL4) regiones significativas para los parámetros de sólidos solubles totales, vitamina C, fenoles totales, flavonoides y capacidad antioxidante del fruto. Los QTLs encontrados, excepto para vitamina C, fueron consistentes al menos en dos de los cuatro años de estudio, por lo que se deduce que el control genético del contenido en antioxidantes es relativamente independiente del medio. Estos resultados tienen gran interés desde el punto de vista práctico porque serán la base para aplicar la selección asistida por marcadores en la mejora de la calidad del fruto en poblaciones de melocotonero.

Abstract

Fruit quality is a very broad selection criterion in the *Rosaceae* family, and agronomic and biochemical traits are important components of the nutritional quality. Polyphenols, among other bioactive compounds, are abundant micronutrients in our diet and fundamental traits in the prevention of degenerative and cardiovascular diseases. In peach, phenolic compounds are a major source of antioxidants to influence the nutritional quality of the fruit.

In this study, biochemical and agronomic traits were analyzed in a F1 population of nectarine (75 genotypes) derived from the cross 'Venus' x 'Big Top' and a peach-F1 population (130 genotypes) derived from the cross 'Babygold 9' x 'VAC-9510'. The results showed a high variability among genotypes for all traits analyzed. The average values for the different quality parameters were within the range observed in the two parents and some of them showed a continuous variation typical of quantitative traits.

Furthermore, positive correlations were observed between total soluble solids (SSC) and firmness of the fruit, of great importance from the commercial point of view for the selection of genotypes with high content of SSC and less susceptible to mechanical damage. Total phenols and flavonoids also showed positive correlations with antioxidant capacity (RAC), demonstrating that these bioactive compounds have a predominant role in the RAC of the fruit. The study allowed to highlight eight genotypes in the population 'Venus' x 'Big Top' and six genotypes in the population 'Babygold 9' x 'VAC-9510' with higher content of total phenolics, flavonoids and RAC.

Volatile compounds (VOCs) as responsible for the aroma play a significant role in the perception of fruit flavor and influence the quality of the peach. In the population 'Venus' x 'Big Top', the characteristics of VOCs analysis were studied using gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS). Seventy seven compounds were identified, including acids, aldehydes, alcohols, C6 compounds, esters, ketones, lactones and terpenes. The total volatile composition was relatively consistent in the samples analyzed, but the relative amount varied significantly among genotypes. The principal component analysis (PCA) of the major VOCs identified genotypes with high content of volatiles typical of the aroma of peach (lactones, esters and terpenes). The methodology confirmed the presence of two volatiles (4-Methyl-5-penta-1, 3-dienyltetrahydrofuran-2-one and 2.4 heptadienal) recently described in peach.

In the peach industry, one of the most important problems in the postharvest handling is chilling injury (CI) because it adversely affects the market. To identify genotypes with low susceptibility to CI, the population 'Babygold 9' x 'VAC-9510' was evaluated for two

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consecutive years. Major symptoms of CI were determined, such as mealiness, graininess, browning, red pigmentation (bleeding) and loss of flavor. This population showed variability for all symptoms evaluated, increasing the severity of them with a longer duration of storage (2 or 4 weeks at 5 ºC). After 2 weeks of storage, the main symptoms observed were bleeding and mealiness, but less severely expressed than on other peach progenies. After 4 weeks of storage, the main symptom of CI was flesh browning. The correlations observed between the antioxidant compounds and symptoms of CI indicate the importance and influence of these bioactive compounds in the further development of the damage. These effects must be taken into consideration in breeding programs to select new varieties with high content of bioactive compounds and low susceptibility to chilling injury.

Finally, in order to understand the genetic control of characters covering the content in biochemical compounds and, therefore, involved in the organoleptic quality of fruit in peach, a genetic map with SSR and SNPs markers was carried out in the population 'Venus' x 'Big Top'. Analysis of QTLs identified significant regions for SSC, vitamin C, total phenolics and flavonoids contents, and RAC on linkage group 4 (GL4). The QTLs found, except for vitamin C, were consistent in at least two of the four years of study, demonstrating that the genetic control of antioxidant content is relatively independent of the growing conditions. These results are of great interest from the practical point of view since it will be the basis for implementing marker-assisted selection for fruit quality in peach breeding programs.

Résumé

La qualité des fruits est un critère de sélection très large chez les *Rosacées* fruitiers, et les caractères agronomiques et biochimiques sont des composantes importantes de la qualité nutritionnelle des fruits. Les polyphénols, parmi d'autres composés bioactifs, sont des micronutriments abondants dans notre alimentation et sont fondamental dans la prévention des maladies dégénératives et cardio-vasculaires. Dans la pêche, les composés phénoliques sont une source majeure des antioxydants avec influence sur la qualité nutritionnelle des fruits.

Dans cette étude, nous avons analysé des caractères biochimiques et agronomiques dans une population F1 (75 génotypes) provenant d'un croisement entre deux variétés de nectarine 'Venus' x 'Big Top' et une population F1 de pêcher (130 génotypes) dérivé d'un croisement entre les deux variétés 'Babygold 9' x 'VAC-9510'. Les résultats ont montré une grande variabilité entre les génotypes pour tous les caractères analysés. Les valeurs moyennes pour les différents paramètres de qualité étaient dans l'intervalle observé chez les deux parents et certains d'entre eux ont montré une variation continue typique de caractères quantitatifs.

Par ailleurs, des corrélations positives ont été observées entre les solides solubles totaux (SSC) et la fermeté du fruit, qui sont d'une grande importance du point de vue commercial pour la sélection de génotypes avec haute teneur en SSC et moins sensibles aux dégâts mécaniques. Les phénols totaux et les flavonoïdes ont également montré des corrélations positives avec la capacité antioxydante (RAC), démontrant que ces composés bioactifs ont un rôle important dans la RAC du fruit. L'étude a permis de preseleccionner huit génotypes dans la population 'Venus' x 'Big Top' et autres six génotypes dans la population 'Babygold 9' x 'VAC-9510' avec une teneur plus élevée en phénols totaux, flavonoïdes et RAC.

Les composés volatils (COVs) comme responsables de l'arôme jouent un rôle important dans la perception de la saveur des fruits et influencent la qualité de la pêche. Dans la population 'Vénus' x 'Big Top' on a étudié les volatiles en combinant la microextraction en phase solide avec la chromatographie en phase gazeuse couplée à la spectrométrie de masse (HS-SPME-GC-MS). Soixante-dix-sept composés ont été identifiés, y compris des acides, des aldéhydes, des alcools, des composés C6, des esters, des cétones, des lactones et des terpènes. La composition en volatiles était relativement constante dans les échantillons analysés, mais les concentrations ont varié entre les génotypes. L'analyse des composantes principales (PCA) des principaux volatiles identifiés ont montré des génotypes avec teneur élevée en composés

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représentatifs de l'arôme de pêche (lactones, esters et terpènes). La méthodologie a confirmé la présence de deux volatiles (4-méthyl-5-penta-1 ,3-dienyltetrahydrofuran-2-one et de 2,4 heptadienal) qui ont été décrit récemment dans le pêcher.

Les problèmes à considérer dans le stockage des pêchers sont les dégâts de froid (DF), car ils affectent la commercialisation du produit. Pour identifier des génotypes avec peu sensibles aux DF, la population 'Babygold 9'x 'VAC-9510' a été évaluée durant deux années consécutives. La population a montré une variabilité pour tous les symptômes évalués et la durée de stockage a augmenté la sévérité de ces symptômes. Après 2 semaines de stockage au froid, les principaux symptômes observés étaient la pigmentation rouge de la chair et l'aspect farineux, mais moins sévère que d'autres descendances de pêchers. Après 4 semaines de stockage, le principal symptôme de la DF était le brunissement de la chair. Les corrélations observées entre les composés antioxydants et les symptômes de DF indiquent l'importance et l'influence des composés phénoliques dans le développement ultérieur de ces dégâts. Ces résultats doivent être pris en considération dans les programmes de sélection dont le but d'avoir des fruits avec meilleur contenu en composés bioactives et peu susceptibles aux dégâts du froid.

Finalement, afin d'étudier le contrôle génétique des composés biochimiques des fruits chez le pêcher, une carte génétique avec des marqueurs SSRs et SNPs a été réalisé dans la population 'Venus' x 'Big Top'. L'analyse des QTLs a identifié des régions importantes dans le groupe de liaison 4 (GL4) pour les solides solubles totaux, la vitamine C, les phénols totaux, les flavonoïdes et la capacité antioxydante des fruits. Des QTLs pour ces caractères ont été identifié, exception faite pour la vitamine C, étaient stables au moins durant deux ans d'étude. Ces résultats sont d'un grand intérêt de point de vue pratique, car ils seront la base pour la mise en œuvre de la sélection assistée par marqueurs dans l'amélioration de la qualité des fruits dans les populations de pêchers.

CAPÍTULO 1

Introducción General

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1.1. El melocotonero

1.1.1. Descripción taxonómica y breve historia de la especie

El melocotonero [*Prunus persica* (L.) Batsch] pertenece a la familia *Rosaceae* subfamilia *Prunoidae*, subgénero *Amygdalus* y género *Prunus*. La familia *Rosaceae* es importante por su elevado número de especies (más de 3000 especies en 110 géneros), también por su valor económico y su amplia distribución (Takhtajan, 1997). El género *Prunus* es uno de los más importantes de la familia, por sus más de 200 especies y por la gran importancia económica que tienen algunas de ellas, como el almendro [*Prunus dulcis* (Mill.) D.A. Webb], el melocotonero [*P. persica* (L.) Batsch], el albaricoquero [*P. armeniaca* L.], el ciruelo europeo [*P. domestica*], el ciruelo japonés [*P. salicina*], el cerezo [*P. avium*] y el guindo [*P. cerasus*]. Además de la especie *P. persica* cultivada por su fruta, se conocen 4 especies muy próximas que tienen melocotones de baja calidad, pero son compatibles con *P. persica*, por lo que suelen utilizarse como patrones, con resistencia a hongos e insectos: *Prunus davidiana* (Carr.) Franch., *Prunus mira* Koehne, *Prunus kansuensis* Rehd. y *Prunus ferganensis* (Kost. & Rjab) Kov. & Kost (Bassi y Monet, 2008).

El melocotonero se considera como la especie modelo dentro del género *Prunus* (Abbott et al., 2002). Es una especie diploide (2n=16), con un periodo juvenil corto (2-3 años) y un genoma relativamente pequeño de aproximadamente 230 Mbp con ocho cromosomas haploides (Ahmad et al., 2011), el doble del tamaño del de *Arabidopsis* ($5,9 \times 10^8$ bp o 0,61 pg/ núcleo diploide) (Baird et al., 1994).

El melocotonero es un frutal originario de China donde las referencias de su cultivo se remontan hasta unos 3000 años (Lurie y Crisosto, 2005). Después, los romanos lo llevaron a Persia (actual Irán) a través de las rutas comerciales, llegando a ser conocido allí como fruta pérsica, de ahí su nombre botánico: *Prunus persica*. Hacia el año 330 a.C. se introdujo en Grecia, y durante la Edad Media su cultivo se extendió por toda Europa. En el siglo XVI, los españoles lo llevaron a México y en el siglo XVIII lo introdujeron en California (Lurie y Crisosto, 2005) siendo, en la actualidad, el área más productiva de melocotonero después de China, Italia y España. En el siglo XIX se constata que el cultivo de melocotonero aparece ya como en su expansión definitiva.

El melocotonero es un frutal cultivado en zonas templadas, entre los 30 y 45° de latitud Norte y Sur. Entre las limitaciones climáticas se encuentran especialmente las temperaturas extremas en zonas frías (-35ºC) y las heladas primaverales. También se

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requieren unas exigencias mínimas en frío invernal para la floración y el buen cuajado del fruto. Todas las combinaciones de tipos de fruto en el melocotonero - melocotón o nectarina, de hueso libre o adherido; de carne amarilla o blanca; de acidez baja, media o alta - están disponibles como fruta fresca en el mercado, de abril hasta octubre en el hemisferio Norte, y de noviembre hasta marzo, en el hemisferio Sur (Lurie y Crisosto, 2005).

El fruto es una drupa (pericarpo membranoso, mesocarpo pulposo, endocarpo leñoso), de forma más o menos globosa con un surco longitudinal bien marcado y una cavidad alrededor del pedúnculo. El epicarpo puede ser adherente a la pulpa o fácilmente separable (Figura 1.1).
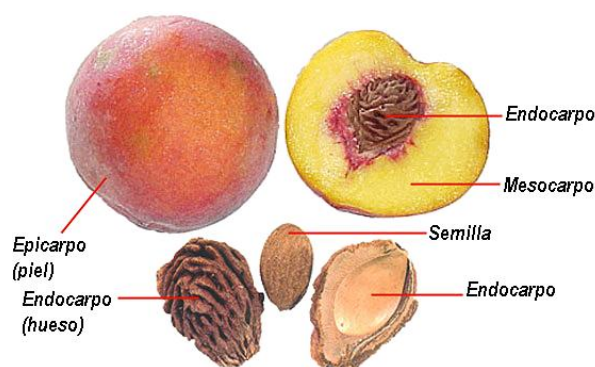
Figura 1.1. Estructura de un fruto en drupa mostrando las distintas partes del mismo. (http://www.euita.upv.es/varios/biologia/web_frutos/Estructura.htm).

1.1.2. Grupos pomológicos

La especie *Prunus persica* se divide habitualmente por los botánicos en diferentes «variedades botánicas» (IOPI, *International Organization for Plant Information*) según sus frutos tengan piel pubescente o lisa (melocotón o nectarina), y forma globular o plana (Figura 1.2). A diferencia del melocotón, las nectarinas no tienen pubescencia en la piel, que es un carácter controlado por un único gen (Lill et al., 1989; Lurie y Crisosto, 2005). Además, las células de la piel en las nectarinas tienen espacios intercelulares más pequeños que en los melocotones y por tanto son más densas (Lurie y Crisosto, 2005). Actualmente para clarificar la situación 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 en Sudamérica también recibe la denominación de *duraznos*.

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* 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 de nectarina, de carne blanca o amarilla.

Figura 1.2. Frutos de las diferentes variedades botánicas de *Prunus persica*. De izquierda a derecha: melocotón, nectarina y paraguayo.

1.1.3. Importancia del cultivo

El melocotonero es la tercera especie frutal de mayor producción a nivel mundial después del manzano y del peral (Arús et al., 2012). El cultivo tiene una producción mundial de 20,3 millones de toneladas con una superficie cultivada de 1,5 millones de hectáreas en 2010 (FAOSTAT, 2012; http://faostat.fao.org) (Figura 1.3). Sin embargo, el consumo de melocotón (2 kg de fruta per capita por año) sigue siendo bajo comparado con otro frutos como la manzana (16 kg) o el plátano (9 kg) (Crisosto, 2006).
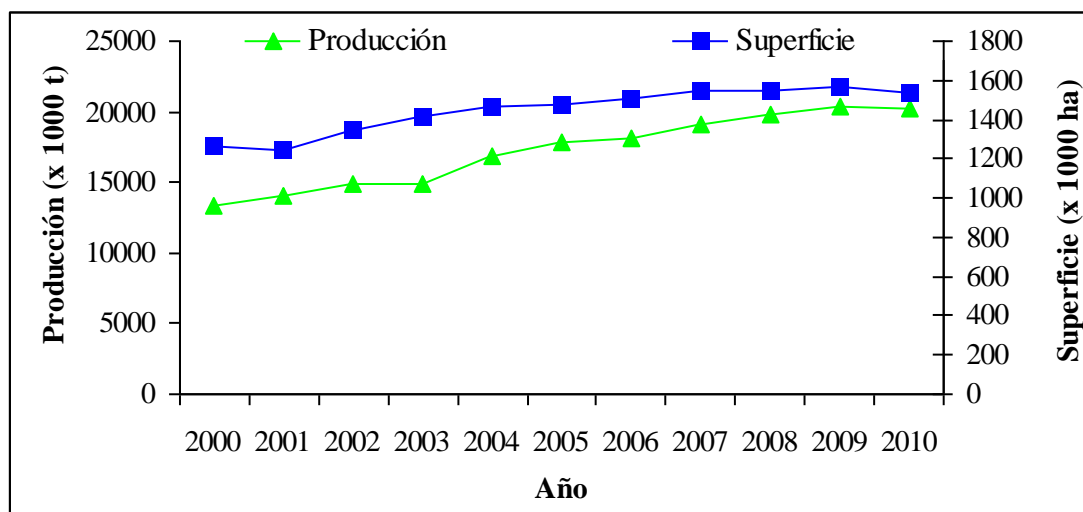
Figura 1.3. Evolución mundial de la producción y de la superficie cultivada de melocotonero en el periodo 2000-2010 (FAOSTAT, 2012).

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Los principales países productores son China, Italia, España, EE.UU, Grecia y Francia (Figura 1.4). España es el tercer productor a nivel mundial después de China, Italia y por delante de Estados Unidos, con una producción de 1,13 millones de toneladas en 2010.
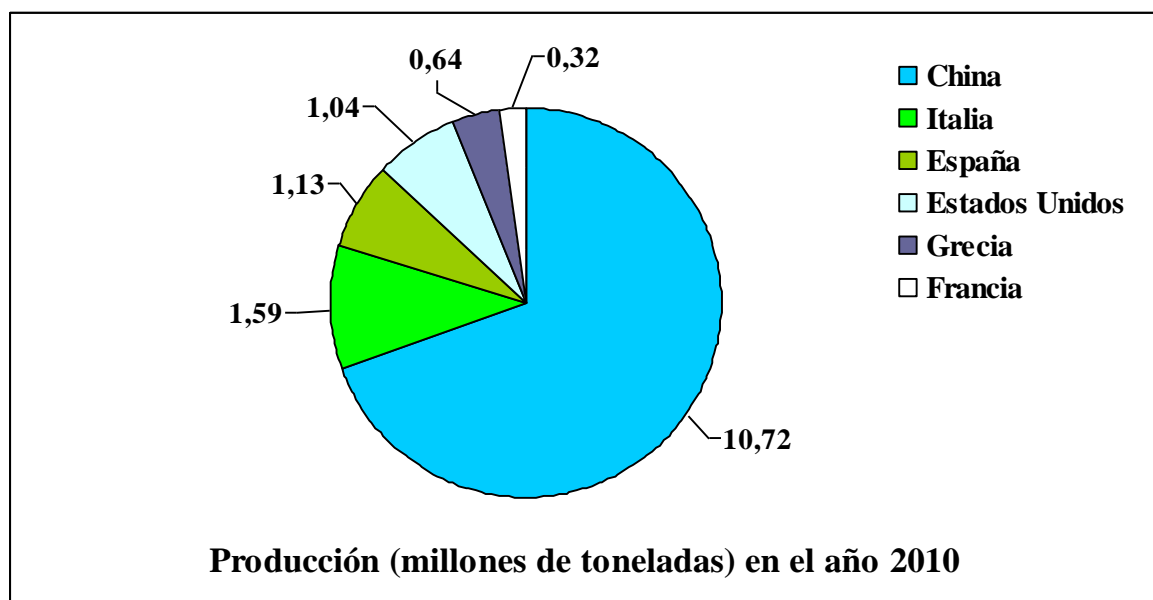
Figura 1.4. Principales países productores de melocotonero en el año 2010 (millones de t) (FAOSTAT, 2012).

En la Unión Europea (UE), el melocotonero ocupa el segundo lugar tras el manzano, tanto en superficie (237.743 ha en 2010) como en producción (3,8 millones de toneladas en 2010) (FAOSTAT, 2012) no habiéndose modificado apenas en los últimos 10 años. La mayor concentración del cultivo se encuentra en los países europeos mediterráneos (Italia, España, Grecia y Francia). España es el segundo país máximo productor europeo después de Italia. Entre los frutales de hueso y pepita, la producción anual media española de melocotón es la más importante, seguida de la del manzano y del peral (Iglesias y Casals, 2011). Dentro de las especies frutales de hueso, el melocotonero y el almendro en España se consideran como frutales de gran importancia socio-económica y representan la mayor superficie cultivada, con 542.100 ha para el almendro y con 73.000 ha para el melocotonero en 2010 (Figura 1.5).

El sector productor español es el que presenta la mayor tasa de renovación varietal de la Unión Europea y ha apostado decididamente por la plantación de nuevas variedades, hecho favorecido por los diferentes planes de reconversión varietal implementados conjuntamente por el Ministerio de Agricultura, Alimentación y Medio Ambiente (MAGRAMA) y las distintas comunidades autónomas (Iglesias y Casals, 2011).

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Superficie cultivada en el año 2010
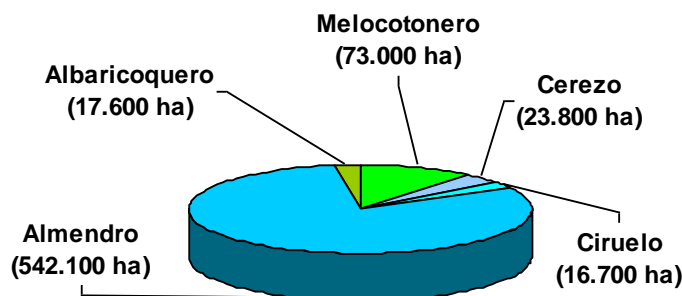
Producción en el año 2010
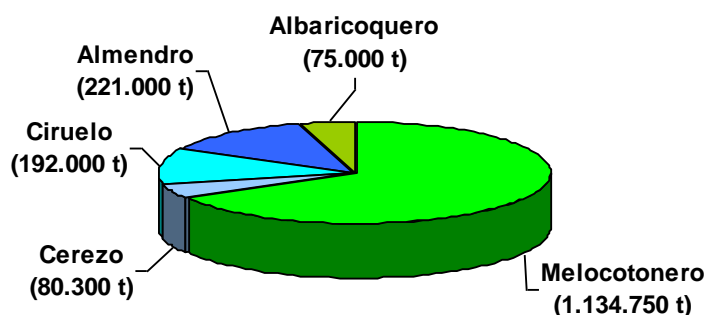
Figura 1.5. Superficie cultivada y producción de los principales cultivos de frutales de hueso en España en el año 2010 (FAOSTAT, 2012).

Las principales comunidades autónomas productoras de melocotón son: Cataluña, Aragón, Región de Murcia, Andalucía y Extremadura (Figura 1.6).
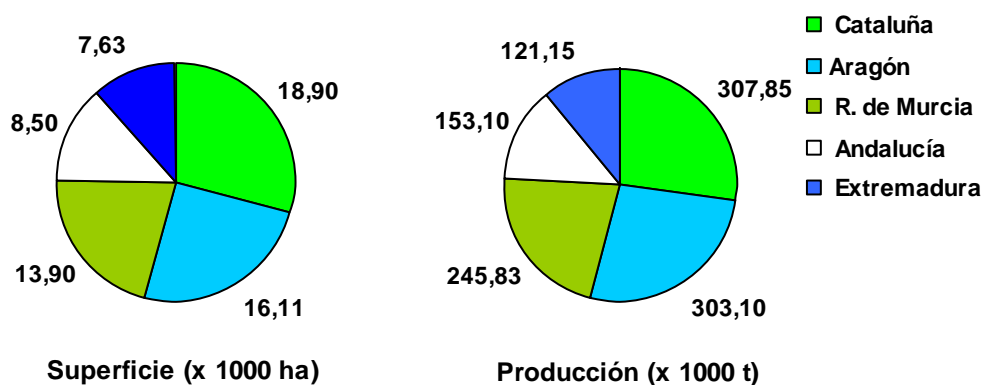
Figura 1.6. Superficie cultivada (x 1000 ha) y producción de melocotonero (x1000 t) en las comunidades autónomas españolas en el año 2009 (MAGRAMA, 2012).

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En cuanto a la tipología del fruto y en base a las producciones del período 2008-2010, la nectarina es el grupo más importante con el 38% de la producción, seguida por el melocotón amarillo (34%) y el melocotón rojo que junto al paraguayo representó el 28% (Figura 1.7) (Iglesias y Casals, 2011). La evolución según la tipología de fruto en el período 1991-2010, muestra un notable incremento de la producción de nectarina, un aumento moderado de melocotón rojo y un descenso muy significativo de la producción de melocotón amarillo, que en 1992 representaba el 67% de la producción total. En el caso de la nectarina, el 76% corresponde a variedades de carne amarilla y el 24% a variedades de carne blanca, mientras que para el melocotón estos porcentajes son del 86% y del 14% para la carne amarilla y blanca, respectivamente (Iglesias et al., 2010).
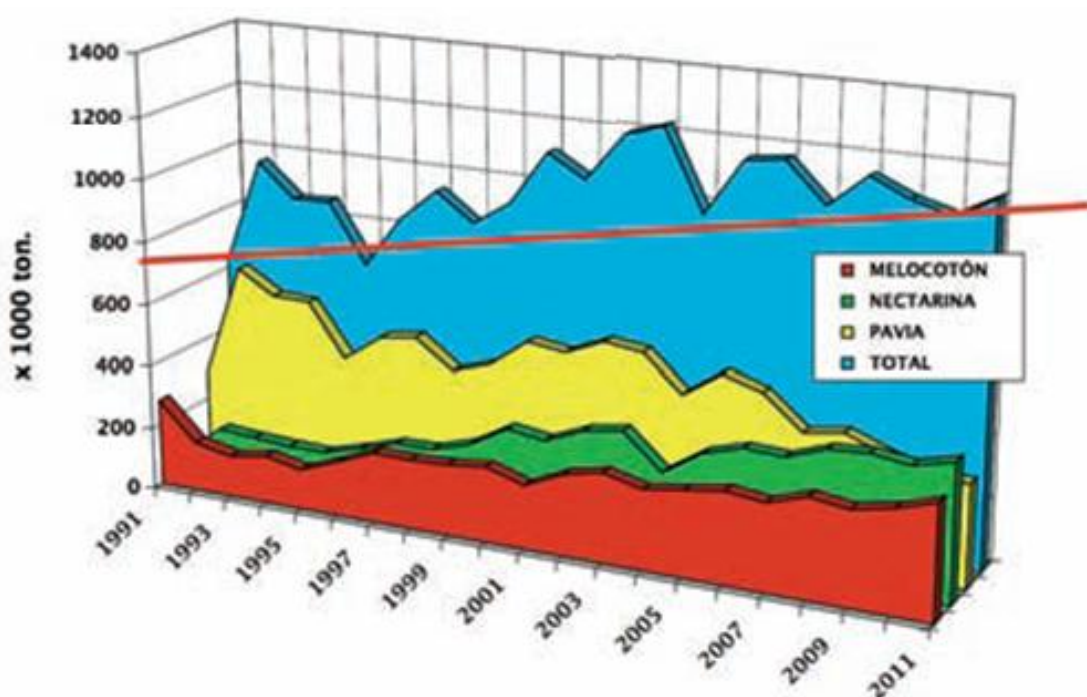
Figura 1.7. Evolución de la producción de los principales grupos varietales en España en el período 1991-2011 (Fuente: Europech'11; Iglesias et al., 2011).

Uno de los aspectos que caracterizan al sector económico del melocotón en España es su creciente competitividad en los mercados internacionales, principalmente de centro, norte y este de Europa. España, desde el año 2006, es el primer exportador de melocotón de Europa y la media anual en el período 2004-2009 alcanzó las 215.000 toneladas, siendo Francia, Alemania y Portugal los principales países importadores. En el caso de la nectarina, las exportaciones en el mismo período alcanzaron las 269.000 toneladas, siendo así superiores a las del melocotón (Iglesias y Casals, 2011).

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El hecho más significativo que caracteriza al cultivo de melocotonero es la gran disponibilidad de nuevas variedades, lo que supone una innovación constante en tipologías de fruto y fechas de recolección (Iglesias y Casals, 2011). En este sentido, los objetivos de los programas de mejora han sido muy diversos y complementarios, intentando, en general, solucionar los problemas agronómicos de las diferentes áreas de producción. La mayor innovación se ha producido en la mejora de la presentación de los frutos, en especial en lo referido a la coloración, calibre, forma y buena aptitud a la manipulación. Las características cualitativas/gustativas han constituido otro objetivo importante y, en la actualidad, se dispone de variedades dulces, semi-dulces, equilibradas, ácidas y muy ácidas, siendo las dos primeras las de mayor aceptación por parte de los consumidores (Iglesias y Casals, 2011).

1.2. Aspectos que condicionan la calidad del fruto en melocotón

La palabra «calidad» proviene del latín *qualitas*, que significa atributo, propiedad o naturaleza básica de un objeto. Sin embargo, en un sentido abstracto su significado es «grado de excelencia o superioridad» (Kader et al., 1985). Bruhn (2007) indicó que uno de los parámetros determinantes de la calidad en el momento de la compra es el sabor, además del valor nutritivo, la inocuidad y el precio.

La calidad de la fruta es un concepto que engloba propiedades sensoriales, valor nutritivo (Layne y Bassi, 2008), y que determina el valor económico en la mayoría de las especies frutales. Esta calidad está relacionada con variables internas (firmeza, contenido en azúcares y en ácidos orgánicos, defectos internos, etc.) y otras variables externas (color, forma, tamaño y defectos o daños externos) (García-Ramos et al., 2005).

El manejo de las prácticas pre y postcosecha es de gran importancia en la industria frutícola, ya que el estado de madurez, el almacenamiento y la distribución va a determinar la calidad del producto final, evaluado en términos de satisfacción del consumidor (Zhang et al., 2008). Por lo tanto, en el sector productivo del melocotón, uno de los mayores objetivos de selección es encontrar el compromiso entre calidad organoléptica y madurez en la cosecha (Fideghelli et al., 1998), creando nuevos genotipos que expresen el sabor y el aroma antes de que el fruto alcance un grado de madurez muy avanzado.

Por ello, en los últimos años, la falta de calidad interna del fruto y por tanto una menor aceptación por parte del consumidor, han provocado que hoy en día el objetivo principal de la mayoría de los programas de selección y mejora sea la obtención de nuevos cultivares que superen a los tradicionales, no sólo en la apariencia externa, sino en las

propiedades que hagan que la fruta tenga una mayor calidad organoléptica y nutricional y un mayor contenido en sustancias saludables.

1.2.1. Fases del desarrollo del fruto

La curva de crecimiento del fruto en los frutales de hueso es en forma de doble-sigmoidea (Figura 1.8). Tonutti et al. (1997) presentaron esta curva de crecimiento dividida en 4 fases: la primera (S1), es una fase de división y expansión celular (crecimiento del pericarpo); la segunda (S2), es una fase de desarrollo del endocarpo y está caracterizada por el crecimiento lento (endurecimiento del hueso y desarrollo del embrión); la tercera (S3), es una fase de aumento de tamaño del fruto debido al alargamiento de las células; y la cuarta fase (S4), se caracteriza por el aumento de la producción de etileno y por la finalización del proceso de madurez.
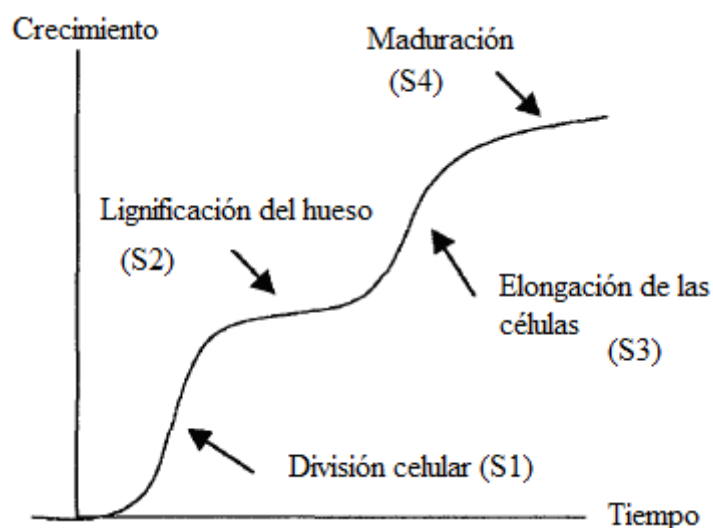
Figura 1.8. Fases de crecimiento del fruto de melocotón (Tonutti et al., 1997).

La maduración (S4), es la fase más determinante en términos de calidad de fruta. Está caracterizada por cambios en el color, firmeza, acidez y contenido en sólidos solubles (Ferrer et al., 2005), aspectos que influyen sobre el aroma, la vida útil y la calidad postcosecha del melocotón (Crisosto et al., 1995). En la madurez fisiológica, la sacarosa alcanza su nivel máximo en el mesocarpo y se observan valores bajos de ácido quínico (Chapman et al., 1991). También los niveles de ácido málico y cítrico fluctúan durante la maduración, siendo máxima la relación málico/cítrico en la madurez del melocotón (Chapman y Horvat, 1990).

1.2.2. Contenido en sólidos solubles y acidez en frutos

El carácter dulce y ácido son factores importantes que afectan la aceptabilidad del fruto por parte del consumidor y están muy correlacionados. Varios autores han asociado la alta aceptabilidad del melocotón por parte del consumidor con altas concentraciones de sólidos solubles (Kader, 1995). La sacarosa, la glucosa y la fructosa son los azúcares más abundantes en el melocotón (Génard et al., 2003), y en el fruto maduro constituyen el 60% de la concentración de los sólidos solubles totales (SSC). En cuando a los ácidos orgánicos, los más abundantes en los frutos de hueso son el ácido málico, cítrico y quínico (Wills et al., 1983), que contribuyen a la acidez total de la fruta (Esti et al., 1997).

Las variedades de melocotón y nectarina pueden ser clasificadas como ácidas, sub-ácidas o de baja acidez, según las diferencias en el metabolismo de los ácidos orgánicos (Tabla 1.1).

Tabla 1.1. Clasificación de las variedades de melocotonero en función de la acidez total del fruto.

Grupo	Acidez valorable (g. ác. mál./100ml)	Acidez valorable (mEq./100 ml)
Subácida / muy dulce	<0,3	<5
Dulce / semidulce	0,3-0,6	5-9
Equilibrada	0,6-0,8	9-12
Ácida	0,8-1,0	12-15
Muy ácida	>1,0	>15

Abreviaciones: ác. mál: ácido málico. Fuente: Iglesias y Echeverría (2009b).

1.2.3. Firmeza del fruto y adherencia al hueso

La firmeza es uno de los principales parámetros para estimar el grado de madurez de un fruto, ya que la maduración mejora y ablanda la textura del mismo, lo que asociado a los cambios en el sabor y el color hace que alcance la máxima calidad comestible. Por lo tanto, tener una fruta madura y con firmeza aceptable es uno de los principales objetivos de los programas de selección y mejora en melocotonero.

En California, Crisosto (2002) observó que los frutos con 26,5–35,3 N de firmeza podían ser considerados buenos para comprar *"ready to buy"* y los frutos con 8,8–13,2 N de firmeza podían considerarse maduros *"ready to eat"*. La disminución de la firmeza de la fruta comienza durante el proceso de maduración (fase S4) y se hace por solubilización y despolimerización de los polisacáridos de la membrana celular (Ortiz et al., 2010). Los melocotones de pulpa o carne fundente (*Melting Flesh, MF*) disminuyen la firmeza al inicio de la madurez y tienen un rápido ablandamiento (*melting*) al final de la misma

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(Pressey et al., 1971). El fenotipo pulpa fundente está asociado con un alto incremento en la pectina soluble y progresiva despolimerización de la misma (Fishman et al., 1993). En estos cultivares, se produce un incremento en la expresión génica y de la actividad de enzimas modificantes de las paredes celulares, en particular de la endo-poligalacturonasa (endo-PG), exo-poligalacturonasa (exo-PG) y pectin-metilesterasa (PME) (Trainotti et al., 2003; Brummell et al., 2004). En los cultivares de pulpa no fundente (*Non Melting Flesh,* NMF), el fruto se mantiene relativamente firme normalmente por una baja actividad de las endo-PGs (Fishman et al., 1993).

En cuanto al control de la consistencia de la pulpa, se ha propuesto el gen *M* como responsable del ablandamiento o *Melting Flesh*. Aquellos genotipos portadores del alelo dominante *M* (genotipos: *MM* y *Mm*), tienen un proceso de ablandamiento del fruto más rápido en las últimas fases de maduración del fruto, que suele coincidir con el pico climatérico. En cambio, aquellos frutos de carne dura, cuyo genotipo corresponde al homocigoto recesivo (genotipo: *mm*) "*Non Melting Flesh*", no pasan por esta fase y se mantienen más firmes durante el proceso de maduración (Lester et al., 1996).

Los frutos de carne blanda generalmente son de hueso libre (*Freestone*, F) y los de carne dura de hueso adherente (*Clingstone*, C), aunque se conocen algunos genotipos de carne blanda y hueso adherente, aunque no es lo más frecuente (Okie, 1988). Por tanto, la combinación de ambos caracteres da lugar a los siguientes fenotipos de frutos: melocotones de carne dura y hueso adherente (CNMF, genotipo: *mmff*) (ej. 'Babygold 9'; 'Spring Prince'; 'Queencrest'; 'Dr. Davis'), melocotones de carne blanda y hueso libre (FMF, genotipo: *MmFf*) (ej. 'Georgia Belle'; 'Venus') y melocotones de carne blanda y hueso adherido (CMF, genotipo: *mmFf*) (ej. 'Big Top'). No se conoce ningún caso de melocotón de carne dura y hueso libre (*mmF*) (Van Der Heyden et al., 1997). Algunos autores han observado diversas gradaciones y han clasificado algunas variedades como 'semifreestone' (ej. 'Snow Bride', 'Raritan', etc) o 'semi-clingstone' (ej. 'Tropic Beauty', 'Flordaprince', 'Sunwright') (Bailey y French, 1949).

1.2.4. Aroma y compuestos volátiles

El aroma del fruto es resultado de una compleja mezcla de un gran número de compuestos volátiles que contribuyen a la calidad sensorial de cada fruto dependiendo de la especie y la variedad (Sanz et al., 1997). El perfil aromático propio de cada especie resulta de la identidad de los compuestos emitidos, su concentración y su intensidad

aromática, medida en unidades de olor que se definen como el cociente entre la concentración de un compuesto y su umbral de percepción olfativa (Buttery, 1993).

El aroma es un carácter de calidad muy importante en la fruta ya que influye sobre la aceptación del consumidor. Sin embargo, hay que considerar que el melocotón cuando se comercializa no llega a desarrollar todo su aroma debido a que se cosecha temprano, y/o se producen cambios por el almacenamiento prolongado de la fruta a bajas temperaturas (Defilippi et al., 2009). En el aroma del melocotón se han identificado más de 110 compuestos volátiles distintos, de los cuales las lactonas, especialmente γ-decalactona, y δ-decalactona (Wang et al., 2010), se han descrito como los compuestos determinantes del aroma característico, aunque actúan en asociación con aldehídos, alcoholes C6, y terpenos (Zhang et al., 2010).

La variabilidad en los compuestos volátiles depende de la variedad, del estado de madurez, de las condiciones de almacenaje y de la distribución de los compuestos dentro del fruto (Aubert y Milhet, 2007; Wang et al., 2010). Dentro del tejido del fruto, está demostrado que la piel produce compuestos en cantidades más elevadas que la pulpa. La formación de compuestos volátiles es un proceso dinámico que aumenta después del inicio del climaterio y continúa tras la recolección de la fruta hasta que comienza la senescencia (Infante et al., 2008). La producción de compuestos volátiles tiene como origen ácidos grasos, aminos, compuestos fenólicos y terpenoides, y los productos más interesantes son aldehídos, ésteres, cetonas, terpenoides y compuestos a base de sulfuro. En frutos inmaduros, los compuestos mayoritarios son C6-aldehidos (*trans*-2-hexanal) y C6-alcoholes (*cis*-3-hexanol), que dan aroma verde, y según el avance del estado de madurez las lactonas, particularmente γ- y δ-lactonas, aumentan significativamente (Zhang y Jia, 2005).

En frutos maduros se han detectado aumentos en benzaldehídos y en linalool. El cis-3-hexenylacetate es un buen marcador del aroma del melocotón (De Santis et al., 2001). Las lactonas (γ-decalactona y δ-decalactona) y otros productos de peroxidación de ácidos grasos no saturados son los productos que confieren el aroma típico del melocotón (Takeoka et al., 1988). Los terpenoides y las lactonas pueden ser más importantes en nectarinas que en melocotón (Engel et al., 1988).

Los ácidos grasos son los precursores de algunos compuestos volátiles y son metabolizados en dos rutas principales: la β-oxidación y la ruta de lipoxigenasa (LOX) (Defilippi et al., 2009). Las enzimas implicadas en el proceso de síntesis de compuestos volátiles están bajo la regulación del etileno durante la madurez de la fruta (Zhu et al.,

2005). La regulación bioquímica y genética de los compuestos volátiles de la fruta ha cobrado últimamente más atención, pero por el momento hay pocos genes descritos como responsables del control genético del aroma y se conoce relativamente poco sobre su regulación (Zhu et al., 2005).

1.2.5. Etileno y maduración de la fruta

La madurez es un proceso complejo influenciado por factores tanto internos como externos. Los eventos asociados a la madurez están causados por cambios en la expresión génica que conduce a alteraciones en el color, la textura y el aroma del fruto (Gray et al., 1994). Los frutos se clasifican en 2 grupos: 1) climatéricos, la madurez está acompañada de un pico respiratorio y de una alta producción de etileno (C_2H_4) (Prinsi et al., 2011), y 2) no climatéricos, aquellos frutos en los que la respiración no muestra un cambio remarcable durante la maduración y la producción de etileno es menor y estable.

Los melocotones son frutos climatéricos que se caracterizan por presentar una rápida maduración debido al aumento de la respiración y la producción de etileno, lo que provoca que al mantenerlos a temperatura ambiente tras la recolección se deterioren rápidamente. El etileno es una hormona natural que regula muchos de los aspectos relacionados con el crecimiento, desarrollo, maduración y senescencia de los frutos en coordinación con la expresión génica (Kader, 1985). Esta hormona está sintetizada a partir del aminoácido metionina vía dos enzimas intermediarias S-adenosil-L-metionina (AdoMet) y 1-aminociclopropano-1-carboxilato (ACC) (Figura 1.9). El etileno tiene bajos niveles durante las primeras fases de crecimiento del fruto y aumenta durante la maduración (S4).
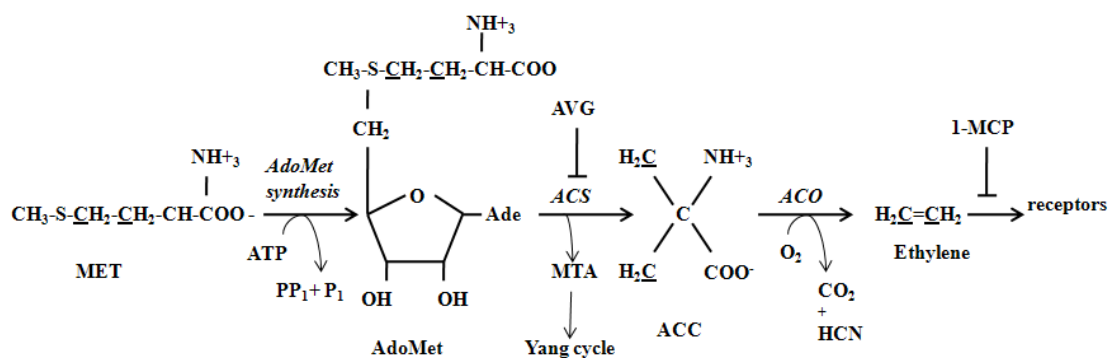
Figura 1.9. Biosíntesis del etileno. Las enzimas están en cursiva. El átomo de carbono donde deriva el etileno está subrayado. Los inhibidores AVG y 1-MCP inhiben la ACC sintasa y la fijación del etileno al receptor, respectivamente (Gray et al., 1994).

Para controlar la producción de etileno y por tanto prolongar la vida útil del producto se han desarrollado compuestos comerciales que interfieren en la síntesis del etileno o su percepción; el aminoetoxivinilglicina (AVG) inhibe la actividad de ACC-sintasa, y el 1-metilciclopropeno (1-MCP) bloquea los receptores de etileno evitando su acción (Lurie, 2008).

1.3. Calidad organoléptica y nutricional

La calidad sensorial y el valor nutritivo de la fruta en el melocotonero tienen un papel importante en la satisfacción del consumidor (Colaric et al., 2005). El sabor, aroma, firmeza y apariencia son considerados entre los componentes sensoriales más importantes (Colaric et al., 2005). El sabor de los frutos está determinado por las concentraciones y el tipo de sólidos solubles y de ácidos orgánicos (Dirlewanger et al., 1999).

En el melocotonero, hay una relación entre la madurez fisiológica del fruto en el árbol y el desarrollo de caracteres clave responsables de su calidad (Ziosi et al., 2008). Una cosecha tardía puede mejorar la calidad organoléptica del fruto, ya que los azúcares y el aroma aumentan cuando el fruto está en el árbol, mientras que los ácidos disminuyen en la última fase de la maduración del fruto (Etienne et al., 2002; Ziosi et al., 2008). Sin embargo, las variedades de pulpa fundente que maduran rápido, se cosechan al inicio de la madurez, y no suelen alcanzar el máximo de aroma cuando llegan al consumidor (Ziosi et al., 2008). Los caracteres más importantes que se persiguen en los nuevos cultivares son, aparte de un buen aroma, una baja acidez, un sabor dulce y una firmeza aceptable para disminuir los daños provocados por el transporte, lo que va a garantizar una alta calidad del producto final y su aceptabilidad por parte del consumidor.

El melocotón es una fruta de verano que cada vez tiene más interés por su valor nutricional (Wolfe et al., 2008). Se han estudiado sus propiedades nutricionales, y se ha observado que los frutos alcanzan su mejor calidad nutricional cuando han terminado el periodo de madurez en el árbol (Kader et al., 1999). En los últimos años, la fruta ha alcanzado la calificación de 'alimento funcional' debido a su bajo contenido en calorías y alto contenido en vitaminas, fenoles, carotenoides, minerales y fibras, con un papel importante en la prevención de riesgos oxidativos (Orazem et al., 2011). El papel significativo en la nutrición humana, se debe fundamentalmente a que la fruta es una fuente de vitaminas [C (ácido ascórbico), A, tiamina (B1), niacina (B3), piridoxina (B6), ácido fólico (B9), E] y de minerales (Wargovich, 2000). Además, la fruta es una fuente de compuestos fenólicos que tienen una serie de beneficios para la salud, como la prevención

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y/o el tratamiento de una serie de patologías como el cáncer y enfermedades cardiovasculares (Tavarini et al., 2008). Muchos estudios epidemiológicos han relacionado el aumento del consumo de fruta con la disminución del riesgo de algunas enfermedades degenerativas como el cáncer (Dauchet y Dallongeville, 2008; Hegedús et al., 2010), responsables de la alta mortalidad en los países occidentales (Müller et al., 2010).

1.4. Compuestos bioquímicos con influencia en la calidad del fruto

1.4.1. Compuestos antioxidantes

El melocotón contiene ácido ascórbico, carotenoides (provitamina A), y compuestos fenólicos, que son fuentes de antioxidantes (Byrne, 2002). Estas sustancias no solo tienen un papel en la apariencia y el gusto de la fruta sino un papel saludable actuando como antioxidantes que capturan radicales libres implicados en enfermedades degenerativas (Rice-Evans et al., 1996). Los radicales libres son compuestos inestables que se producen durante el funcionamiento normal de las células (Lee et al., 2003) pero pueden causar daños en las membranas celulares (Nijveldt et al., 2001).

1.4.1.1. Ácido ascórbico (Vitamina C)

La vitamina C es una de las más importantes para la nutrición humana. Como el cuerpo humano no sintetiza ácido ascórbico, más del 90% de la misma en la dieta humana es aportada por la ingesta de frutas y verduras (Hernández et al., 2006). La ingesta diaria recomendada de la vitamina C para adultos es de 60 mg/día (Food and Nutrition Board, 2000). La función más conocida de la vitamina C es como agente antiescorbuto (Magiorkinis et al., 2011) y participa en la síntesis de colágeno (Libby y Aikawa, 2002).

La vitamina C se considera como uno de los antioxidantes naturales más eficaces y menos tóxicos. Posee las características de lo que podría considerarse un secuestrador ideal de radicales libres protegiendo al ADN, las proteínas y los lípidos de la oxidación (Pavlovic et al., 2010). La vitamina C ha sido reconocida y aceptada por la *US Food and Drug Administration* (FDA) como uno de los cuatro antioxidantes dietéticos más importantes, los otros tres son la vitamina E, la vitamina A cuyo precursor es el β-caroteno, y el selenio. Como tal, es eficaz frente a los radicales superóxido e hidroxilo, el peróxido de hidrógeno y el oxígeno singlete (Halliwell et al., 1995). El ácido ascórbico es un potente reductor, pierde con facilidad átomos de hidrógeno y se transforma en ácido dehidroascórbico, que también posee actividad de vitamina C.

Las prácticas culturales y las condiciones climáticas (Lee y Kader, 2000) pueden modificar el contenido de vitamina C en los frutos cosechados. De la misma manera, el

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grado de madurez en la cosecha o el método de cosecha, también afectan al contenido en vitamina C (Kader, 1988).

1.4.1.2. Fenoles totales

Estos compuestos son metabolitos secundarios de las plantas, con diferentes actividades y estructuras químicas. Los polifenoles son un amplio grupo de compuestos divididos en diferentes clases (proantocianidinas, flavonoides, flavonas, flavonoles, flavanones, isoflavonas, etc) (Manach et al., 2004). Los compuestos fenólicos poseen un anillo aromático de benceno, con uno o más grupos hidróxilos incluyendo derivados funcionales (ésteres, metil ésteres, glicósidos, etc.) (Tsimidou, 1998). La naturaleza de los polifenoles varía desde moléculas simples como los ácidos fenólicos, hasta compuestos altamente polimerizados, como los taninos. Dentro de este grupo, los fenoles simples, como el fenol, cresol, timol y resorcinol están ampliamente distribuidos entre todas las especies vegetales. Igualmente, los ácidos fenólicos, tales como el gálico, vainilínico, fidroxibenzoíco, y los aldehídos como la vainilina, también son abundantes en las plantas (Belitz y Grosch, 1988).

Los compuestos fenólicos dan a la fruta importantes características de calidad deseables, como el color y las propiedades antioxidantes, pero también otras no deseables como el amargor y la astringencia (Bravo, 1998). Los fenoles contribuyen a la capacidad antioxidante de la fruta, teniendo así efectos saludables en la dieta (Heinonen et al., 1998). También favorecen la estabilidad frente a la oxidación de las proteínas de baja densidad (LDL), lo que protege contra enfermedades cardiovasculares (Steinberg et al., 1989). Sin embargo el contenido en estos compuestos antioxidantes de la fruta varía entre piel y pulpa. En manzano, melocotonero y ciruelo, la piel de la fruta representa el 6-9% del peso fresco del fruto, pero contiene de 2 a 5 veces más compuestos fenólicos que la pulpa, y por lo tanto es una fuente importante de compuestos fenólicos (Wargovich et al., 2012).

1.4.1.3. Flavonoides

Se han identificado alrededor de 9000 flavonoides (Williams et al., 2004) siendo los polifenoles más distribuidos en las plantas. Los flavonoides, con estructura básica C6-C3-C6, incluyen a las antocianinas, los flavonoles, las flavonas, las flavanonas, las chalconas, las isoflavonas y los flava-3-oles.

Los «flavonoles» se encuentran repartidos abundantemente en todos los alimentos de origen vegetal, especialmente en las frutas. *Las «flavanonas»* como la hesperidina y la

naringenina se encuentran prácticamente restringidas a frutos cítricos. *Las «chalconas» son compuestos minoritarios*, que se encuentran en manzana y fresa principalmente (floretina). *Las «proantocianidinas» y las «antocianidinas»* se localizan en las semillas de las uvas y en las cerezas respectivamente, a las que confieren el color rojo y rojo azulado. *Las flavonas* no suelen encontrarse en frutas pero sí en cereales y en muchas plantas herbáceas.

En las plantas, los flavonoides (Figura 1.10) se encuentran en estado libre o más frecuentemente unidos a azúcares formando heterósidos porque tienen mayor estabilidad química. Dentro de todos estos grupos de flavonoides, las flavonas, los flavonoles y sus glucósidos son los compuestos más abundantes en vegetales (Hertog et al., 1993). Todos estos compuestos tienen una actividad antioxidante que depende del número de grupos hidroxilo que tienen en sus estructuras (Rice-Evans et al., 1996). Estos compuestos polifenólicos muestran un gran espectro de actividades biológicas en los seres humanos: propiedades cardiotónicas, y prevención de enfermedades neurodegenerativas (Pluma et al., 1998).
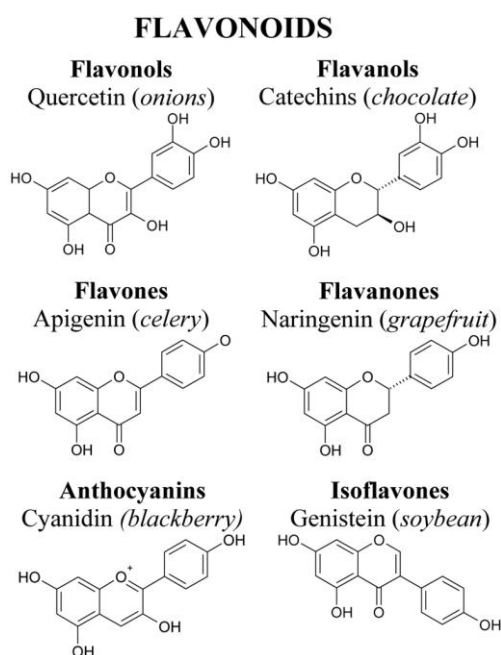
Figura 1.10. Estructura de los flavonoides (Manach et al., 2009).

Los hábitos alimenticios son muy diversos en el mundo. La ingesta promedio de flavonas y flavonoles se sitúa entre los 20 y 26 mg/día (Hollman and Katan, 1998), excediendo a la de otros antioxidantes en la dieta, como el β-caroteno (2-3 mg/día) y la vitamina E (7-10 mg/día), mientras que es aproximadamente un tercio de la vitamina C (70-100 mg/día). Así pues, los flavonoides representan una contribución importante al potencial antioxidante en la dieta humana.

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Las antocianinas son metabolitos secundarios de las plantas que pertenecen a la familia de los flavonoides. La ingestión diaria en la dieta está estimada en 12,5 mg/día/persona en EE.UU. (Wargovich et al., 2012). Su estructura se caracteriza por un esqueleto básico de quince átomos de carbono (C6-C3-C6) de tipo 2-fenil benzopirona (Figura 1.11). Sus derivados privados del azúcar se denominan antocianidinas y son más estables bajo la forma heterosídica que bajo la forma aglicona. El mecanismo de acción antioxidante de las antocianinas es donar átomos de hidrógeno a los radicales libres, consiguiendo cortar la reacción de oxidación (Huang et al., 2005). La contribución de las antocianinas a la pigmentación de los alimentos vegetales está claramente reconocida, a través de las antocianidinas, responsables de los colores rojo, azul, violeta, naranja y púrpura de la mayoría de las plantas y de sus productos (Shahidi y Naczk, 1995) y contribuyen también a la calidad visual de la fruta (Mazza y Miniati, 1993). Las más frecuentes en plantas, de las 600 identificadas en la naturaleza, son pelargonidina (rojo-naranja), cianidina (rojo-púrpura) y delfinidina (azul-púrpura).
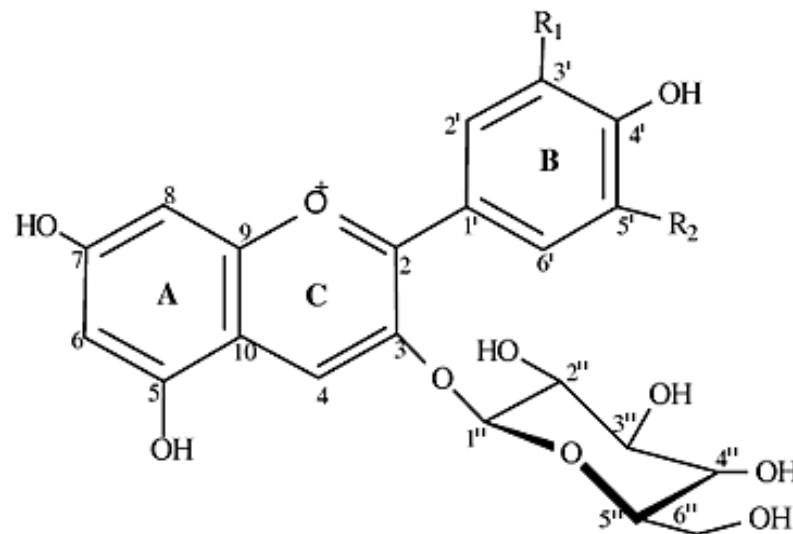
Figura 1.11. Estructura química de las antocianinas (Wu y Prior, 2005).

La principal antocianina identificada en melocotón es la cyanidin 3-glucoside con contribución de cyanidin 3-rutinoside (Ishikura, 1975). La tabla 1.2 presenta el contenido en antocianinas de algunas variedades de melocotonero.

Tabla 1.2. Contenido en antocianinas en distintas variedades de melocotonero.

Genotipos (n)	Antocianinas (mg C3G/100 g PF)	Referencias
(n=20) Variedades en California	pulpa: 0-23 piel: 34-273	Tomás-Barberán et al. (2001)
(n=8) Variedades de melocotón de pulpa roja	1-36	Cevallos-Casals et al. (2006)
(n=19) Variedades de melocotón de pulpa blanca, amarilla, roja	1-266	Vizzotto et al. (2007)
(n=218) Genotipos en segregación	0,1-31	Cantín et al. (2009b)
(n=20) Variedades en California	0,5-7	Byrne et al. (2009)

Fuente: Wargovich et al. (2012). Abreviaciones: (n) número de genotipos estudiados, Equivalentes de cyanidin 3-glucoside (C3G), PF: peso fresco

1.4.1.4. Capacidad antioxidante (RAC)

La actividad antioxidante de los compuestos fenólicos tiene interés desde un punto de vista tecnológico y nutricional (Berra et al., 1995). Así, los compuestos fenólicos intervienen como antioxidantes naturales de los alimentos, por lo que la obtención y preparación de alimentos con un alto contenido en estos compuestos supone una reducción en la utilización de aditivos antioxidantes, a la vez que se obtienen alimentos más saludables, e incluso podrían llegar a clasificarse como alimentos funcionales. Desde un punto de vista nutricional como ya se ha descrito anteriormente, esta actividad antioxidante se asocia con su papel protector en las enfermedades cardiovasculares y en el cáncer, así como en procesos de envejecimiento, por lo que está siendo intensamente estudiado mediante ensayos *in vivo* e *in vitro* (Tsimidou, 1998). Aunque los antioxidantes son reconocidos como fitoquímicos importantes, no existe una información en el etiquetado de "antioxidantes totales" como indicador nutricional debido a la falta de métodos estándar de cuantificación (Ou et al., 2002).

La capacidad antioxidante de frutos en diferentes especies puede variar en relación con las moléculas antioxidantes presentes (Wang et al., 1996) aunque las variaciones pueden venir determinadas también por el genotipo de la misma especie (Tavarini et al., 2008), incluso varían según el tipo fundente/no fundente, según el cultivar (Scalzo et al., 2004), factores genéticos y/o ambientales (Tabla 1.3).

Tabla 1.3. Capacidad antioxidante de distintas variedades de melocotonero.

Genotipos (n)	RAC en (mg Trolox/100 g PF)	Referencias
(n=20) Variedades en California	46-1.006 (pulpa)	Gil et al. (2002)
20) Variedades en California	230-1.789 (piel)	
(n=8) Variedades de melocotón de pulpa roja	440-1.784	Cevallos-Casals et al. (2006)
(n=4) Variedades de melocotón de pulpa blanca	540-1.096	Vizzotto et al. (2007)
(n=6) Variedades de melocotón de pulpa amarilla	437-1.128	Vizzotto et al. (2007)
(n=9) Variedades de melocotón de pulpa roja	2.787-13.505	Vizzotto et al. (2007)
(n=218) Genotipos en segregación	227-630	Cantín et al. (2009b)
(n=20) Variedades en California	350-2.250	Byrne et al. (2009)

Fuente: Wargovich et al. (2012). Abreviaciones: (n) número de genotipos estudiados, PF: peso fresco

Existen varios métodos para determinar la RAC (Relative Antioxidant Capacity): 2, 2- azinobis (2,2-azinobis (3-ethilbenzotiazolina-6-ácido sulfónico) (ABTS) (Miller y Rice-Evans, 1997), 2,2-difenil-1-picrylhidrazil (DPPH) (Brand-Williams et al., 1995), "reducción del hierro/poder antioxidante" (FRAP) y capacidad de absorción de radicales oxigenados (ORAC) (Cao et al., 1993). Los métodos más utilizados son ABTS y DPPH, porque presentan una excelente estabilidad en ciertas condiciones, aunque también muestran diferencias. La forma de medir la capacidad antioxidante es por comparación con la capacidad antioxidante del Trolox durante un tiempo de reacción determinado (10 min) (Tavarini et al., 2008). La determinación de la capacidad antioxidante se puede medir usando el 2, 2-diphenyl-1-picrahydrazyl (DPPH) (Huang et al., 2005) que se reduce con un radical presente en la muestra (Figura 1.12).
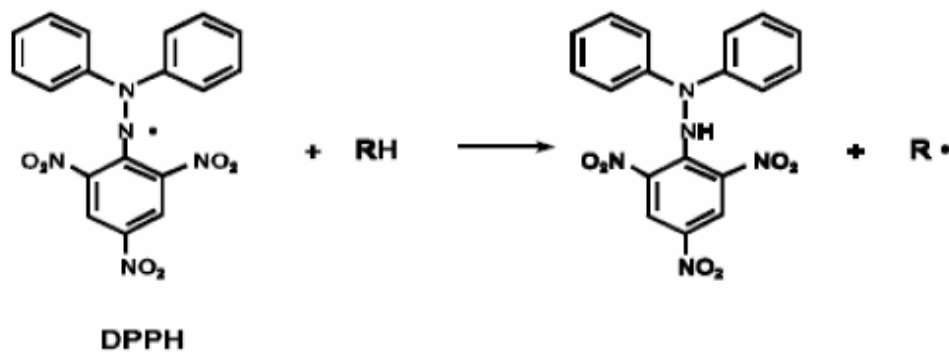
Figura 1.12. Estructura de DPPH y su reducción por un antioxidante (Prakash, 2001).

1.4.2. Azúcares totales

El melocotonero está muy valorado como fruta de mesa debido a su atractivo color, a su sabor y también porque es fuente de azúcares, vitaminas y minerales (Kumar et al., 2010). La calidad del melocotón depende en gran medida de su contenido en azúcares totales y ácidos orgánicos (Wu et al., 2003). Los principales azúcares individuales en melocotón son sacarosa, glucosa, fructosa y sorbitol. La sacarosa es máxima en la madurez y puede alcanzar el 50% de la materia seca, mientras que el sorbitol representa sólo el 8% de la materia seca (Moing, 2000). El contenido en azúcares individuales se modifica durante el proceso de maduración del melocotón (Orazem et al., 2011). Dirlewanger et al. (1999) observaron que el contenido en sacarosa estaba negativamente correlacionado con el contenido en ácido málico, en cítrico y químico, mientras que la glucosa y la fructosa estaban positivamente correlacionadas con el contenido de estos ácidos. La sacarosa se acumula rápidamente durante la fase S3, alcanzando un pico en la fase S4 si se deja la fruta terminar su maduración en el árbol (Vizzotto et al., 1996). La relación porcentaje glucosa/ fructosa puede cambiar de 1/1 durante la fase (S3) a 1/0,8 durante la fase (S4) (Souty et al., 1998) debido al consumo de glucosa en el proceso de respiración del fruto. Por esto, los factores previos a la cosecha son muy importantes para la acumulación de los distintos azúcares en el fruto.

1.5. Calidad postcosecha y daños por frío

El melocotonero es una fruta climatérica que produce etileno y por tanto continúa el proceso de respiración y maduración después de la cosecha, lo que hace que se deteriore rápidamente si está a temperatura ambiente. Por tanto, el almacenamiento del melocotón a bajas temperaturas (0-5ºC) es necesario para minimizar el ablandamiento y prolongar su vida útil para la comercialización (Lurie y Crisosto, 2005). Sin embargo, este procedimiento tiene limitaciones debido al desarrollo de síntomas de decaimiento interno (IB, *internal breakdown*), síntomas también llamados daños por frío (DF) (Dagar et al., 2010). Algunos de los síntomas de daños por frío se desarrollan más después de sacar la fruta a temperatura ambiente y no se perciben hasta que la fruta llega al consumidor, por lo que la comercialización está limitada por la baja aceptabilidad por parte del consumidor (Crisosto et al., 1995).

Los daños por frío se caracterizan por dos tipos principales de síntomas: a) desórdenes texturales, y b) desórdenes en la coloración de la pulpa (Lurie y Crisosto, 2005). Entre los desórdenes texturales se destacan los síntomas conocidos como lanosidad

(*woollyness*) o harinosidad (*mealiness*) (Van Mollendorf, 1987) y pulpa coriácea (*leatheriness*) (Luza et al., 1992); y entre los desórdenes vinculados a la coloración, se destacan el pardeamiento de la pulpa o de la cavidad del hueso y el enrojecimiento o sangrado de la pulpa (Lurie y Crisosto, 2005).

La investigación postcosecha está orientada a mantener la máxima calidad del fruto, y a minimizar las pérdidas de cultivos entre la producción y el consumo. Las pérdidas en calidad y cantidad entre la cosecha y el consumo pueden oscilar entre el 5 y el 25% en países desarrollados, y entre el 20 y el 50% en países en vías de desarrollo, dependiendo del producto, la variedad y las condiciones de manejo (Kader, 2007). La respiración y la producción de etileno, junto a los cambios de composición en la fruta, la transpiración y la pérdida de agua son los principales factores biológicos responsables de la pérdida de calidad postcosecha de frutas y hortalizas.

Crisosto et al. (1999) mostraron que los cultivares de hueso adherente y con pulpa no fundente (CNMF) son menos susceptibles a los daños por frío. En cuanto a los cultivares de hueso libre y pulpa fundente (FMF) y los cultivares de hueso adherido y pulpa fundente (CMF) tienen el potencial de desarrollar harinosidad en la fruta (Peace et al., 2006). Algunos cultivares son más susceptibles que otros, indicando que los síntomas de daños por frío tienen un significativo componente genético (Peace et al., 2006; Cantín et al., 2010a).

Varios estudios han relacionado las alteraciones de la textura de la pulpa de la fruta harinosa con las modificaciones de las paredes celulares, con poca atención al efecto que pueden tener los compuestos antioxidantes en el desarrollo de daños por frío. Tsaltani et al. (2010) mostraron que algunos compuestos fenólicos a altas concentraciones son también sustancias de pardeamiento de la pulpa de la fruta. También Chang et al. (2000) indicaron que estos compuestos pueden influir en el pardeamiento de los alimentos porque los fenoles son el origen de las reacciones de pardeamiento catalizadas por la enzima polifenol oxidasa (PPO). El desarrollo, durante el almacenamiento, de sabores no característicos del melocotón en los genotipos de carne no fundente está relacionado, en parte, con las altas concentraciones de compuestos fenólicos solubles (Karakurt et al., 2000). Varios estudios han mostrado que la expresión de los síntomas de daños por frío, sobre todo el pardeamiento interno de la fruta, se desarrolla más en frutos guardados a una temperatura entre 2,2 y 7,6 ºC que los almacenados a 0 ºC pero por encima de la temperatura de congelación (Crisosto et al., 1999).

La combinación de tecnologías postcosecha, junto con el correcto control y aplicación de la pre-refrigeración, acondicionamiento y conservación de la fruta puede permitir alcanzar un máximo de mantenimiento de la calidad de los productos y prolongar su vida útil, a la vez que se reducen las pérdidas económicas ocasionadas por las malas prácticas en el procesado (Navarro-Neila et al., 2008).

1.6. Control genético de los caracteres que definen la calidad de la fruta

Conocer el control genético de los caracteres que más influyen en la calidad organoléptica de la fruta interesa en gran medida para poder manejarlos eficientemente en los programas de mejora de melocotonero. Sin embargo, muchos de estos caracteres son cuantitativos, con una heredabilidad baja y con un control poligénico, por lo que deben utilizarse métodos de análisis genético.

El mapeo de características cuantitativas a través de la identificación de *loci* de caracteres cuantitativos «*Quantitative Trait Loci*» (QTL), se considera una herramienta muy importante dentro de la mejora genética de plantas (Cerón-Rojas y Sahagún-Castellanos, 2007). Dentro del genoma, los QTLs se identifican basándose en el principio de asociación entre los marcadores moleculares polimórficos y el fenotipo de los individuos de una población de mejora (Mora et al., 2008).

El melocotonero es la especie del género *Prunus* que mejor se ha caracterizado genéticamente y se considera como especie modelo en la familia de las *Rosaceae*. El gran valor económico del melocotonero, la autocompatibilidad que permite fácilmente realizar una población F2, la posibilidad de recortar la fase juvenil después de la plantación a 2-3 años, el pequeño tamaño de su genoma y la colinearidad del mismo con otras especies, ha permitido realizar estudios comparativos en la familia rosáceas (Abbott et al., 2002). En el melocotonero se han descrito numerosos genes que controlan importantes caracteres de calidad de fruta (Abbott et al., 2007). Muchos caracteres implicados en la calidad del fruto y de herencia mendeliana están estudiados y mapeados, como melocotón/nectarina (*G/g*) y color de pulpa (*Sc/Sc*) (Bliss et al., 2002), fundente/no fundente (*M/m*) (Warburton et al., 1996), y hueso libre/hueso adherente a la pulpa (Dettori et al., 2001). También caracteres cuantitativos o QTLs que controlan algunos caracteres como el contenido en azúcares (sacarosa, fructosa, glucosa y sorbitol) y contenido en ácidos orgánicos (málico, cítrico, y quínico), que determinan en gran parte la calidad del fruto de melocotonero, también han sido mapeados y localizados en algunas poblaciones (Dirlewanger et al., 1999; Etienne et al., 2002).

1.6.1. Mapeo genético

El mapeo genético se puede definir como la disección genética de fenotipos complejos de material en segregación, facilitada por el uso de marcadores según unos diseños experimentales y análisis estadísticos apropiados (Angaji, 2009). El mapeo empieza con la recogida de los datos fenotípicos y genotípicos de una población en segregación, seguida por el análisis estadístico para ver los posibles marcadores donde la variación alélica tiene una correlación con el fenotipo (Salvi y Tuberosa, 2005).

Collard et al. (2005) han generalizado la construcción de un mapa genético en tres etapas: obtención de una población en segregación, identificación y genotipado de los marcadores polimórficos y análisis estadístico.

Los mapas de ligamiento en *Prunus* están más desarrollados en melocotonero que en otras especies (Dirlewanger et al., 2006). Inicialmente y en paralelo, el uso del cruzamiento interespecífico melocotón x almendro permitió la construcción de los primeros mapas saturados (Tabla 1.4) (Foolad et al., 1995; Bliss et al., 2002; Jáuregui et al., 2001). El mapa considerado en la actualidad de referencia del género *Prunus,* se construyó utilizando una población F2 de almendro ('Texas') × melocotonero ('Early Gold') (TxE), mapeando 235 RFLPs y 11 isoenzimas en 8 grupos de ligamiento (GLs), (Joobeur et al., 1998; Aranzana et al., 2003). Posteriormente, se añadieron otros 264 SSRs (Howad et al., 2005), (0,63 cM/marcador, 826 marcadores distribuidos en 524 cM). El mapa está sesgado, por el alto nivel de heterocigosis y la incompatibilidad de las dos especies: casi el 46% de los *loci* mapeados tienen una segregación asimétrica en la progenie. Se observó también una translocación reciproca entre los GLs 6 y 8 (Jáuregui et al., 2001; Pozzi y Vecchietti, 2009). El mapa TxE permitió la realización de una población altamente polimórfica, conveniente para estudios de ligamiento, estableció una terminología común para los grupos de ligamientos, y dio un grupo de marcadores transferibles de posiciones conocidas del mapa para usarlo en genómica comparativa (Dirlewanger et al., 1998; Lu et al., 1998; Sosinski et al., 1998; Dettori et al., 2001; Gillen and Bliss, 2005; Yamamoto et al., 2001, 2005; Blenda et al., 2007). En cuanto al melocotonero, Chaparro et al. (1994) construyeron, en una población intraspecífica F2, el primer mapa con 83 marcadores de tipo RAPD (*Random Amplified Polymorphic DNA*), un isoenzima y cuatro caracteres morfológicos (Tabla 1.4). Posteriormente, se construyeron varios mapas utilizando distintos tipos de marcadores (RFLPs/RAPDs/SSRs/AFLPs; http://www.bioinfo.wsu.edu/gdr/; Dirlewanger et al., 1998; Lu et al., 1998; Sosinski et al.,

1998; Dettori et al., 2001; Gillen and Bliss., 2005; Yamamoto et al., 2001, 2005; Foulongne et al., 2003; Blenda et al., 2007; Fan et al., 2008; Ogundiwin et al., 2009).

Tabla 1.4. Mapas inter- e intra-específicos de melocotonero.

Población	Número de marcadores	T×E Anchura	Distancia total	Referencias
'Texas' (*) x 'Early Gold' F2 2004	817	817	519 cM	Joobeur et al., 1998; Aranzana et al., 2003; Dirlewanger et al., 2004; Horn et al., 2005 Lalli et al., 2005
Prunus Bin Map	613	-	-	Howad et al., 2005 Cabrera et al., 2009
Ciruelo x Almendro-Melocotonero** 2004	166	-	524.8 cM	Dirlewanger et al., 2004
'NC174RL' × 'Pillar'	88	0	396 cM	Chaparro et al., 1994
'N J Pillar' × KV77119	47	2	332 cM	Sosinski et al.,1998.
'Padre' (*) × '54P455'	161	23	1144 cM	Foolad et al., 1995; Bliss et al., 2002
'Ferjalou-Jalousia' x 'Fantasia'2004	249	49	712 cM	Dirlewanger et al., 1998;2004 Etienne et al., 2002
'Lovell' × 'Nemared' 1998	153	1	1297 cM	Lu et al., 1998 Sosinski et al.,1998
GxN 'Garfi' (*) x 'Nemared' F2 2001	51	-	474 cM	Jáuregui et al., 2001
'IF731' × '*P. ferganensis*' BC1 2005	216	71	665 cM	Dettori et al., 2001; Verde et al., 2005
Melocotonero AxJ 2005	178	45	571 cM	Yamamoto et al., 2005
Melocotonero PMP2 2005	177	0	-	Gillen and Bliss, 2005
'New Jersey Pillar' × KV 77119 (NJxKV), 'Suncrest' × 'Bailey' (ScxB), 'Lovell' × 'Nemared' (LxN)	218	0	1297 cM	Lu et al., 1998 Sosinski et al.,1998
'Summergrand' × 'P1908 *P. davidiana*'	153	57	874 cM	Foulongne et al., 2003a
'Contender' x 'Fla92-2c'	126	96	535 cM	Fan et al., 2008
'Dr. Davis' x 'Georgia Bell'	211	158	818 cM	Ogundiwin et al., 2009
'Guardian' x 'Nemaguard'	172	-	737 cM	Blenda et al., 2007

Todos los mapas son de melocotonero excepto (*) que es almendro y (**) ciruelo x almendro-melocotonero

Muchos de los mapas derivados de cruzamientos inter e intra-específicos están anclados en el mapa de referencia de *Prunus* TxE (Pozzi y Vecchietti, 2009; Arús et al., 2012). El uso más importante del mapeo genético es para identificar las porciones del genoma que tienen genes y QTLs asociados a caracteres de interés (Collard et al., 2005).

1.6.2. Análisis de QTLs

El mapeo de QTLs está basado en el principio de que los genes y los marcadores segregan con recombinación de cromosomas (*crossing-over*) durante la meiosis, para analizarlos en la población (Peterson, 1996; Collard et al., 2005).

Las regiones del genoma que contienen los genes asociados a un carácter cuantitativo particular son llamadas como «*locus de un carácter cuantitativo*» o QTL (*Quantitative Trait Loci*) (Collard et al., 2005). Los QTLs son *loci* genéticos donde diferentes alelos funcionales segregan y causan efectos significativos en un carácter cuantitativo (Salvi y Tuberosa, 2005).

La mayoría de los trabajos de búsqueda y análisis de QTLs indican que hay muchos QTLs con un efecto mínimo y sólo unos pocos tienen un efecto significativo (Brem y Kruglyak, 2005), por lo que es difícil identificar QTLs con efecto mayor en el fenotipo (Salvi y Tuberosa, 2005). Los métodos para la detección y el mapeo de QTLs se basan en el análisis de la asociación entre fenotipos y alelos de marcadores (Collard et al., 2005). El análisis de QTLs implica: 1) la selección y el cruzamiento de líneas parentales que difieren en uno o más caracteres cuantitativos, y 2) el análisis de la segregación de la descendencia para relacionar cada QTL con un marcador de ADN conocido, o intervalo de marcadores (Pérez-Panadés et al., 2003). Existen varios procedimientos para caracterizar los QTLs (Mora et al., 2008). Uno de ellos es el mapeo por intervalos (Lander y Botstein, 1989). Este método considera dos marcadores moleculares adyacentes al QTL (marcador-QTL-marcador) (Rojas y Castellanos, 2007).

Los marcadores son utilizados para dividir la población en diferentes grupos de genotipos basándose en la presencia o ausencia de un *locus* particular y para determinar si existe una diferencia significativa entre grupos respecto al carácter que se está midiendo (Tanksley, 1993; Young, 1996). Una diferencia significativa entre la media fenotípica de los grupos, dependiendo del tipo de población, indica que el *locus* del marcador está ligado a un QTL que controla el carácter en cuestión (Collard et al., 2005).

1.6.3. Factores que influyen sobre la detección de QTLs

Hay muchos factores que influyen en la correcta detección de QTLs que están segregando en una población. Los factores más destacados son las propiedades genéticas de los QTLs que controlan el carácter, los factores ambientales, el tamaño de la población estudiada y el error experimental (Collard et al., 2005). Las propiedades genéticas de los QTLs incluyen la magnitud del efecto de los QTLs individuales ya que se detectan sólo los QTLs con alto efecto fenotípico, y lo cercano que se encuentre el marcador al QTL (Collard et al., 2005). Los QTLs que estén muy próximos (aprox. 20 cM o menos) se detectan como un único QTL en poblaciones de poco tamaño (<500) (Tanksley, 1993). Además, los factores ambientales pueden tener una gran influencia sobre la expresión de los caracteres cuantitativos. Los experimentos que son réplicas entre ubicaciones y en diferentes años de estudio nos permiten estudiar la influencia del factor ambiental sobre los QTLs que controlan los caracteres de interés (Price y Courtois, 1999). Otro factor muy importante a considerar es el tamaño de la población utilizada en el mapeo genético. A mayor tamaño, más posibilidades de detectar QTLs con efecto menor (Haley y Andersson, 1997; Collard et al., 2005). Otra limitación puede ser el error experimental, que es debido a errores del genotipado o de la evaluación de los caracteres fenotípicos. Los errores del genotipado y los datos perdidos pueden afectar al orden y las distancias entre marcadores en los grupos de ligamiento (Collard et al., 2005). También habría que considerar la heredabilidad del carácter. A menor heredabilidad, mayor dificultad para detectarlo por lo que la población tiene que ser mayor. Un criterio para aceptar o rechazar el efecto del QTL es la probabilidad de significación. Si el límite de probabilidad adoptado es muy exigente, será más difícil detectar efectos pequeños, o el tamaño de la población deberá ser mayor.

1.6.4. Análisis de QTLs en *Prunus*

La aproximación de localizar genes candidatos está basada en la identificación y mapeo de secuencias de ADN correspondientes a genes estructurales o reguladores cuyas funciones biológicas tienen efectos sobre caracteres de interés (Illa et al., 2011). Esta aproximación se usó inicialmente para la búsqueda de genes relacionados con la resistencia a enfermedades. En *Prunus*, se ha utilizado para identificar el *locus* de la auto-incompatibilidad floral, el gen de la *endoPG* responsable del carácter fundente vs no fundente de la textura del melocotonero (Illa et al., 2011).

Existen varios estudios en los que se han localizado distintos QTLs en el género *Prunus* (ver Tabla 1.5 como resumen y Arús et al., 2012). Dirlewanger et al. (1998),

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Etienne et al. (2002) y Quilot et al. (2004b) encontraron QTLs implicados en el control de compuestos físico-químicos de calidad de fruta, como azúcares y contenido en ácidos orgánicos, peso del fruto, acidez, fecha de floración y de madurez del fruto. Otros QTLs para pH, acidez y contenido en ácido málico y cítrico están mapeados cerca del gen *D* (GL5), responsable de la baja acidez del fruto (Dirlewanger et al., 1998, 1999). Para la acidez valorable, se han detectado tres QTLs en los GLs 1, 5 y 6. Se han mapeado QTLs para peso fresco del fruto y productividad en el GL 6, cerca del locus *S* que controla la forma del fruto. Por otro lado, también se han detectado QTLs con efecto mayor para la fecha de floración en el GL4, fecha de madurez, color de la piel del fruto, y sólidos solubles en los GL2 y 6 (Quilot et al., 2004b; Eduardo et al., 2011). Eduardo y colaboradores (2011) detectaron un alto efecto pleiotrópico de la fecha de maduración del fruto en los caracteres de calidad como el peso del fruto, la acidez, los sólidos solubles y el color, utilizando una población de melocotonero derivada de un cruzamiento intra-específico entre las variedades 'Contender' x 'Ambra' (Tabla 1.5).

En cuanto a los daños por frío, se han localizado tanto QTLs como genes candidatos en distintos grupos de ligamiento (Tabla 1.5). Genes que codifican para la enzima endopoligalacturonasa, que controla los caracteres hueso libre y pulpa fundente (Peace et al., 2005), y leucoanthocanidin dioxygenasa, co-localizan con el QTL mayor asociado con pardeamiento de la pulpa (Ogundiwin et al., 2008). También se han detectado QTLs en relación con el control de daños por frío en la población 'Georgia Belle' x 'Dr. Davis' (Peace et al., 2005). También Ogundiwin et al. (2009) y Cantín et al. (2010a) identificaron 3 zonas en el grupo de ligamiento GL4 asociadas al pardeamiento y a la susceptibilidad a los daños por frío en distintas poblaciones de mejora en melocotonero.

Veintiocho genes candidatos (Dirlewanger et al., 2004a) y veintiocho QTLs (Arús et al., 2012) responsables de la floración, características de hojas y frutos, arquitectura de la planta y resistencia a enfermedades, están localizados en el mapa de referencia TxE, permitiendo la identificación de muchos marcadores moleculares transferibles (RFLPs, SSRs, etc.) en estas zonas del genoma donde se han encontrado estos genes o QTLs. Illa y colaboradores (2011) han identificado 273 genes candidatos relacionados con el aroma, la acidez, firmeza, color, desarrollo del fruto, y se han mapeado en el mapa de referencia de melocotonero (TxE).

Tabla 1.5. QTLs implicados en el control de distintos caracteres relacionados con la calidad del fruto.

Caracteres	GL	Mapa	Referencias
pH	5, 6, 8	'Ferjalou-Jalousia' x 'Fantasia'	Dirlewanger et al. (1998)
Ácido quínico	1	=	=
Sacarosa	5, 6	=	=
Fructosa	3, 4, 5, 8	=	=
Sorbitol	1, 6	=	=
Glucosa	8	=	=
SSC	1, 5, 6	=	=
Peso del fruto y locus *S*	6	'Ferjalou-Jalousia' x 'Fantasia'	Dirlewanger et al. (1999) Etienne et al. (2002)
locus *D*	5	=	=
TA	1, 5, 6	=	=
Ácido málico	1, 5, 6	=	=
Ácido cítrico	5, 6, 9	=	=
pH	2	'IF731' x *P. ferganensis*	Quarta et al. (2000) Verde et al. (2002)
Azúcares totales	1, 2	=	=
Resistencia mildiú	7	=	=
Fecha floración	4	=	=
Fecha maduración	2, 6	=	=
Color piel	2, 6	=	=
SSC	1, 2, 6	=	=
Melocotón/nectarina	5	*P. davidiana* x 'Summergrand'	Quilot et al. (2004b)
Fecha maduración	4	=	=
Fecha floración	1, 2	=	=
Peso del fruto	4, 5, 7	=	=
Color de la piel	5	=	=
Color pulpa	1, 3	=	=
TA	2, 3, 4, 5	=	=
Dulzor	1, 3, 5	=	=
SSC	4, 5	=	=
Sacarosa	6, 7	=	=
Glucosa	2, 4, 5, 7	=	=
Fructosa	1,2, 4, 7	=	=
Sorbitol	2, 4, 5	=	=
Azúcares totales	1, 5, 6	=	=
Ácido quínico	4, 5, 6, 7	=	=
Ácido cítrico	1, 3, 4, 7	=	=
Ácido málico	2, 3, 5, 6	=	=
Daños por frío	1, 4, 5	'Georgia Belle' x 'Dr. Davis'	Peace et al. (2005)
Daños por frío	1, 2, 4, 5	'Dr. Davis' x 'Georgia Belle'	Ogundiwin et al. (2009)
Daños por frío	4	'Venus' x 'Big Top'	Cantín et al. (2010a)
Fecha maduración	4	'Contender' x 'Ambra'	Eduardo et al. (2011)
Peso del fruto	1, 2, 4, 6	=	=
Color de la piel	4, 6	=	=
pH	4	=	=
TA	4	=	=
SSC	4	=	=

CAPÍTULO 2

Objetivos

Objetivos

El objetivo principal de la tesis ha sido estudiar en dos poblaciones de mejora de melocotonero la variabilidad fenotípica en los caracteres agronómicos y de calidad del fruto (contenido de antioxidantes, de azúcares totales y de compuestos volátiles), y localizar QTLs implicados en el control genético de estos caracteres.

Entre los objetivos específicos se encuentran:

1) Evaluación de la diversidad fenotípica en dos poblaciones de melocotonero. Estudio e interacción entre los caracteres agronómicos y contenido en antioxidantes y azúcares totales del fruto en las poblaciones 'Venus' x 'Big Top' y 'Babygold 9' x 'VAC-9510'.

2) Evaluación, identificación y cuantificación de compuestos volátiles del fruto en la población de nectarina 'Venus' x 'Big Top'. Estudio y comparación de los perfiles aromáticos en los parentales y en la progenie.

3) Evaluación de la calidad postcosecha en cuanto a la susceptibilidad a los daños por frío del fruto en la población 'Babygold 9' x 'VAC-9510'. Influencia de los niveles de compuestos antioxidantes en el desarrollo de daños por frío.

4) Mapeo genético en la población 'Venus' x 'Big Top' mediante marcadores SNPs y SSRs y localización de QTLs implicados en el control genético de los caracteres bioquímicos estudiados en fruto, con especial interés en los caracteres organolépticos más importantes del melocotonero.

CAPÍTULO 3

Evaluation of antioxidant compounds and total sugar content in a nectarine [Prunus persica (L.) Batsch] progeny

Evaluation of antioxidant compounds and total sugar content in a nectarine [*Prunus persica* (L.) Batsch] progeny

Abstract

Epidemiological studies suggest that consumption of fruit rich in phenolic compounds is associated with health-protective effects due to their antioxidant properties. For these reasons quality evaluation has become an important issue in fruit industry and in breeding programs. Phytochemical traits such as total phenolics, flavonoids, anthocyanins, L-ascorbic acid, sugar content and relative antioxidant capacity (RAC) were analyzed over four years in flesh fruit of an F1 population "Venus" × "Big Top" nectarines. Other traits such as harvesting date, yield, fruit weight, firmness, soluble solids concentration (SSC), pH, titratable acidity (TA) and ripening index (RI) were also determined in the progeny. Results showed high variability among genotypes for all analyzed traits. Total phenolics and flavonoids showed significant positive correlations with RAC implying that both are important antioxidant bioactive compounds in peaches. We found genotypes with enhanced antioxidant capacity and a better performance than progenitors, and in consequence the best marketability.

3.1. Introduction

Peach [*Prunus persica* (L.) Batsch] production has an important place in the world (18.6 million tons in 2009) with a cultivated area of around 1.6 million ha (FAOSTAT, 2011). Peaches and nectarines are, after apples, the second most important fruit crop in the European Union (EU), with a production of 4.1 million tons in 2009 and a cultivated area of around 245,191 ha (FAOSTAT, 2011). Spain is the third largest producer in the world, after China and Italy, and the second in Europe, with a production of 1.2 million tons in 2009 and a cultivated area of 72,000 ha (FAOSTAT, 2011).

Peaches are a popular summer fruit and there has been an increasing interest in their nutritional value (Wolfe et al., 2008). Many epidemiological studies suggest that increased fruit consumption decreases the risk of several degenerative diseases including atherosclerosis, heart and brain disorders, and different types of cancer (Hegedús et al., 2010) which are still responsible for the highest mortality rate in Western countries (Müller et al., 2010). In particular, the consumption of peaches can suppress reactive oxygen species (ROS) in human plasma and provide protection from chronic diseases (Tsantili et al., 2010). Fruits have recently been accepted as a functional food, because of its low caloric content along with its high level of antioxidant and nutritional compounds, such as

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vitamins, phenols, minerals or fiber that could play an important role in preventing oxidative stress (Orazem et al., 2011). The phenolic compounds (anthocyanins, flavonoids, *etc.*) give fruits with both desirable qualities like color and antioxidant properties and undesirable qualities like astringency and bitterness (Bravo, 1998). Slimestad et al. (2009) reported that there is a correlation between taste (astringency, bitterness) and content of phenolic compounds which have an important role in the natural defense mechanisms and health benefits of fruits. Moreover, Koh et al. (2009) reported that flavonoids are particularly interesting as they are potent *in vitro* antioxidants and are thought to play key roles in many of the processes underlying vascular dysfunction.

In recent years, there has been an increased interest in breeding programs identifying and quantifying phenolic substances in fruit in order to evaluate their potential health-promoting properties, as well as their visual appearance (pigmentation and browning) and taste (astringency) (Orazem et al., 2011; Cantín et al., 2009b). Peaches could be of great interest as an important antioxidant source and intake of these fruits may provide health-promoting advantages (Rossato et al., 2009).

Apart from the antioxidant evaluation, peach breeding programs have stressed the importance of taste in the selection of new cultivars (Crisosto and Crisosto, 2005). Orazem et al. (2011) reported that the edible quality of peaches depends to a great extent on their sweetness, and that the amounts of sucrose, sorbitol and malic acid correlate positively with the taste and aroma of peach fruit. Sweetness and acidity are the most important factors affecting consumer acceptability of stone fruits and these factors are strictly correlated (Rossato et al., 2009). Sucrose, glucose and fructose are the main sugars in peaches (Génard et al., 2003; Cantín et al., 2009a), and in ripe fruit, these sugars comprise about 60% of the soluble solids concentration (SSC). The relative concentrations of these sugars also influence sweetness, as fructose is 2.3 times and 1.7 times sweeter than glucose and sucrose, respectively (Kulp et al., 1991).

The main objective of this work was to evaluate, in a nectarine segregating F1 population derived from "Venus" × "Big Top" over a 4-year study, the existing phenotypic diversity of antioxidant compounds and total sugar content among genotypes, and to study the relationships among agronomic and biochemical fruit quality traits. The correlations between biochemical and agronomic traits will be very useful in peach breeding programs. The ultimate objective of this study was to select superior genotypes with enhanced antioxidant capacity in fruits that will benefit consumers with health-promoting properties.

3.2. Experimental section

3.2.1. Plant material

The progeny assayed was a segregant F1 population of 75 seedlings obtained from a controlled cross, between *Prunus persica* cvs. "Venus" (female parent) and "Big Top" (male parent). "Venus" is a freestone, melting and yellow flesh nectarine cultivar, whereas "Big Top" is a clingstone, melting and yellow flesh nectarine cultivar. The segregant population is entirely melting flesh, either cling- or freestone. 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 × 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. Samples were harvested over four years (2007–2010).

3.2.2. Agronomical and basic fruit quality parameters

During the years 2007–2010, agronomic and fruit quality traits were measured individually in each seedling tree. Harvesting date and annual yield were evaluated in each independent seedling. Harvesting date ranged from first-July to mid-August, depending on the genotypes. Fruits were handpicked at commercial maturity and assessed by peel fruit color and flesh firmness. Fruits were considered ripe in the tree when their growth had stopped, exhibited orange-red ground color, began softening, and were easily detached. Yield (kg/tree) was measured and a representative fruit sample (20 fruits) was taken for fruit quality evaluations (Cantín et al., 2009b). Fruit weight was 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 and data were expressed in Newtons. The 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 initial pH and titratable acidity (TA) were measured by automatic titration system with NaOH 0.1 N to pH 8.1 (862 Compact Titrosampler); data are given as g malic acid per 100 g FW, since this is the dominant organic acid in peach. The ripening index (RI) was calculated as the ratio between SSC and TA.

3.2.3. Phytochemical extraction

For all analyses only fruit flesh was used, as it is usually consumed. Twenty representative fruits were peeled with a sharp knife, flesh was weighted, immediately frozen separately in liquid nitrogen, and stored at −20 °C until analysis. For vitamin C determination, samples at harvest were kept in 5 mL of 5% metaphosphoric acid for preservation of ascorbic acid, frozen in liquid nitrogen and stored at −20 °C until analysis. Samples were homogenized with a polytron in 5 mL 5% metaphosphoric acid and centrifuged at 20,000 g for 20 min at 4 °C, filtered with Miracloth and the supernatant was used for vitamin C analysis. For phenolic compounds, frozen fruit material (5 g) was homogenized in a polytron with 10 mL of extraction solution, consisting of 0.5 N HCl in methanol/Milli-Q water (80% v/v). The mixture was then centrifuged for 20 min at 4 °C and 20,000 g. Supernatant was recovered and the volume measured. This hydroalcoholic extract was used for total phenolics, flavonoids, anthocyanins and antioxidant capacity assays. For the determination of sugars, the frozen fruit material (5 g) was homogenized in a Polytron with 10 mL of extraction solution consisting of ethanol/Milli-Q water (80% v/v). The mixture was centrifuged at 20,000 g for 20 min at 4 °C. The supernatant was recovered and processed to be assayed by high-performance liquid chromatography (HPLC) as described by Cantín et al. (2009a) with some modifications.

3.2.4. Antioxidant compounds analysis

Vitamin C, total phenolics, flavonoids, anthocyanins and relative antioxidant capacity were evaluated with colorimetric methods and measured using a spectrophotometer (Beckman Coulter DU 800) as described by Cantín et al. (2009b) and methods therein. In order to avoid interferences, other analysis could be recomended for specific determinations of anthocyanins, flavonoids and total phenolics (Tabart et al., 2010; Escarpa and González, 2001). Standard calibration curves were daily prepared. For vitamin C determinations, absorbance was measured at 525 nm and the amount of vitamin C was expressed as mg of ascorbic acid (AsA) per 100 g fresh weight (FW). For total phenolics content, the colorimetric method based on the chemical reduction of the Folin-Ciocalteau reagent was used. Absorbance was measured at 725 nm and the content was expressed in milligrams of gallic acid (3,4,5-Trihydroxy-benzoic acid) equivalents (GAE) per 100 g of FW. Total flavonoids content was determined measuring absorbance at 510 nm and the results were expressed as milligrams of catechin equivalents (CE) per 100 g of FW. The total anthocyanins content was evaluated measuring in the hydroalcoholic extract the

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absorbance at 535 and 700 nm. The anthocyanins concentration was calculated using the molar extinction absorptivity coefficient ε = 25,965/cm M and was expressed in mg of cyanidin 3-glucoside equivalents (C3GE) per kg of FW. The relative antioxidant capacity (RAC) was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). The absorbance was measured after 10 min of reaction at 515 nm and RAC was expressed as μg of Trolox equivalents per g of FW.

3.2.5. Total sugars analysis

To estimate the variation in sugar profile among genotypes, sugar composition and quantification were measured as described by Cantín et al. (2009a). For the analysis, 250 μL of the homogenized extract was incubated at 80 °C for 20 min in 200 μL of 800 mL/L ethanol, with 5 g/L manitol added as an internal standard. Samples were purified using ion exchange resins (Bio-Rad Barcelona, Spain) (Jiménez et al., 2011). Twenty μL was injected into the HPLC system (Aminex HPX-87C column, 300 mm × 7.8 mm; Bio-Rad, Barcelona, Spain) with a refractive index detector (Waters 2410). The solvent was deionized water running at a flow rate of 0.6 mL per min at 85 °C. Sugar quantification was performed with Millenium 3.2 software (Waters) using standards of analytical grade (Panreac Quimica SA, Barcelona, Spain). Sugar concentrations were expressed as g per kg FW.

3.2.6. Statistical analysis

All traits were measured or scored for each genotype separately over the four year period, and means of four years were calculated. All statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL). To obtain basic statistics for the entire plant material studied, minimum and maximum values, mean and mean standard error (SE), were calculated for each trait. Data for each genotype were averaged, and mean values were used as estimated genotypic values. Finally, correlations were calculated with raw data of the four years, according to Pearson's test at $P \leq 0.01$. Principal component analysis (PCA) was applied on the antioxidant compounds and basic agronomical traits in the studied population as an attempt to identify superior genotypes based on their antioxidant compound contents. The component matrix was evaluated and orthogonal factors were rotated using variance maximizing (Varimax).

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3.3. Results and discussion

3.3.1. Agronomical and basic fruit quality traits

Traits were evaluated in both parents and each seedling separately over four years (2007–2010) of study (Table 3.1). Mean values of fruit weight, firmness, soluble solids content (SSC), pH, titratable acidity (TA) and the ripening index ratio (RI = SSC/TA) were calculated for parents ("Venus" and "Big Top") as well as for the 75 individual seedling progeny. Results showed high variability among genotypes for the different agronomic and fruit quality traits evaluated. Means for all analyzed traits were inside the interval values obtained for the parents and exhibited continuous variation, which is typical of quantitative or polygenic inheritance. The fruit weight varied greatly among genotypes (190.2 ± 3.8 g) as a consequence of the variability in tree production and fruits number for each seedling. Fruit weight is a major quantitative inherited factor determining yield, fruit quality and consumer acceptability (Dirlewanger et al., 1999). The mean value for yield in the progeny for 2007–2010 was 6.9 ± 0.3 kg per tree with high variability among genotypes, which may occur due to year and genotype (data not shown). These values were similar to those obtained by Cantín et al. (2010b) in the same population during three years. Milatović et al. (2010) reported that yield of the peach tree depends on different factors, such as density of flower buds and flowers, fruit set, fruit size, winter and late spring freeze damage, precipitation amount, and orchard management. The variation for agronomic and basic biochemical fruit quality traits among years of study showed lower yield for years 2008 and 2010 (5.9 and 5.4 kg, respectively) compared with the mean value of four years (6.9 ± 0.3 kg) and consequently higher fruit weight for these two years (209.0 and 214.2 g, respectively) than the average weight (190.2 ± 3.8 g). This variability in annual yield was mainly due to rains damage which occurred in the full blooming of the population limiting the number of available fruits. The pH, fruit firmness and SSC showed low variability among years (data not shown).

Table 3.1. Agronomical and basic fruit quality traits in the "Venus" × "Big Top" population. For progenitors data are means ± SE of two years (2009–2010). For the progeny (*n* = 75 genotypes), data are means ± SE of four years of study (2007–2010).

Traits	Progenitors		Progeny[a]		
	"Venus"	"Big Top"	Min	Max	Mean ± SE
Fruit weight	178.0 ± 58.3	204.0 ± 39.3	109.2	261.8	190.2 ± 3.8
Firmness	36.1 ± 0.1	49.2 ± 6.9	24.2	50.7	39.2 ± 0.6
SSC	13.9 ± 0.1	14.4 ± 0.1	11.2	17.5	13.9 ± 0.2
pH	3.4 ± 0.1	3.9 ± 0.1	3.2	4.0	3.6 ± 0.1
TA	0.7 ± 0.1	0.4 ± 0.1	0.5	1.1	0.7 ± 0.1
RI	20.3 ± 0.3	35.2 ± 0.3	13.8	35.8	23.8 ± 0.7

[a] Data from 2007 were partially presented in Cantín *et al.* (2010b). Units and abbreviations: Fruit weight (g); Firmness (N); N = Newtons; SSC = Soluble solids content (°Brix); TA = Titratableacidity (g malic acid/100 g FW); RI = Ripening index (SSC/TA).

Regarding flesh fruit firmness, it ranged from 24.2 to 50.7 N with higher variability among seedlings. The two progenitors and some genotypes of the progeny showed firmness values higher than 35 N, which has been defined as the threshold between mature and immature fruit (Valero et al., 2007). Our analysis revealed a mean firmness of 39.2 N (4.08 kg/cm^2) which is lower than the maximum level of fruit firmness for marketing fresh peaches and nectarines, set by the EU at a 6.5 kg/cm^2 (=63.7 N), using a 8 mm diameter probe (Commission Regulation EC, No.1861/2004 of 28 October 2004).

Regarding SSC, the population showed values from 11.2 to 17.5 °Brix with a mean of 13.9 ± 0.2 °Brix, which is greater than the minimum (8 °Brix) established by the EU to market peaches and nectarines (R-CE No.1861/2004). Kader (1999) considered mean values of SSC over 10 °Brix as the minimum value for consumer acceptance for yellow-flesh nectarines, which is the case in our progeny. The variability found in SSC among seedlings can be explained by the quantitative performance of this quality trait (Quilot et al., 2004a).

The pH values varied from 3.2 to 4.0 with a mean value of 3.6 ± 0.1, which are values of normal acidity fruits. The progeny showed acid and non-acid fruits, since fruit with a pH higher than 4.0 at maturity are considered as non-acid (Monet, 1979). The progeny showed variability of TA among genotypes with a mean value of 0.7 ± 0.1 g malic acid per 100 g fresh weight (FW), which is lower than the maximum limit (0.9%) for normal acidity peaches (Hilaire, 2003). "Venus" is an acid nectarine (TA = 0.7%), and "Big Top" is a non-acid nectarine (TA = 0.4%), which explains the segregation of this trait in the progeny.

The ripening index (RI = SSC/TA) ranged from 13.8 to 35.8 among genotypes, depending on their SSC and TA values. In peaches, the RI is a major organoleptic quality trait of the mature fruit and is commonly used as a quality index (Bassi and Selli, 1990). The relationship between TA and SSC has an important role in consumer acceptance of some apricot, peach, nectarine and plum cultivars. Crisosto et al. (2004) reported that in the case of cultivars with TA > 0.9% and SSC < 12.0 °Brix, consumer acceptance was controlled by the interaction between TA and SSC rather than SSC alone. Our results showed only four genotypes with the mean value of TA > 0.9% and the mean value of SSC < 12.0 °Brix (3, 14, 58 and 65).

3.3.2. Correlations between agronomical and basic biochemical traits

Significant correlations were found among pomological traits related to fruit quality. In the progeny, annual yield was positively correlated with fruit weight ($r = 0.278$, $P \leq 0.05$), also a positive correlation was found for SSC and RI ($r = 0.263$, $P \leq 0.05$). Firmness was significantly correlated with SSC ($r = 0.367$, $P \leq 0.01$), pH ($r = 0.385$, $P \leq 0.01$) and RI ($r = 0.347$, $P \leq 0.01$). Similar low correlations were found for Cantín et al. (2010b) when studied 1100 peach genotypes. The positive correlation between firmness and SSC is important since the selection of genotypes with high SSC will aim first at higher firmness and second at lower susceptibility to mechanical damage during handling and packaging (Crisosto et al., 2001). The ripening index showed a high positive correlation with pH ($r = 0.930$, $P \leq 0.01$), and a negative correlation with titratable acidity ($r = -0.315$, $P \leq 0.01$) indicating that in our progeny when most of the fruits are mature pH seems to increase and acidity to decrease. The pH showed a negative correlation with TA ($r = -0.343$, $P \leq 0.01$) in this progeny, as other authors reported in different peach genotypes (Cantín et al., 2009b; Dirlewanger et al., 1999).

3.3.3. Antioxidant compounds content

The antioxidant compounds content in the "Venus" × "Big Top" progeny, showed a high variability among genotypes (Table 3.2). The ascorbic acid (AsA) content ranged from 2.1 to 7.2 mg of AsA/100 g of FW, with a mean value of 4.0 ± 0.1 mg of AsA/100 g of FW. The parents, "Venus" and "Big Top", differed for vitamin C content, and as a consequence the progeny showed high segregation among genotypes. Our results indicate that peach is a good source of vitamin C and highlight the fact that ascorbic acid content is an important part of the overall evaluation of peach fruit quality. 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 (Tavarini et al., 2008). Cantín et al. (2009b) reported that total ascorbic acid content in 218 peach genotypes from different progenies varied greatly 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. Preliminary data obtained by these authors in this progeny but only tested during one year of study, showed lower values (2.6 mg of AsA/100 g of FW) when studying a short number of genotypes. Also Gil et al. (2002) quantifying the total ascorbic acid contents of nectarine cultivars from California reported contents from 6 to 8 mg of AsA/100 g of FW in yellow-flesh nectarines and from 5 to 14 mg/100 g of FW in white-flesh nectarines.

Table 3.2. Content of antioxidant compounds in the "Venus" × "Big Top" population. For progenitors, data are means ± SE of two years (2009–2010). For the progeny (*n* = 42–75 genotypes), data are means ± SE of four years of study (2007–2010).

	Progenitors		Progeny		
Compounds	**"Venus"**	**"Big Top"**	**Min**	**Max**	**Mean ± SE**
Vitamin C	3.0 ± 0.7	4.9 ± 0.7	2.1	7.2	4.0 ± 0.1
Total phenolics	22.1 ± 8.0	26.4 ± 9.8	22.5	49.2	32.6 ± 0.7
Flavonoids	7.6 ± 3.8	7.8 ± 4.6	5.9	33.8	12.5 ± 0.6
Anthocyanins	2.1 ± 0.1	5.9 ± 2.2	1.2	9.5	3.2 ± 0.2
RAC	386.1 ± 18.5	521.4 ± 47.4	292.4	835.8	464.2 ± 12.5

Units: Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of FW); Anthocyanins (mg C3GE/kg of FW); RAC; Relative Antioxidant Capacity (µg Trolox Equivalents/g of FW). Abbreviations: AsA = Ascorbic acid; C3GE = Cyanidin-3-glucoside equivalents; CE = Catechin equivalents; GAE = Gallic acid equivalents.

Total phenolics ranged from 22.5 to 49.2 mg of gallic acid equivalent (GAE) per 100 g of FW. The amount of total phenolics in our progeny fell within the range reported in the literature for peach fruits, namely 14–77 mg GAE/100 g of FW. Tavarini et al. (2008) reported similar total phenolics amounts (14–50 mg GAE/100 g of FW) in peach cultivars and other similar results were reported by Gil et al. (2002) in yellow-flesh nectarines (18 to 54 mg GAE/100 g of FW).

Regarding flavonoids, it ranged from 5.9 to 33.8 mg catechin equivalent (CE) per 100 g of FW in our progeny with an average of 12.5 ± 0.6 mg of CE/100 g of FW. These results, revealed flavonoids content similar to that obtained by Cantín et al. (2009b) in peach and nectarine progenies ranging from 1.8 to 30.9 mg of CE/100 g of FW, with an average of 8.8 mg of CE/100 g of FW. It is remarkable that the "Venus" × "BigTop" progeny showed higher total phenolics and flavonoids content when compared with the parents. This fact could be very interesting in the peach genotype selection process, mainly

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selecting fruits rich in flavonoids, since the consumption of flavonoid-rich foods holds the potential to limit neurodegeneration preventing age-dependent loses in cognitive performance (Vauzour et al., 2008).

The anthocyanins content ranged from 1.2 to 9.5 mg cyanidin-3-glucoside equivalents (C3GE) per kg of FW, showing less variability among genotypes due to the similar flesh color of seedlings and lower concentrations compared to the study of Cantín et al. (2009b) who reported that in some progenies total anthocyanins greatly varied among genotypes (0.1–26.7 mg of C3GE/kg of FW) depending on the red pigmentation of the flesh.

The relative antioxidant capacity (RAC) ranged from 292.4 to 835.8 (µg Trolox Equivalents (TE) per g of FW) showing a high variability among genotypes (mean value was 462.2 ± 12.5 µg TE/g of FW). This could be explained by the fact that the antioxidant capacity of fruits varies in relation to the antioxidant molecules present in the different species but variations can also occur within the genotypes of a single species (Gil et al., 2002). Cantín et al. (2009b) reported values of RAC (227.3 to 629.9 µg of TE/g of FW, with an average of 405 µg of TE/g of FW) in the same range of these results even slightly lower. In general, antioxidant compounds content presented here were higher than those found for these authors when less genotypes of the same progeny were analyzed.

To evaluate the influence of the different environmental conditions on the fruit antioxidant compounds content, data related to 2007, 2008, 2009 and 2010 were separately evaluated (Table 3.3).

Table 3.3. Annual amounts of antioxidant compounds in the "Venus" × "Big Top" progeny. Data are means ± SE of four years of study (2007–2010) ($n = 42$–75 genotypes).

Compounds	2007	2008	2009	2010	Mean ± SE
Vitamin C	2.8 ± 0.1	3.9 ± 0.2	6.3 ± 0.2	3.2 ± 0.2	4.0 ± 0.1
Total phenolics	36.9 ± 1.7	44.2 ± 0.7	21.2 ± 0.6	23.3 ± 0.8	32.6 ± 0.7
Flavonoids	12.9 ± 1.0	21.7 ± 1.2	6.5 ± 0.4	8.1 ± 0.6	12.5 ± 0.6
Anthocyanins	2.2 ± 0.2	1.7 ± 0.1	4.0 ± 0.5	4.6 ± 0.4	3.2 ± 0.2
RAC	380.6 ± 14.0	617.0 ± 23.0	322.6 ± 11.0	444.8 ± 10.0	464.2 ± 12.5

Units: Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of FW); Anthocyanins (mg C3GE/kg of FW); RAC; Relative Antioxidant Capacity (µg Trolox Equivalents/g of FW). Abbreviations: AsA = Ascorbic acid; C3GE = Cyanidin-3-glucoside equivalents; CE = Catechin equivalents; GAE = Gallic acid equivalents.

An interesting year-to-year and genotype-to-genotype variability differences in the antioxidant compounds were outlined. The vitamin C content showed higher values in 2009, whereas total phenolics, flavonoids and RAC showed comparable values among

years. The anthocyanins content showed similar mean values in 2009 and 2010 but higher than those observed in the previous two years of the study, this could be due to the period of maturity of fruit and the harvest date which were more similar in those years. In 2008, the total phenolics, flavonoids content and the antioxidant capacity of the flesh fruit were notably higher than in the other years. These changes found in the antioxidant compounds content could be as a result of growing conditions, pre and postharvest conditions and genetic factors affecting the antioxidant compounds content. Harvesting date showed significant negative correlation with flavonoids ($r = -0.331$, $P \leq 0.01$) and sucrose content ($r = -0.310$, $P \leq 0.01$) indicating that harvesting time could present variability among years and consequently influence the antioxidant and total sugar content among genotypes.

The antioxidant content in the analyzed fruits should be attributed in part to the important role of the rootstock on fruit quality as Giorgi et al. (2005) reported. Moreover, the environmental conditions to which the genotypes are subjected, and the annual climatic changes may partly explain the different accumulation patterns of antioxidant compounds. As a consequence, only the evaluation of several years of harvest may lead to an accurate assessment in the selection of new peach-rootstock combination (Giorgi et al., 2005; Tulipani et al., 2011).

All the antioxidant traits studied, except for flavonoids and anthocyanins (data not shown), showed a normal distribution (Figure 3.1) which is typical of quantitative characters.

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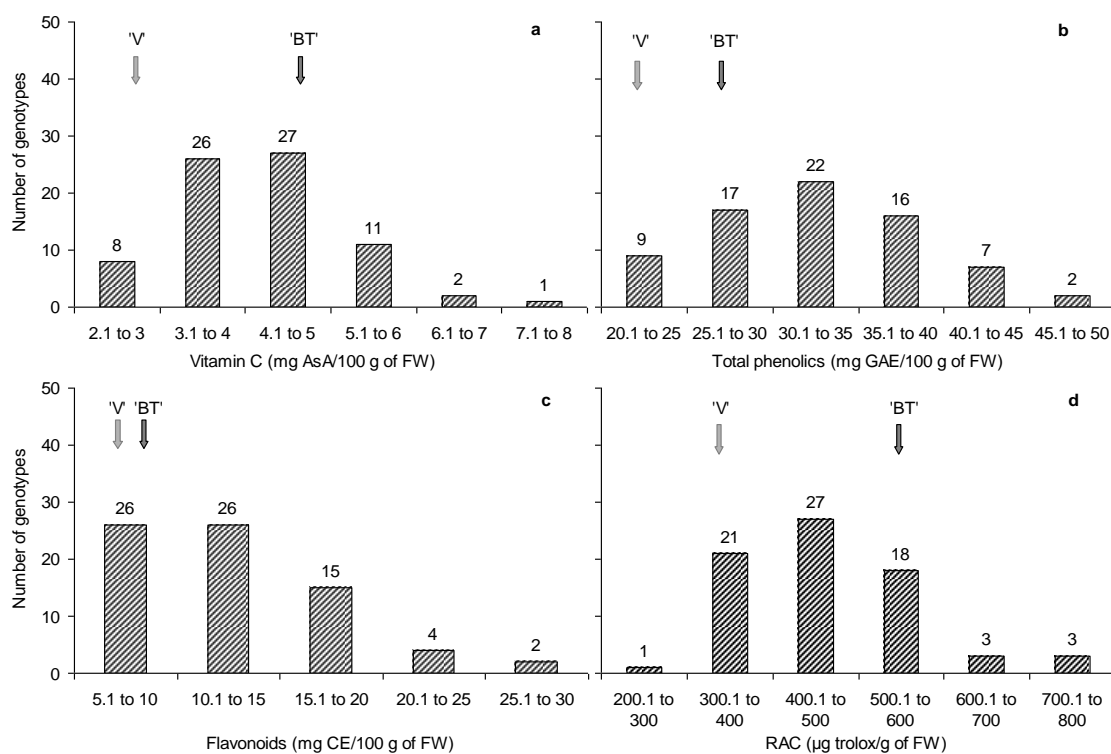
Figure 3.1. Segregation of (a) vitamin C, (b) total phenolics, (c) flavonoids, and (d) antioxidant capacity (RAC), in the "Venus" × "Big Top" progeny. Data are means (*n* = 42–75 genotypes) of four years of study (2007–2010). Arrows show the values for the parents "Venus" ('V') and "Big Top" ('BT').

Segregation was also observed when comparing the progeny with its parents, and some genotypes showed even higher values than "Venus" and "Big Top". For vitamin C, at least ten genotypes (12, 27, 37, 38, 39, 40, 42, 43, 52 and 74) showed higher vitamin C contents. For total phenolics, most of the progeny (5, 10, 18, 20, 23, 32, 35, 37, 40, 43, 44, 47, 61 and 74) showed higher contents compared with "Venus" and "Big Top". The same thing occurred in the flavonoids content and these genotypes (10, 23, 28, 35, 37, 40, 43, 44, 47, 61 and 74) showing higher contents compared with the progenitors. For the relative antioxidant activity, seventeen genotypes (9, 10, 18, 20, 21, 23, 24, 27, 32, 35, 36, 37, 43, 44, 47, 61 and 74) showed values higher than the progenitors. In general, at least nine genotypes (18, 27, 32, 35, 37, 43, 44, 47 and 74) can be highlighted due to their higher contents of antioxidant compounds.

3.3.4. Total sugar content

The sucrose, glucose, fructose and sorbitol contents in the "Venus" × "Big Top" progeny were analyzed separately (Table 3.4), as they play an important role in peach flavor quality (Robertson et al., 1988).

Table 3.4. Sugar content (g per kg FW) in the "Venus" × "Big Top" population. For progenitors data are means ± SE of two years (2009–2010). For the progeny (*n* = 42–75 genotypes), data are means ± SE of four years of study (2007–2010).

	Progenitors		Progeny		
Sugar content	**"Venus"**	**"Big Top"**	**Min**	**Max**	**Mean ± SE**
Sucrose	41.0 ± 5.7	85.1 ± 17.0	40.7	102.3	58.4 ± 1.2
Glucose	10.0 ± 0.4	8.9 ± 0.8	8.3	23.4	12.2 ± 0.3
Fructose	13.4 ± 0.5	10.9 ± 1.7	8.9	19.1	12.4 ± 0.2
Sorbitol	8.6 ± 3.8	6.5 ± 1.7	1.7	19.5	6.6 ± 0.5
Sucrose/glucose	4.0 ± 0.4	9.5 ± 2.1	3.2	7.6	4.9 ± 0.1
Glucose/fructose	0.7 ± 0.1	0.8 ± 0.1	0.8	1.2	0.9 ± 0.1
% Sorbitol	5.6 ± 1.9	2.9 ± 0.4	1.1	8.7	3.5 ± 0.2
Total sugars	73.0 ± 9.6	111.5 ± 14.1	67.4	138.9	89.7 ± 1.6

The studied population exhibited considerable phenotypic variation in sugar contents among genotypes. Mean values for all sugars were inside the interval values obtained for the parents, except for glucose that were higher, and these contents exhibited continuous variation, which is typical of quantitative or polygenic inheritance. Sucrose was the major sugar present in the evaluated genotypes, with 58.4 ± 1.2 g per kg FW, followed by fructose, glucose and sorbitol. The sorbitol content varied greatly among genotypes, ranging from 1.7 to 19.5 g per kg FW. Consequently, the percentage of sorbitol in the sugar composition was significantly different among genotypes, ranging from 1.1 to 8.7%. Colaric et al. (2005) reported that sorbitol was the attribute most related to peach aroma and taste among carbohydrates and organic acids.

Wu et al. (2005) reported that sucrose in peaches is dominant at maturity, followed by the reducing sugars (glucose and fructose) and then sorbitol. In our progeny the mean levels of glucose and fructose were quite similar (mean glucose/fructose ratio = 0.98) and about five times lower than the mean value for sucrose (mean sucrose/glucose ratio = 4.9). Some researchers reported glucose and fructose in comparable amounts (Wu et al., 2005). However, a slight variation in glucose/fructose ratio (from 0.8 to 1.2) was detected in the studied seedlings. Identifying genotypes with low glucose/fructose ratio might be of particular interest, since the relative concentrations of these sugars influence sweetness, as fructose is 2.3 times and 1.7 times sweeter than glucose and sucrose, respectively (Kulp et

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al., 1991). In agreement, Robertson and Meredith (1988) found that "high quality" peaches contained lower glucose/fructose ratios than "low-quality" peaches.

Total sugar content (the sum of sucrose, glucose, fructose and sorbitol) in peeled fruits ranged from 67.4 to 138.9 g per kg FW with an average of 89.7 ± 1.6 g per kg FW. Total sugar content is an important quality trait in fruit breeding programs, since it has been reported to be highly related to the aroma and taste of peaches and nectarines (Colaric et al., 2005). The "Venus" cultivar showed lower content than that observed by Colaric et al. (2005). Quilot et al. (2004a) reported that for total sugar content, variation among trees, among fruits of the same tree, and among years are not negligible in comparison with the variation among genotypes. Cantín et al. (2009a) studying 205 genotypes from different progenies reported that the average content of total sugars in the peeled fruit was 72.1 g per kg FW in peaches and 77.1 g per kg FW in nectarines.

The annual variation for sugars compounds and total sugar content (Figure 3.2) showed small variation among years except for 2008. This year high values of sucrose, glucose and fructose were obtained leading to high total sugar content in agreement with the high SSC found (15.5 °Brix).
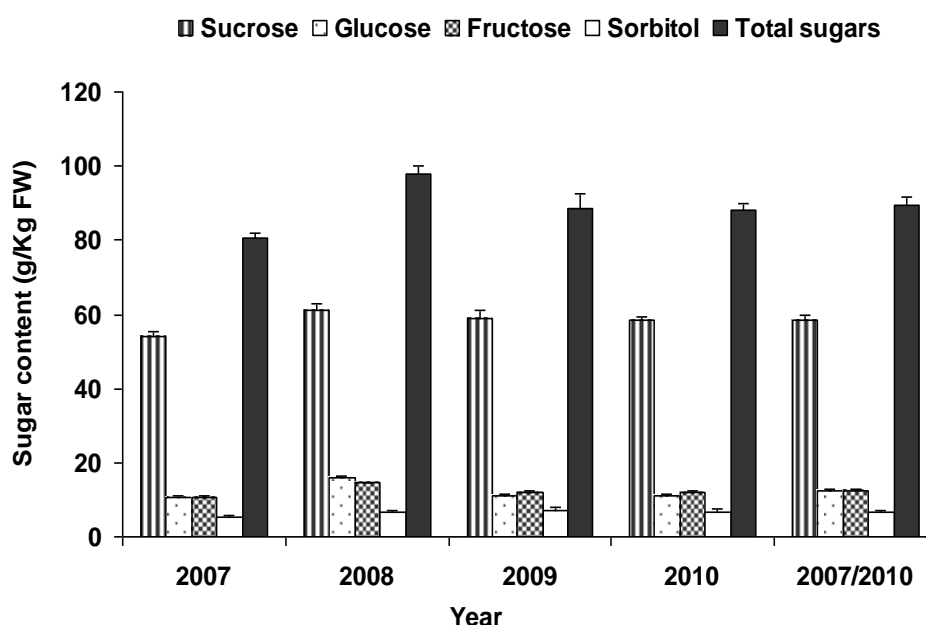
Figure 3.2. Annual amounts of sugar compounds (g per kg of FW) in the "Venus" × "Big Top" progeny. Data are means ± SE of four years of study (2007–2010) (n = 42–75 genotypes).

3.3.5. Correlations between phytochemicals traits

We found significant positive correlations ($P \leq 0.01$) of relative antioxidant capacity *versus* total phenolics (Table 3.5), flavonoids, and vitamin C ($r = 0.738$, $r = 0.851$ and $r = 0.455$, respectively), implying that they are important bioactive compounds for the antioxidant activity of peaches, in accordance with Cantín et al. (2009b). The DPPH assay for the RAC determination explains the correlation found with total phenolics. The high positive correlation found between total phenolics and flavonoids content ($r = 0.807$, $P \leq 0.01$) (Table 3.5), indicates that flavonoids are an important group of phenolic compounds in peaches and nectarines with high antioxidant activity. Moreover, total sugars showed positive significant correlations with total phenolics ($r = 0.398$), vitamin C ($r = 0.350$), and RAC ($r = 0.384$) at $P \leq 0.05$. Pirie and Mullins (1977) reported a good correlation in grapes between sugar content in berries and levels of phenolic substances, due to the role of sugars in the regulation of phenolic biosynthesis. Linear regression between RAC and total phenolics and flavonoids were also high ($r = 0.738$ and $r = 0.851$, respectively at $P \leq 0.01$, data not shown). Similarly, Gil et al. (2002) reported a strong correlation ($r = 0.93$–0.96) between total phenolics and antioxidant activities in fresh nectarine and peach fruits. It is well established that a strong and positive relationship exists between total phenolics and total anthocyanins content and RAC, suggesting that breeders can select for higher phenolics.

Table 3.5. Correlation coefficients between some phytochemical traits in the "Venus" x "Big Top" progeny.

Traits	Flavonoids	Total phenolics	RAC	SSC	Total Sugars
Vitamin C	0.420 **	0.374 **	0.455 **	0.579 **	0.350 **
Flavonoids		0.807 **	0.851 **	0.482 **	ns
Total phenolics			0.738 **	0.524 **	0.398 **
RAC				0.597 **	0.384 **

** $P \leq 0.01$; ns, not significant. Abbreviations: RAC; Relative Antioxidant Capacity.

3.3.6. Principal component analysis for agronomical and biochemical traits

A Principal Component Analysis (PCA) was performed on agronomical and biochemical data in the "Venus" × "Big Top" progeny (Figure 3.3). A four component model accounted for more than 70% of total variance, with the first two components, PC1 and PC2, explaining 21.9% and 19.4% of total variance, respectively. Progeny displayed a great variability (Figure 3.3a). PC1 discriminated between parental acid "Venus" and non-acid "Big Top". An examination of PC1 loadings (Figure 3.3b) suggested that this

separation was mainly due to basic biochemical traits (TA, pH and RI). Genotypes on the positive side of PC1 were in general less acid, showed higher firmness and accumulated more sugars and less anthocyanins than individuals on the negative side. An examination of PC2 loadings (Figure 3.3b) suggested that separation on this component was mainly due to antioxidant traits (flavonoids, total phenolics and RAC) and in a less extent to sugar compound accumulation (glucose, fructose and sorbitol). Analysis confirmed the higher contents in total phenolics, flavonoids and RAC for some genotypes (individuals on the positive side of PC2, especially (18, 27, 32, 35, 37, 43, 44 and 47) than progenitors. The PCA shows a close relationship between flavonoids, total phenolics and RAC as well as between RI, pH and firmness. The results obtained in this progeny were coherent and reflected the known correlations between bioactive and agronomical traits as described in others studies (Cantín et al., 2009a, 2010b).

To summarize, the progeny showed a great phenotypic variance for all the pomological and antioxidant studied traits. High variability was found in yield, fruit weight, firmness, SSC, TA, ascorbic acid, total phenolics, flavonoids, anthocyanins, antioxidant capacity, and total sugars which indicate that there is an important genetic potential to develop new nectarine cultivars with high fruit quality. On the other hand, the significant correlations found between some agronomical and quality attributes could be of interest for quality oriented fruit breeding programs. The study also emphasizes the usefulness of PCA in evaluating the fruit quality of new breeding releases and studying relationships among pomological traits.

Our results lead us to the conclusion that the antioxidant capacity of peach is characterized by huge levels of variations, much explained by the genotype, but harvest conditions and season may also be significant factors. Most of the progeny showed higher total phenolics and flavonoids content than parents. This fact could be of importance for selection of specific traits in the progeny. The phenotypic variation found in all studied traits will allow selecting superior genotypes with higher antioxidant content than the existing commercial varieties and this will naturally be beneficial for health.

Figure 3.3. Principal component analysis of agronomical and biochemical traits in the "Venus" × "Big Top" progeny. Analysis was performed using mean data of four years of study (2007–2010). PC1/PC2 scores plot (a) explaining 41.3% of the total variance. Symbols: (■) parents "Venus" ('V') and "Big Top" ('BT'), (●) progeny. PC1/PC2 loadings plot (b) generated from PCA analysis. Symbols: (■) agronomical and basic biochemical traits, (●) antioxidants, (▲) sugars.

CAPÍTULO 4

Variability in the aroma profile of an F1 nectarine [Prunus persica (L.) Batsch] population

Variability in the aroma profile of an F1 nectarine

[*Prunus persica* (L.) Batsch] population

Abstract

Aroma has gained special attention in recent years as one of the most important fruit quality traits. The reason is that volatile compounds play a significant role in flavour perception of peach and nectarine fruits, as they are responsible for their distinctive aroma. This study was conducted in an F1 population (75 genotypes) derived from the cross 'Venus' x 'Big Top' nectarines. To characterize the volatile profile of fruits in the entire progeny, the headspace-solid phase microextraction technology combined with gas chromatography-mass spectrometry (HS-SPME-GC-MS) was applied. Over 77 volatiles compounds were successfully profiled, including 10 carboxylic acids, 10 aldehydes, 9 alcohols, 3 C6 compounds, 3 esters, 12 ketones, 9 lactones, 12 terpenoids and 9 other compounds. Results showed that profiles in volatiles compounds varied greatly among all genotypes. Our results confirmed the presence of two compounds that were recently described for the first time in peach cultivars (4-Methyl-5-penta-1,3-dienyltetrahydrofuran-2-one and 2,4 heptadienal). The relative amount of each volatile varied greatly between parents and as a consequence among the genotypes in the progeny. The principal component analysis of the volatiles identified a number of genotypes with high content of some lactones and terpenoids which are considered to impact peach aroma. Significant correlations between levels of specific volatiles and agronomical and biochemical traits were observed.

4.1. Introduction

Aroma is one of the most important traits that are used to evaluate fruit quality in addition to, being an essential factor to attract consumers to eat peaches (Chai et al., 2012). Infante et al. (2008) reported that the characteristic aroma of peach fruits results from the volatile compounds released from their skin and pulp, which confer a complex mixture of organic compounds. The volatile profile has been described to change with ripening, genetic background, storage conditions, maturity stage and/or ripening conditions. Volatile compounds contributing to the aroma of fresh fruit represent only 0.01 to 0.001% of the fruit fresh weight, but still have a major effect on its quality (Zhang et al., 2007). Although over 110 volatile compounds have been identified in peaches (Aubert and Milhet, 2007; Wang et al., 2010; Sánchez et al., 2012), only a few are considered to have an impact in aroma and these include lactones, C6 aldehydes and alcohols, and esters (Zhang et al.,

2010). Thus, while lactones and esters provide fruity notes, C6 compounds contribute with green sensory notes to the aroma of ripen fruit (Zhang et al., 2010). More specifically γ- and δ-decalactone, have been reported to play an important role in the overall aroma and they represent the characteristic peach aroma compounds (Visai and Vanoli 1997; Aubert et al. 2003; Zhang and Jia, 2005). Other lactones found in peach: γ-octalactone and γ-decalactone have also pleasant aroma descriptions such as fruity or coconut-like, and contribute to the overall peach aroma with those notes (Sánchez et al., 2012, and references therein). However, all these lactone compounds act in association with other compounds, such as C6 aldehydes, aliphatic alcohols, and terpenes, such as *trans*-2-hexenal, *cis*-3-hexenol, and linalool, which are responsible for the spicy, grassy and floral characteristics of peach flavour (Eduardo et al., 2010). The overall aroma properties of fruits depend upon the combination of volatiles produced, as well as on the concentration and potency of the individual volatile compounds (Defilippi et al., 2009).

The variability in aroma volatiles is highly dependent on the specific cultivar, the processing, stage of maturity and/or ripening and storage conditions. Furthermore, the distribution of the volatiles varies within the fruit (Aubert and Milhet, 2007; Wang et al., 2010). Among fruit tissues, it has been shown that the skin produces greater amount of volatiles than internal tissues; this higher capacity for aroma production was attributed to either an abundance of fatty acid substrates or to a higher metabolic activity (Defilippi et al., 2009). Distribution of lactones was also found to be different in skin and pulp (Aubert and Milhet, 2007). In many cases, stone fruits are harvested in the early stages of development to ensure that they withstand mechanical stress imposed during postharvest packing and handling which often results in aroma alteration or even aroma loss, this being the most common reason for consumer dissatisfaction (Defilippi et al., 2009). C6-aldehydes (*trans*-2-hexenal) and C6-alcohols (*cis*-3-hexenol) were the major components in fruits when harvested before the optimal ripening stage as could be assessed by the low ethylene production and high respiration rates. With fruit maturity, the C6-C12 lactonic compounds, particularly γ- and δ-lactones became the dominant volatile constituents, which increased significantly by the climacteric stage (Zhang and Jia, 2005). Infante et al. (2008) reported that volatile formation during fruit ripening is a dynamic process as could be revealed by the volatile composition changing both qualitatively and quantitatively even after harvest. Aubert et al. (2003) investigated the changes in the volatile composition during maturation and artificial ripening of yellow-fleshed nectarines and reported that the levels of volatile compounds, in particular, lactones and C13 norisoprenoids, were found to

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be the same in the artificially ripened nectarines compared with the tree-ripe nectarines. Engel et al. (1988) reported that nectarine fruits contain significantly higher amounts of δ-decalactone than peaches. Robertson et al. (1990) reported that white-fleshed peaches contain more linalool than yellow-fleshed cultivars.

In this study, headspace-solid phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) was applied to characterize the volatile compound complement of fruits from a nectarine progeny population. The purpose of this work was to evaluate phenotypic variation by comparing the volatile profiles in the parents and the progeny. This information will be useful in breeding programs to select genotypes with specific aroma.

4.2. Materials and methods

4.2.1. Plant material

The population used in this study was a segregant F1 progeny of 75 seedlings resulting from a cross between 'Venus' and 'Big Top'. 'Venus' is a freestone, melting yellow flesh nectarine cultivar, whereas 'Big Top' is a clingstone, melting yellow flesh nectarine cultivar. The segregant population thus obtained was entirely melting flesh, but segregating for cling- or freestone. Seedlings were established (one tree per genotype) in an experimental orchard at the Experimental Station of Aula Dei-CSIC (northern Spain, Zaragoza) in 2002 and were grown under standard conditions of irrigation, fertilization and pest and disease control. Since 2004, different agronomic and quality traits have been evaluated in this population (Cantín et al., 2009a; 2010a). Fruits were hand-picked at commercial maturity which was assessed by peel fruit colour and flesh firmness. Fruits were considered ripe in the tree when their growth had stopped, exhibited yellow ground colour, had begun softening, and were easily detached. Fruit weight, pH, TA, soluble solids content (SSC), ripening index (RI), flesh firmness, and antioxidants were analyzed (see Chapter 3).

4.2.2. Sample preparation procedure

Volatile compounds content was determined in fruits from each seedling genotype and from the two parental genotypes. From each genotype/tree of the population, twenty representative fruits were selected at harvest, and 5 g samples of peeled flesh fruit were frozen in liquid nitrogen. Samples were ground to powder in liquid nitrogen to get both replicates (A) and (B) for each seedling, and stored separately at -80ºC until further

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analysis. A reference sample was made by mixing 0.5 g nectarine fruit powder from all individual genotypes. This mixture was routinely analyzed every day of experimentation as an external control in order to monitor the stability of the analytical system. For the volatiles extraction, 0.5 g of the frozen tissue powder was weighed in a 7 ml vial, which was then sealed, and incubated at 30 ºC for 10 min. Then, 500 μL of 100 mM EDTA-NaOH (pH=7.5) solution was added to the sample (0.5 g) to reach a final EDTA concentration of 50 mM. Solid $CaCl_2.2H_2O$ (1.1 g) was then immediately added to give a concentration of 5 M. Samples were sonicated for 5 min after closing the vials. One mL aliquot of this mix was then transferred into a 10 mL crimp cap vial (Waters), capped and used for GC-MS analysis. Each of parents and the 75 nectarine fruit samples were analyzed using two replicated aliquots.

4.2.3. Head Space-Solid Phase Microextraction (HS-SPME) analysis

Head space solid phase microextraction was the method used for capturing volatiles. For head space analysis, a 65 μm polydimethylsiloxane–divinylbenzene (PDMS/DVB) SPME fiber (Supelco Inc. Bellefonte, PA, USA) was used. The fiber was selected because of its reported sensitivity in the analysis of aroma compounds (Kataoka et al., 2000). Immediately after preparation, samples were subjected directly to Head Space Solid Phase Micro-Extraction (HS-SPME). Pre-incubation and extraction were performed at 50ºC for 10 and 20 min, respectively, as described in Sánchez et al. (2012). Desorption was performed for 1 min at 250 ºC in splitless mode (Wang et al., 2009; González-Mas et al., 2011).

4.2.4. Gas Chromatography-Mass Spectrometry conditions

VOCs trapped on the fiber were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) as described in Tukunov et al. (2005) with minor modifications, using an autosampler COMBI PAL CTC Analytics (Zwingen, Switzerland), a 6890N GC Agilent Technologies (Santa Clara, CA, USA) and a 5975B Insert XL MSD Agilent, equipped with an Agilent J&W Scientific DB-5ms fused silica capillary column (5%-phenyl-95%-dimethylpolysiloxane as stationary phase, 60-m length, 0.25 mm i.d., and 1 μm thickness film). Oven programming conditions were 40 ºC for 2 min, 5 ºC/min ramp until 250 ºC and then held isothermally at 250 ºC for 5 min. Helium was used as the carrier gas with a constant flow of 1.2 mL/min. Mass/Z detection was performed by an Agilent mass spectrometer in the EI mode (Ionization Energy, 70 eV; source temperature 230 ºC).

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Data acquisition was performed in scanning mode (mass range m/z 35-220; seven scans per second). Chromatograms and spectra were recorded and processed with the Enhanced ChemStation software for GC-MS (Agilent).

4.2.5. Compounds identification and quantification

Identification of compounds was based both on the comparison between the Mass Spectra (MS) for each putative compound with those of the NIST 2005 Mass Spectral library and also with the match to the Gas Chromatography (GC) retention time and MS custom library which have been generated using commercially available compounds (González-Mas et al., 2011). Compounds used as reference were of analytical grade and purchased from Sigma-Aldrich Química (Madrid, Spain). Only those compounds/peaks confirmed by both mass spectrum and retention time in each and every chromatogram were considered. For relative quantification, the peak area was integrated from the extracted ion chromatogram corresponding to a specific ion previously selected for each compound. A mixture of samples representing all analyzed genotypes was injected regularly as part of the injection series and was used as a reference for correction for temporal variation and fiber aging. Finally, corrected results for each compound were expressed as $\log_2$ of the ratio between sample signal relative to the signal in the reference sample.

4.2.6. Statistical analysis

For the Principal Component Analysis (PCA), the complete dataset including all replicates were considered. For this analysis, the $\log_2$ of the ratio (samples signal / reference signal) was used. For PCA and dendrogram, the program SPSS version 19 was used (SPSS Inc., Chicago, IL). Finally, correlations VOCs and biochemical traits were calculated with raw data of the four years, according to Pearson's test at $P \leq 0.01$.

4.3. Results and discussion

4.3.1. Identification and relative quantification of nectarine VOCs

A total of seventy-seven volatile compounds were identified (Table 4.1) and relatively quantified (Table 4.2) in the nectarine population. Most of these compounds have been previously reported in peach fruit by other authors (Zhang et al., 2010; Wang et al., 2010; Sánchez et al., 2012) and are included among the 100 or so volatile compounds already identified in peach. The volatile compounds observed in the 'Venus' x 'Big Top' population included ten carboxylic acids, ten aldehydes, nine alcohols, three C6

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compounds, two esters, twelve ketones, ten lactones, twelve terpenoids, and nine other compounds belonging to other classes. The C6 compounds including aldehydes, commonly known as "green" compounds: hexanal, *cis*-3-hexenal, and *trans*-2-hexenal have been described as having a floral, grassy and green odour. Compounds as benzaldehyde, linalool, γ-hexalactone, γ-decalactone and δ-decalactone have been previously reported as major compounds in peach/nectarine (Eduardo et al., 2010).

Table 4.1. List of the 77 volatile compounds detected in fruits of the nectarine 'Venus' x 'Big Top' population.

Retention time (min)	Family	Compounds
16.720	Acids	Butanoic
18.222		Pentanoic
21.749		Propenoic
22.070		Hexanoic
23.754		Acetic
25.441		Heptanoic
25.684		Valeric
27.649		Octanoic
30.838		Nonanoic
33.835		Decanoic
5.425	Alcohols	Ethanol
17.478		1-Pentanol
18.461		1-Hexanol
23.372		5-Methyl-5-octen-1-ol
27.056		1,6-Octadien-3-ol
29.123		Cyclohexanol
29.201		p-Menth-1-en-8-ol
34.506		1-Butanol
34.780		cis-p-Mentha-2,8-dien-1-ol
11.944	Aldehydes	Pentanal
15.936		Hexenal
20.300		Lilac aldehyde D
22.653		Benzaldehyde
23.683		Octanal
24.087		2,4-Heptadienal
25.533		Benzeneacetaldehyde
27.195		Nonanal
29.120		2-Nonenal
32.416		2-Dodecenal
15.842	C6 compounds	3-Hexenal
18.076		2-Hexenal
31.490		Cyclohexene
23.546	Esters	4-Hexen-1-ol, acetate
24.897		Geranyl tiglate
40.923		5-Methyl-3-phenylcyclopent-2-en-1-one
19.932	Ketones	3-Nonen-2-one
22.931		5-Hepten-2-one
29.361		4-Decanone
29.461		Bicyclo[2.2.1]heptan-2-one

34.931		m-Ethylacetophenone
36.100		2-Buten-1-one
36.459		But-3-*en*-2-one
37.521		Geranyl acetone
38.066		8,8,9-Trimethyl-deca-3,5-diene-2,7-dione
40.923		5-Methyl-3-phenylcyclopent-2-en-1-one
43.045		Benzophenone
46.191		Methanone
25.774	Lactones	γ -Hexalactone
29.038		γ -Heptalactone (2(3H)-Furanone
32.351		γ -Octalactone (2(3H)-Furanone
36.660		4-Methyl-5-penta-1,3-dienyltetrahydrofuran-2-one
37.971		γ -Jasmolactone
38.212		2H-Pyran-2-one
38.335		2(3H)-Furanone
38.427		γ -Decalactone
39.128		δ-Decalactone
22.516	Terpenoids	*cis*- α -Terpineol
23.179		β –Myrcene
24.671		1,2-Cyclohexanediol
24.812		p-Cymene
25.012		Limonene
26.949		Terpinolene
27.084		Linalool
30.287		Thymol
30.653		α-Terpinol
35.127		4-Hydroxy- α -ionone
36.106		β-Damascenone
38.883		β-Ionone
6.179	Other compounds	2-Pentene, (E)-
9.056		Pentane
9.719		Furan
22.774		2H-Pyran
25.896		Cyclopropane
27.897		2,3-Epoxycarane
29.272		1-Oxaspiro[2.5]octan-4-one
30.982		4-(Prop-2-enoyloxy) octane
33.211		Tetradecane

Recently, Sánchez et al. (2012) identified and quantified a total of 110 volatile compounds including: alcohols, ketones, aldehydes, esters, lactones, carboxylic acids, phenolics and terpenoids in peach fruit samples from different genetic backgrounds, locations and maturity stages. The lactone putatively identified as 4-Methyl-5-penta-1,3-dienyltetrahydrofuran-2-one and the aldehyde identified as 2,4-heptadienal, recently described as new components of the volatile profile in peach fruit, were also found in our nectarine progeny. Almost all the volatile compounds showed dramatic changes in the levels of accumulation among the different progeny genotypes. The different aromatic profile of the progenitors 'Venus' and 'Big Top' was the reason to generate a large segregation of volatiles compounds in the progeny (Figure 4.1; Table 4.2). Most reports in

the literature have focused on the evolution of peach and nectarine aromas during ripening (Wang et al., 2009, and references therein). During fruit maturation, lactones with long side chain (γ-decalactone, δ-decalactone), benzaldehyde and linalool levels increased, reaching the highest amounts in mature fruit (Toselli et al., 2010 and references therein).
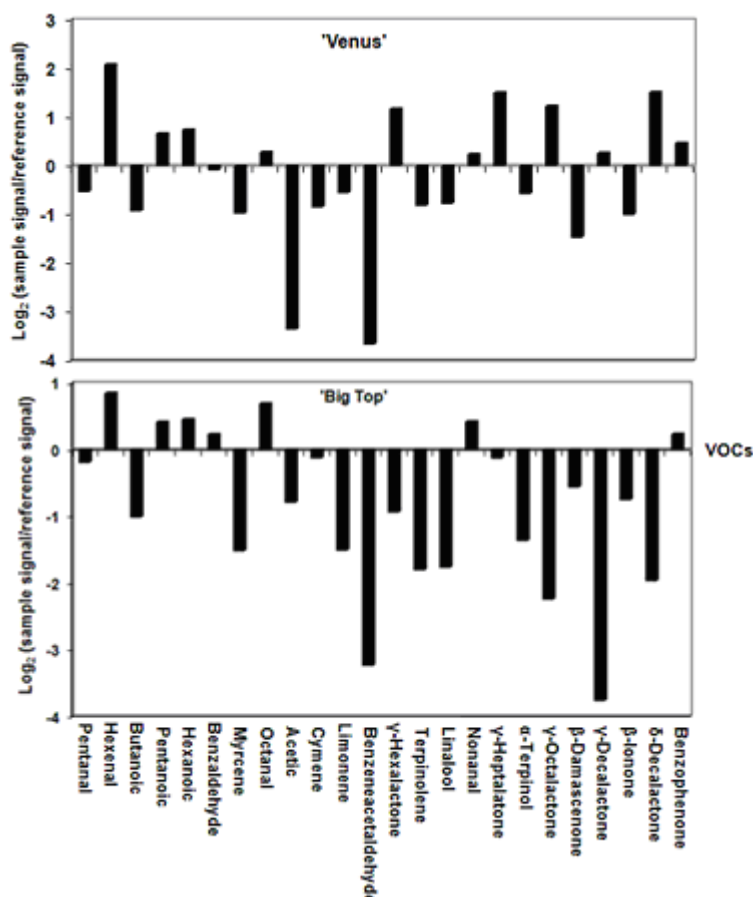
Figure 4.1. Aromatic profile of the two progenitors 'Venus' and 'Big Top'. The quantification of volatile compounds is done as log$_2$ (sample signal/reference signal). The volatiles are compared to the reference sample (value=o).

Acids, Aldehydes and Terpenoids

Nine acids, ten aldehydes and twelve terpenoids were identified in the 'Venus' x 'Big Top' population (Table 4.1). Recently, Sánchez et al. (2012) reported that the aldehyde 2,4-heptadienal, initially described in plum and apricot cultivars, was also found in peach fruits. Chai et al. (2012) reported that terpenoids, mainly the monoterpenols, contribute to the fruity characteristics of fruits aroma, most of which are described as very pleasant. Visai and Vanoli (1997) reported that nectarines produced large amounts of terpenoids (mainly linalool and terpinolene). Linalool aldehyde is one of the major esters present in mature peaches and nectarines, increasing in levels significantly during fruit

maturation and reaching values significantly higher in nectarine than in white-fleshed peaches (Wang et al., 2009).

Alcohols and Ketones

In this work, nine alcohols and twelve ketones were found (Table 4.1). The relative abundance of these volatile compounds was higher than those in 50 peaches and nectarines cultivars evaluated by Wang et al. (2009) supporting that volatile profile is highly dependant on genetic background. On the other hand, the VOC pattern differs not only between peaches and nectarines cultivars, but also among the different varieties and is also affected by management practices (Toselli et al., 2010).

Alcohols contribute to the green and herbaceous notes (Chai et al., 2012). Their content in our samples was relatively low because fruits were harvested at commercial maturity.

C6 compounds

Four C6 compounds were found (Table 4.1), being hexanal and 2-hexenal the major C6 compounds identified in the progeny. C6 compounds have been described as major compounds in immature peaches and nectarines; and their levels decrease with the fruit maturation (Engel et al., 1988; Horvat et al., 1990). Wang et al. (2009) reported that the C6 compounds are products of enzyme-catalyzed breakdown of unsaturated fatty acids. The high contents of these compounds found in some genotypes of our population could indicate that this metabolic pathway is quite active in our fruit even when they were at the ripe stage.

Lactones and Esters

Nine lactones and three esters were found in our population (Table 4.1). In all genotypes, γ-heptalactone, γ-octalactone, γ-decalactone and δ-decalactone were major components, although all lactones characterizing the typical peach and nectarine aroma in fruits, were identified in our nectarines (Table 4.1). Levels of δ-decalactone are normally positively correlated with γ-decalactone content (Wang et al., 2009) and are the 'character impact' compounds of peaches (Engel et al., 1988), appearing toward the end of the maturation.

Table 4.2. Relative quantification of volatile compounds detected in fruits of all nectarine 'Venus' x 'Big Top' population. Results were expressed as the ratio of $\log_2$ (sample signal/reference signal) with comparison of reference sample.

Target compounds	Retention time	Means	Desvt	Min	Max
Furan	9.719	0.33	0.53	-0.64	2.00
Pentanal	11.944	-0.44	0.58	-1.66	1.05
3-Hexenal	15.842	-0.05	0.85	-1.73	2.27
Hexanal	15.936	-0.28	1.73	-3.72	3.58
2-Hexenal	18.076	-0.23	0.63	-1.90	1.06
Pentanoic acid	18.222	0.58	0.36	-0.43	1.60
Hexanoic acid	22.070	0.55	0.27	-0.48	1.21
Benzaldehyde	22.653	-0.13	0.36	-1.63	0.76
5-Hepten-2-one	22.931	-0.10	0.40	-0.90	1.00
β-Myrcene	23.179	-0.82	1.35	-3.75	2.61
Octanal	23.683	0.38	1.38	-1.12	6.08
Acetic acid	23.754	-0.52	1.29	-5.15	2.16
2,4-Heptadienal	24.087	-0.26	0.80	-1.95	1.48
p-Cymene	24.812	-0.12	0.79	-1.57	1.59
Limonene	25.012	-0.72	1.19	-3.12	2.75
Benzenacetaldehyde	25.533	-0.30	1.22	-3.52	2.29
γ-Hexalactone	25.774	-0.36	0.89	-3.02	0.90
Terpinolene	26.949	-0.69	1.34	-3.73	2.80
Linalool	27.084	-0.80	1.64	-4.62	3.23
Nonanal	27.195	-0.21	0.44	-1.52	0.74
Octanoic acid	27.649	0.23	0.38	-1.73	0.83
γ-Heptalactone (2(3H) Furanone	29.038	-0.12	0.64	-1.53	1.01
2-Nonenal	29.120	-0.09	0.33	-0.63	0.89
α-Terpinol	30.653	-0.70	1.35	-3.92	2.89
γ-Octalactone (2(3H) Furanone	32.351	-0.49	1.27	-3.69	1.79
β-Damascenone	36.106	-0.58	0.63	-1.97	0.65
Geranyl acetone	37.521	-0.62	0.55	-1.70	0.74
2H-Pyran-2-one	38.212	-0.88	1.49	-3.43	1.83
γ-Decalactone	38.427	-0.87	2.29	-5.92	5.11
β-Ionone	38.883	-0.49	0.95	-2.46	1.81
δ-Decalactone	39.128	-0.89	1.45	-4.36	2.36
Benzophenone	43.045	0.03	0.39	-1.20	0.92

Sánchez et al. (2012) reported that lactones production on peach fruit are highly correlated between them and are central in the interactions with other volatiles groups including linear esters. Our genotypes in general did not show high content of esters as other authors showed (Engel et al., 1988) and this could be due to the genetic background or the technique used.

Most of the volatiles compounds identified showed a normal distribution concerning relative content in the progeny (Figure 4.2; Table 4.2) which is typical of quantitative characters. The segregation pattern for the progeny is different for each volatile when comparing the progeny with the parents (Figure 4.2). The broad ranges (relative quantification) and the normal distribution found for some volatiles in the

population have significance in peach breeding since volatile compounds can be genetically controled and aroma plays a central role in fruit quality.
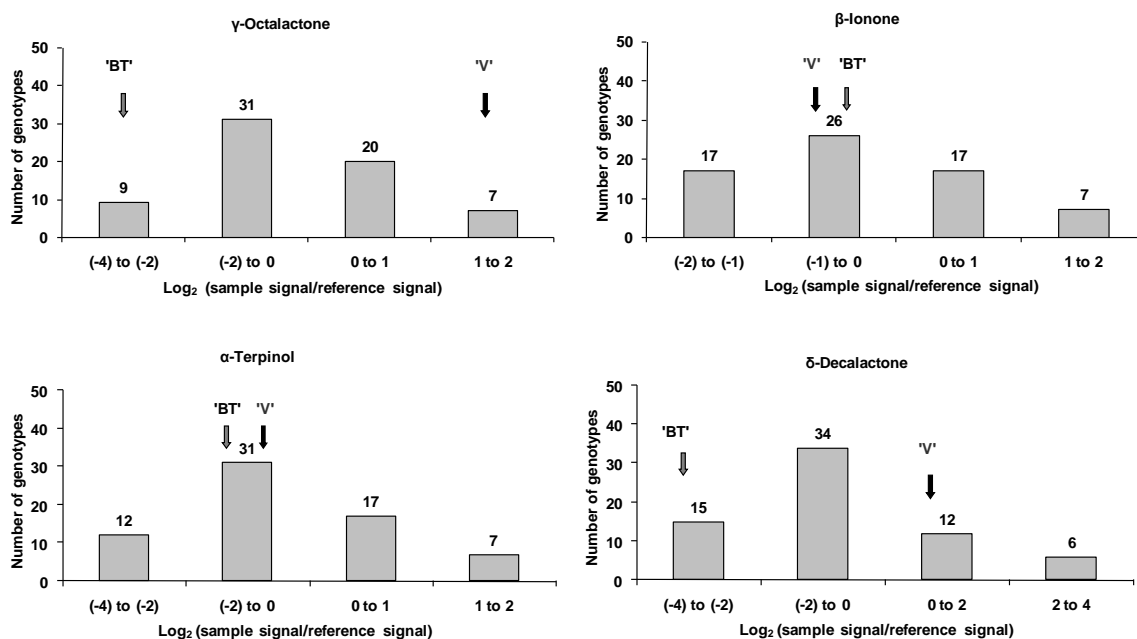
Figure 4.2. Segregation of γ-Octalactone, β-Ionone, α-Terpinol and δ-Decalactone, in the 'Venus' × 'Big Top' population. Data are means (*n* = 67-75 genotypes) of one year of study (2010). Arrows show the values for the parents 'Venus' ('V') and 'Big Top' ('BT').

4.3.2. Correlations between VOCs and fruit quality traits

Pentanal, butanoic acid, furan, 2,4-Heptadienal, p-Cymene and hexanoic acid showed significant positive correlations with titratable acidity (TA) (r=0.366, r=0.321, r=0.467, r=0.577, r=0.423, r=0.450, $P \leq 0.01$, respectively). These lipid derived compounds are related to immature fruits and confer "green" notes to handmade peach juice (Derail et al., 1999) so the correlation found, although low, may indicate some acidity in the mature samples of some genotypes. Significant positive correlations of benzenacetaldehyde and β-damascanone were found with the ripening index (RI) (r=0.499, r=0.556, $P \leq 0.01$, respectively) corroborating that these VOCs are contributors to the fruit mature aroma.

Zhang et al. (2010) reported that among the large number of volatiles present in peach fruits, only a few are considered to have an impact on aroma and those are including lactones, C6 aldehydes and alcohols, and esters. Our progeny did not show any significant correlation between VOCs and firmness, except for the hexanal which presented a low correlation of r=-0.255 ($P \leq 0.05$). The soluble solids content (SSC) was also poorly correlated with volatiles compounds except for terpinolene (r=-0.517, $P \leq 0.01$).

To further investigate how the volatile compounds are related to the antioxidant activity of flesh fruit studied in the progeny among four years, correlation analysis was carried out. Results showed that the γ-heptalactone was correlated with vitamin C (r=0.291, $P \leq 0.01$) and flavonoids (r=0.244, $P \leq 0.05$). Another positive correlation was observed between γ-octalactone, with total phenolics (r=0.311, $P \leq 0.01$), and with flavonoids (r=0.263, $P \leq 0.05$). These correlations could be important for breeding programs in order to get fruits rich in lactones and antioxidants at the same time. Huang et al. (2011) reported that 55 out of the 88 VOCs identified in soybean were significantly positively correlated with antioxidant activity and these authors indicated the probable involvement of volatile compounds in some antioxidant activity. Nevertheless, no significant correlation was found between VOCs and the relative antioxidant capacity (RAC) in our study. This confirms that in the progeny none of the identified compounds (lactone, esters, alcohols, carboxylic acids and terpenoids) were directly involved in the relative antioxidant capacity of our nectarine fruits.

To elucidate genetic diversity among the 'Venus' x 'Big Top' progeny studied concerning volatiles profiles, a dendrogram was constructed (Figure 4.3). The analysis cluster for all genotypes in the nectarine progeny showed two main groups all genotypes. 'Venus' and 'Big Top', were included in two different clades (Group 1 and Group 2) because of their VOCs different profiles (Figure 4.1). The dendrogram showed that most of the population belongs to Group 1 as the 'Venus' female parent. In adition, 'Big Top' showed less relative content in lactones compared to 'Venus', which explain in part the segregation in the progeny for these specific compounds. Some genotypes, such as the individual 43-next to Big Top, showed a VOCs profile very similar to that of this parental.
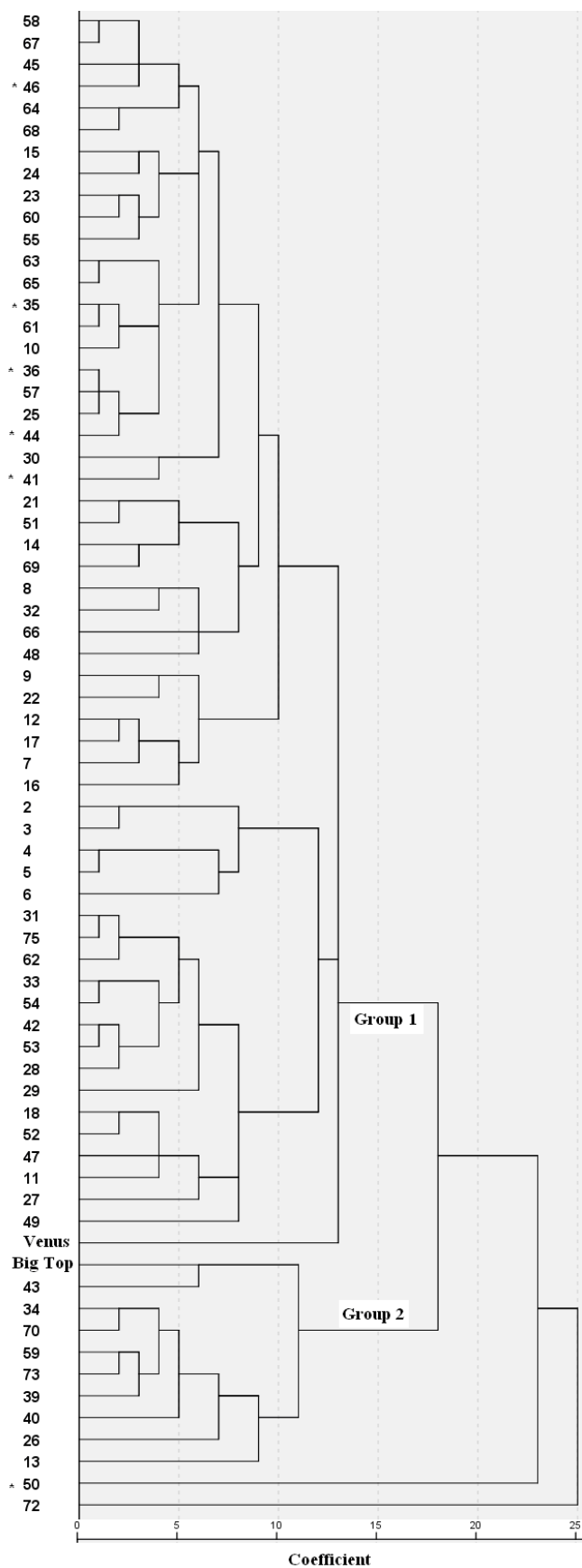
Figure 4.3. Dendrogram of major volatile compounds in the 'Venus' x 'Big Top' population. Cluster analysis was performed using mean data of two replications from one year of study (2010).

4.3.3. Assessment of variability of VOC production in our population by Principal Component Analysis

To establish which compounds were more valuable to define and differentiate the samples according to genotypes of the 'Venus' x 'Big Top' progeny, a principal component analysis (PCA) was carried out using the determinant volatile compounds which produce the peach aroma. The first two principal components explain 39.4% of the variance, and clearly separate both parents and some genotypes from the rest of the progeny (Figure 4.4). The first component, explaining 23.9% of the variance, mainly separates the parental 'Venus' and some genotypes from all the other genotypes. Analysis of the loading plots reveals the volatile compounds responsible of the samples separation (Figure 4.4A). The second component explains about 15.5% of the variance and clearly separates some genotypes with high lactones content from the other genotypes. We observed a high variability of volatile compounds content among genotypes. The progenitors, 'Venus' and 'Big Top', even though they both are nectarines, showed very different aromatic profile which is responsible of the segregation observed in the progeny. The segregation observed among genotypes and both parents could reveal a genetic control of these volatile components (see also Figure 4.1 and 4.2). The cultivar 'Venus' showed high contents of pentanoic acid, hexanoic acid, nonanal, hexanal, octanal, γ-Hexalactone, γ-Heptalactone γ-Octalactone, γ-Decalactone, δ-Decalactone and benzophenone, while the cultivar 'Big Top' presented high contents of hexanal, pentanoic acid, hexanoic acid, benzaldehyde, octanal, heptadienal, nonanal and benzophenone. The PCA analysis confirmed that the most relevant VOCs for the positive axe for PC1 and PC2 are mostly lactones for 'Venus' and for some genotypes (35, 36, 41, 44, 46, 47, 50 and 61).

Although low correlations between VOC and antioxidants were found, the PCA analysis permitted to select two genotypes (35 and 44) for their high contents of antioxidant compounds (Abidi et al., 2011). The combination of techniques permits to select individuals with high content of volatiles apart of the antioxidant compounds content.

In the future, the VOCs profile would be very important to complete the specific organoleptic profile for registration of new cultivars. It is worthy to note that the analytical technique used to characterize VOCs profile in nectarine genotypes will be a powerful tool. The phenotypic variation found in this progeny concerning VOCs profiles and other

antioxidant compounds content, provides a valuable material for new peach breeding programs.
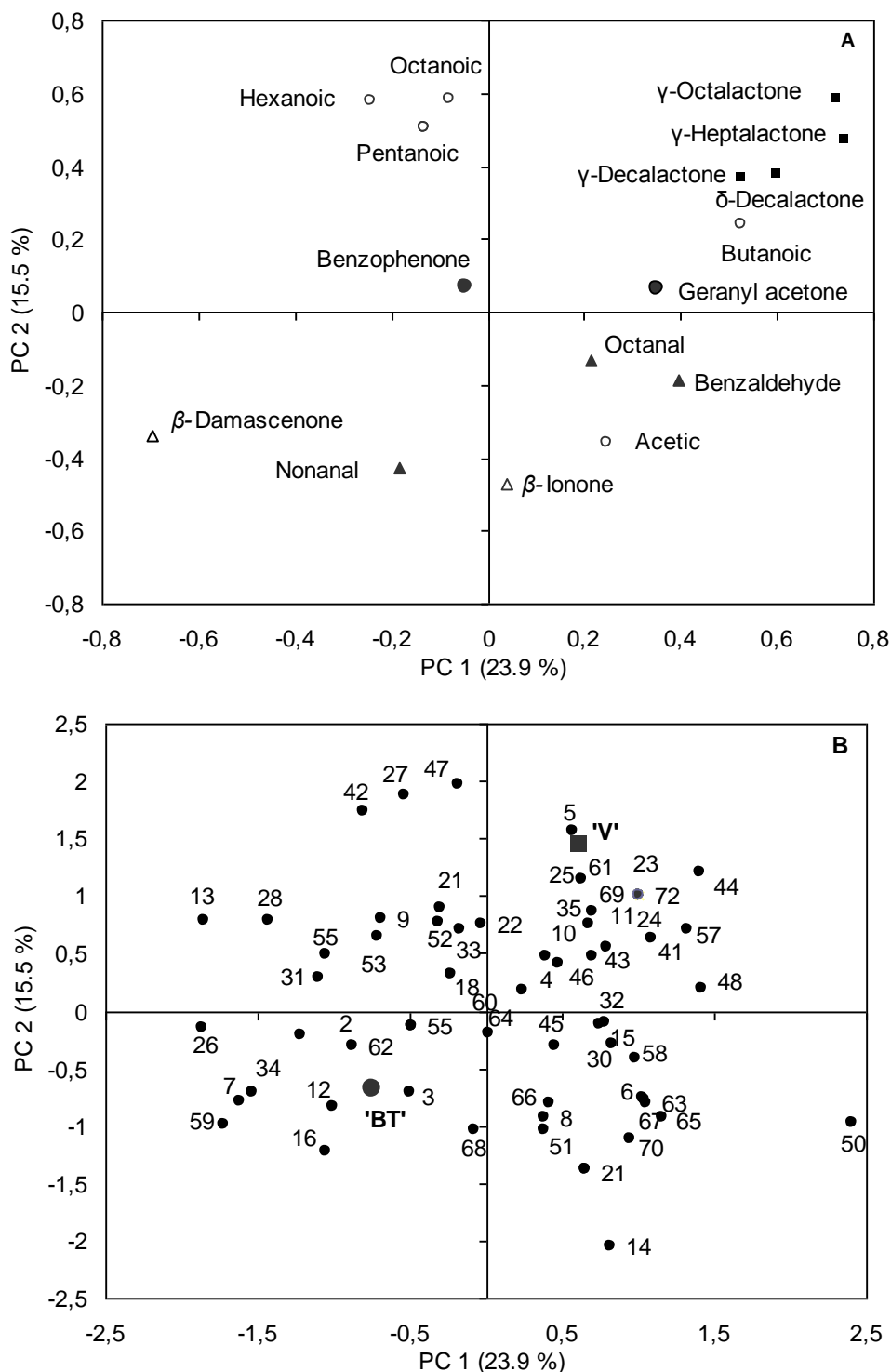
Figure 4.4. Principal component analysis of major volatile compounds in the 'Venus' x 'Big Top' population. Analysis was performed using mean data of two replications from one year of study (2010). PC1/PC2 scores plot (A) explaining 39.4% of the total variance. PC1/PC2 loadings plot (B) generated from PCA analysis. Symbols: A: (o) Carboxylic acids; (▲) Aldehydes; (●) Ketones; (■) Lactones; (Δ) Terpenoids. B: parents 'Venus' ('V') and 'Big Top' ('BT').

CAPÍTULO 5

Effect of antioxidant compounds and total sugar contents on the chilling injury susceptibility of a clingstone peach [Prunus persica (L.) Batsch] progeny

Effect of antioxidant compounds and total sugar contents on the chilling injury susceptibility of a clingstone peach [*Prunus persica* (L.) Batsch] progeny

Abstract

In order to identify genotypes with good organoleptic properties, antioxidant-rich content and low susceptibility to chilling injury (CI), samples of fruit flesh from an F1 population derived from the cross between clingstone non-melting yellow flesh 'Babygold 9' × 'VAC-9510' peach cultivars (*Prunus persica* L. Batsch) were studied over three years. Basic agronomical and biochemical traits were determined in the progeny. Phytochemical traits such as L-ascorbic acid, flavonoids, total phenolics, relative antioxidant capacity (RAC), and sugar contents were also analyzed. Major symptoms of chilling injury, such as mealiness, graininess, flesh browning, red pigmentation (bleeding) and loss of flavor, were evaluated. Results showed a high variability in agronomical and biochemical traits within the progeny. Six genotypes (named as 10, 54, 65, 73, 91 and 120) with high total phenolics and flavonoids contents and RAC were selected. The studied population exhibited also wide phenotypic variation in sugars content with a similar distribution of these compounds among years. The progeny also showed variability for all the evaluated CI symptoms and sixteen genotypes (14, 33, 52, 58, 68, 76, 78, 80, 93, 96, 113, 114, 118,120, 128, and 129) showed low susceptibility to CI. The duration of storage (2 or 4 weeks at 5 ℃) increased the severity of these symptoms but with less intensity compared to other peach progenies. After 2 weeks of cold storage, mealiness and bleeding were the main CI symptoms observed, whereas flesh browning was predominant after 4 weeks. The low significant positive correlations observed between phenolic compounds and CI symptoms indicated their possible influence in the susceptibility to internal browning and should be considered in the current breeding programs selecting fruits with high bioactive compounds and preserving their post-harvest quality.

5.1. Introduction

An important field of research nowadays is the control of 'redox' status by consuming foods with high polyphenolic contents. The main reason for this interest is the recognition of the antioxidant properties of polyphenols, their great abundance in our diet, and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative diseases (Manach et al., 2004). Natural antioxidants present in the diet increase the resistance to oxidative stress

and they may have a substantial impact on human health (Dimitrios, 2006). Fruits are known to have a beneficial effect on human health, with antioxidant, anti-inflammatory and immune-stimulating functions, which can be attributed to the content of phenolic compounds (Madrau et al., 2009; Bak et al., 2012 and references therein).

Fruit quality traits, including flesh firmness, acidity, texture, and content of total sugars and antioxidant compounds, determine consumer acceptance. Such traits are negatively impacted by pre- and post-harvest factors like cold storage, a widely used industrial procedure to extend fruit market life. Peach is the most important fruit crop in Spain but it has a short post-harvest life due to the rapid ripening and microbial decay (Cao et al., 2010). Cold storage remains the main method to inhibit fruit decay and extend post-harvest life, however, the fruit are very sensitive to low temperature and exhibit chilling injury (CI) after long periods of cold storage (Lurie and Crisosto, 2005). If susceptible varieties of peach, nectarine and other stone fruits are held too long at a low temperature, they will not ripen properly when re-warmed and they will suffer physiological disorders collectively known as chilling injury (Crisosto et al., 1999; Cantin et al., 2010a). It manifests itself as dry, mealy, woolly (lack of juice) or hard-textured fruit with no juice (leatheriness), flesh or pit cavity browning, and flesh bleeding or internal reddening (Lurie and Crisosto, 2005). Browning is often seen in mealy fruit, although it can occur in the absence of mealiness, when enzymes such as polyphenol oxidase act on phenolic substrates when they are brought into contact (Crisosto et al., 1999; Peace et al., 2006). Peace et al. (2006) reported that flesh bleeding results from a spread of red pigment, presumably anthocyanins, through the fruit flesh during cold storage or after subsequent ripening. Mealiness is a fruit flesh textural disorder, where affected ripe fruit has a dry grainy feel when chewed (Peace et al., 2006). In simple terms, mealy fruits are dry and soft when ripe, whereas leathery fruits are dry and firm when ripe, or remain firm because of a failure to ripen (Ju et al., 2000; Peace et al., 2006). Crisosto et al. (1999) reported that clingstone non melting flesh (CNMF) cultivars are less susceptible to CI. A clearer statement is that CNMF cultivars cannot get mealy, but some may have a short market life due to high susceptibility to browning or leatheriness. On the other hand, freestone melting flesh (FMF) and clingstone melting flesh (CMF) cultivars have the potential to develop mealiness in their fruit, depending on whether they carry further genes for susceptibility (Peace et al., 2006). The genetic control of CI in peach has been studied and it has been demonstrated that mealiness, browning and bleeding are probably controlled by major genes (Peace et al., 2006; Ogundiwin et al., 2007; Cantín et al., 2010a). Moreover, one

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major quantitative trait locus (QTL) has been detected for each of these symptoms (mealiness, bleeding, browning) in linkage groups (LG) 4 and 5 (Peace et al., 2006; Ogundiwin et al., 2007).

Most research emphasis has been put on texture alterations in relation to cell wall modifications in mealy peaches, with less attention given to other metabolic factors including the effects on CI of antioxidant components and activity in the fruit. Lee et al. (2012) reported that CI development has been associated with enzymatic activity of polyphenol oxidase which leads to oxidative degradation of mono-phenolic compounds to produce the polyphenolic polymers that impart a brown color to fruit flesh. Tsaltani et al. (2010) reported that many phenolic compounds are also browning substrates at higher concentrations. These compounds can take part in modifying the food's coloring at high concentrations because some of them are substrates for undesirable browning reactions, which are catalyzed by the polyphenol oxidase (PPO) enzyme (Chang et al., 2000). Development of CI symptoms was associated with decreased total phenolics, flavonoids and total antioxidant concentrations and reduced juiciness during storage (Tsantili et al., 2010). Wang et al. (2006) observed that a pre-treatment of peaches with salicylic acid (SA, a phenolic compound) alleviated browning symptoms in chilling sensitive peaches by promoting the ascorbate–glutathione cycle. Also Meng et al. (2009) reported that post-harvest treatments with methyl jasmonate (MeJA) reduced browning by increasing peroxidase activity (POD) and thus reducing phenolic concentrations. Meng et al. (2009) demonstrated that MeJA also maintained calcium contents in cell walls at stable levels during cold storage, increased polygalacturonase activity and decreased pectin methylesterase activity during ripening after cold storage, indicating that MeJA could prevent mealiness in peaches. Thus, varieties that have a balance of high phenolic compound content and low PPO activity can be very attractive (Chang et al., 2000). In recent years, a few studies have reported that application of exogenous SA, as a natural and safe phenolic compound and methyl salicylate (MeSA), at non-toxic concentrations, exhibits a high potential in controlling post-harvest losses is effective at increasing resistance to CI in post-harvest horticultural crops, including peach (Cao et al., 2010).

The main objectives of this work were (1) to evaluate the existing phenotypic diversity of antioxidant compounds and total sugar content among genotypes of a CNMF population; (2) to quantify the expression of different CI symptoms in the entire population after two different periods of cold storage; and (3) to study the effect of antioxidant compounds and total sugars content on the expression of different CI symptoms.

5.2. Materials and methods

5.2.1. 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 metaphosphoric acid were purchased from Sigma-Aldrich (Steinheim, Germany).

5.2.2. Plant material

The progeny assayed was a segregant F1 population of 130 seedlings obtained from a controlled cross, between *Prunus persica* cvs. 'Babygold 9' × 'VAC-9510'. Both cultivars and the segregant population are CNMF. 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 × 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. Samples were harvested and analyzed over three consecutive years (2009-2011).

5.2.3. Quality parameters

During the years 2009-2011, agronomic and fruit quality traits were measured individually in each seedling tree. Harvest date and annual yield were also recorded. Harvest date ranged from early-July to mid-August, depending on the genotypes. Fruits were hand picked at commercial maturity and assessed by peel fruit color and flesh firmness. Fruits were considered ripe in the tree when their growth had stopped, exhibited yellow ground color, began softening, and were easily detached. Yield (kg/tree) was measured and a representative fruit sample (40 fruits) was taken for fruit quality evaluations as described by Cantín et al. (2009a). Fruit weight was also scored. Flesh firmness of the fruits was performed by a hand penetrometer. The SSC (ºBrix), initial pH and titratable acidity (TA) of the juice were measured as described in Abidi et al. (2011). The ripening index (RI) was calculated as the ratio between SSC and TA.

5.2.4. Phytochemical extraction

For all analyses only fruit flesh samples were used, as it is usually consumed. Twenty representative fruits were peeled with a sharp knife, 5 g of flesh were weighted, immediately frozen in liquid nitrogen, and stored at -20 ºC until analysis, except for vitamin C that samples were kept in 5 mL of 5 % metaphosphoric acid for preservation of ascorbic acid. Samples were homogenized with a polytron with the correspondent buffer solution and processed as reported in Abidi et al. (2011). The supernatant was recovered and processed to be assayed as described by Cantín et al. (2009a) with some modifications (Abidi et al., 2011).

5.2.5. Antioxidant and total sugar determinations

Vitamin C, total phenolics, flavonoids and relative antioxidant capacity were evaluated with colorimetric methods and measured using a spectrophotometer (Beckman Coulter DU 800) as described by Cantín et al. (2009a) and methods therein. Standard calibration curves were daily prepared. Vitamin C was measured at 525 nm and the amount of vitamin C was expressed as mg of ascorbic acid (AsA) per 100 g fresh weight (FW). For total phenolics content, a colorimetric method based on the chemical reduction of the Folin-Ciocalteau reagent was used. Absorbance was measured at 725 nm and the content was expressed in milligrams of gallic acid equivalents (GAE) per 100 g of FW. Total flavonoids content was determined measuring absorbance at 510 nm and the results were expressed as mg of catechin equivalents (CE) per 100 g of FW. The relative antioxidant capacity (RAC) was determined using DPPH. The absorbance was measured after 10 min of reaction at 515 nm and RAC was expressed as µg of Trolox equivalents (TE) per g of FW.

To estimate the variation in sugar profile among genotypes, sugar composition and quantification were analyzed by HPLC as described by Cantín et al. (2009b) with some modifications described in Abidi et al. (2011).

5.2.6. Chilling injury symptoms evaluation

Chilling injury susceptibility was evaluated in the progeny after storage of samples of 20 fruits per seedling at 5 ºC and 95% RH (relative humidity) according to Crisosto et al. (1999) during 2 or 4 weeks and ripening at room temperature during 2–3 days. Fruits were then evaluated as described in Cantín et al. (2010a) for symptoms of CI such as lack of juiciness (flesh mealiness), flesh graininess, fail to ripening (leatheriness), flesh

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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. When fruits had a dry appearance and little or no juice after hand squeezing were considered mealy. Mealiness, graininess and off-flavor were 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 in the population 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 CI symptoms.

5.2.7. Statistical analysis

All traits were measured or scored for each genotype separately over the three year period. Minimum and maximum values, mean and mean standard error (SE), were calculated for each studied trait in the progeny during the three years using SPSS 19.0 (SPSS Inc., Chicago, IL). Year means were also separated by Duncan's multiple test for each. Data for each genotype were averaged, and mean values were used as estimated genotypic values. Finally, correlations were calculated with raw data of the three years, according to Pearson's test at $P \leq 0.01$.

5.3. Results and discussion

5.3.1. Agronomic and basic fruit quality traits

Agronomic and basic biochemical fruit quality traits were evaluated separately in each seedling over the three years of the study (2009-2011). Mean values of yield, fruit weight, firmness, SSC, pH, TA and RI were calculated from the 130 individual seedlings. All tested parameters were found to show considerable variability among genotypes **(Table 5.1)**. Regarding flesh firmness, values were lower than the maximum level of fruit firmness for commercial peaches fixed by the EU (63.7 N; Commission Regulation EC, No.1861/2004 of 28 October 2004). Regarding SSC, all the progeny showed values over the minimum (8 ºBrix) established by the EU to market peaches and nectarines (R-CE No.1861/2004). Regarding pH values, the progeny can be considered as acid and non-acid fruits, since fruit with a pH higher than 4.0 at maturity are considered as non-acid (Monet, 1979). The progeny showed variability of TA among genotypes with a mean value of 0.5 g

malic acid/100 g of FW. The mean TA observed in the progeny was lower than the maximum limit (0.9 g malic acid/100 g FW) for normal acidity peaches (Hilaire, 2003). These are important data since consumer acceptance is proven to be correlated with SSC and TA, although is also dependent on other cultivar attributes such flavor and aroma (Crisosto and Crisosto, 2005). In peaches, the RI is a major organoleptic quality trait of the mature fruit and is commonly used as a quality index (Bassi and Selli, 1990). In this progeny, the RI showed a high variability among genotypes (14.3-33.6).

Table 5.1. Range, mean and standard error (SE) of agronomical and biochemical fruit quality traits in the 'Babygold 9' x 'VAC-9510' progeny. For the progeny (n = 130 genotypes), data are mean of three years of study (2009-2011).

Traits	Range	Mean	SE
Production	2.0-24.4	11.4	0.3
Fruit weight	138.9-308.5	217.8	3.0
Firmness	21.4-53.9	32.5	0.4
SSC	9.1-14.4	11.0	0.1
pH	3.5-4.2	3.7	0.1
TA	0.3-0.8	0.5	0.1
RI	14.3-33.6	21.5	0.3
Vitamin C	2.8-9.5	5.4	0.1
Total phenolics	11.3-41.7	25.1	0.6
Flavonoids	2.3-18.0	6.4	0.3
RAC	238.4-610.4	395.0	6.2

Units and abbreviations: Production (kg/tree); Fruit weight (g); Firmness (N); N = Newtons; SSC = Soluble solids content (°Brix); TA = Titratable acidity (g malic acid/100 g FW); RI = Ripening index (SSC/TA); Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of FW); RAC; Relative Antioxidant Capacity (µg TE/g of FW). Abbreviations: AsA = Ascorbic acid; C3GE = Cyanidin-3-glucoside equivalents; CE = Catechin equivalents; GAE = Gallic acid equivalents; TE= Trolox equivalent.

Significant year-to-year variations in agronomic and basic biochemical traits were also observed in the progeny (**Table 5.2**). However, the first year of the study (2009) differed significantly from the last two years (2010 and 2011) for yield, fruit weight, firmness and pH. The progeny showed significant differences in 2011 for TA and RI. It is well-known that yield depends on a number of factors, such as density of flower buds and flowers, fruit set, fruit size, winter and late spring freeze damage, precipitation amount, and orchard management (Milatović et al., 2010). Fruit mass is one of the major selection criteria, and this complex trait is known to be greatly influenced by the environment and very often by interactions between genotype and environment so experiment must be repeated over several years or in different locations to take the environmental effect into account (Quilot et al., 2002).

Table 5.2. Annual variability of agronomical and biochemical fruit quality traits in the 'Babygold 9' x 'VAC-9510' progeny. Data are means (of n = 130 genotypes) ± SE (Duncan test at P ≤0.05).

Traits	2009	2010	2011
Production	6.5 ± 0.3[a]	13.8 ± 0.4[b]	14.6 ± 0.5[b]
Fruit weight	199.6 ± 5.7[a]	221.3 ± 3.9[b]	228.0 ± 6.4[b]
Firmness	37.1 ± 0.8[b]	29.8 ± 0.6[a]	31.3 ± 0.9[a]
SSC	11.2 ± 0.3[bc]	10.7 ± 0.1[ab]	11.0 ± 0.2[b]
pH	3.8 ± 0.1[b]	3.7 ± 0.1[a]	3.7 ± 0.1[a]
TA	0.5 ± 0.1[a]	0.5 ± 0.1[a]	0.6 ± 0.1[b]
RI	22.6 ± 0.7[b]	21.5 ± 0.7[b]	19.9 ± 0.4[a]
Vitamin C	5.2 ± 0.2[a]	5.4 ± 0.2[a]	5.7 ± 0.2[a]
Total phenolics	26.1 ±1.0[a]	23.8 ± 0.8[a]	24.1 ± 1.0[a]
Flavonoids	5.8 ± 0.3[a]	5.4 ± 0.2[a]	7.6 ± 0.4[b]
RAC	396.7 ± 13.1[b]	363.9 ± 14.8[a]	415.7 ± 12.1[b]
Sucrose	54.1 ± 1.9[a]	52.6 ± 0.9[a]	51.6 ± 1.0[a]
Glucose	6.3 ± 0.2[a]	7.2 ± 0.1[b]	6.5 ± 0.1[a]
Fructose	8.0 ± 0.3[b]	8.0 ± 0.1[b]	7.5 ± 0.1[a]
Sorbitol	3.2 ± 0.1[c]	2.6 ± 0.1[b]	2.0 ± 0.1[a]
Sucrose/glucose	8.8 ± 0.3[b]	7.5 ± 0.1[a]	8.2 ± 0.2[b]
Glucose/fructose	0.6 ± 0.1[a]	0.9 ± 0.1[b]	0.8 ± 0.1[b]
% de Sucrose	39.4 ± 0.9[b]	39.7 ± 0.9[b]	36.8 ± 0.6[a]
% de Glucose	4.5 ± 0.1[a]	5.2 ± 0.1[c]	4.9 ± 0.1[b]
% de Fructose	6.2 ± 0.2[a]	5.8 ± 0.1[a]	7.7 ± 0.2[b]
% de sorbitol	1.8 ± 0.1[c]	1.8 ± 0.1[b]	1.4 ± 0.1[a]
Total sugars	71.6 ± 2.5[bc]	70.4 ± 1.0[b]	67.6 ± 1.1[ab]

Units and abbreviations: Production (kg/tree); Fruit weight (g); Firmness (N); N = Newtons; SSC = Soluble solids content (°Brix); TA = Titratable acidity (g malic acid/100 g FW); RI = Ripening index (SSC/TA); Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of FW); RAC; Relative Antioxidant Capacity (µg Trolox Equivalents/g of FW); sucrose, glucose, fructose, sorbitol and total sugars (g kg^{-1} FW). AsA = Ascorbic acid; C3GE = Cyanidin-3-glucoside equivalents; CE = Catechin equivalents; GAE = Gallic acid equivalents; TE= Trolox Equivalent; SE= standard error.

5.3.2. Antioxidant compounds content

Vitamin C content ranged in this progeny from 2.8 to 9.5 mg of AsA/100 g of FW (Table 5.1) representing a more than 7-fold variation. 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 (Tavarini et al., 2008; Cantín et al. 2009a; Abidi et al., 2011). The mean amount of total phenolics in our progeny fell within the range reported in the literature for peach fruits, namely 14-77 mg GAE/100 g of FW (Proteggente et al., 2002; Vizzotto et al., 2007; Cantín et al., 2009a; Abidi et al., 2011). It has been reported that climatic conditions and agricultural factors can affect the fruit nutritional composition including bioactive compounds (Valero et al., 2007). However, in this work, all genotypes were grown under the same environmental conditions and cultural practices, and then, the differences in fruit

quality parameters commented above as well as in the content in bioactive compounds may be due to differences in the genotype and year of study. According to this statement, Hegedús et al. (2010) have found 21- to 35-fold differences in total phenolic content and antioxidant activity among a wide range of apricot genotypes grown under the same conditions. Also, Cantín et al. (2009a) found a big influence of genotype on this trait among 15 different peach populations. Regarding flavonoids, the results (mean value of 6.4 mg of CE/100 g of FW) revealed a flavonoids content similar to that obtained by Cantín et al. (2009a) in different peach progenies with an average of 8.8 mg of CE/100 g of FW. The RAC also showed a high variability among genotypes with an average of 395 µg of Trolox equivalent per g of FW. Similar content and variations were showed in other peach populations by Cantín et al. (2009a) (227.3 to 629.9 µg TE/g FW, with an average of 405 µg of TE/g FW) and Abidi et al. (2011) (292.4 to 835.8 µg TE/g FW, with an average of 464.2 µg TE/g FW). The antioxidant capacity of fruits varies in relation to antioxidant moieties present in the different species, although variations can also occur among cultivars within a single species (Gil et al., 2002; Cantín et al., 2009a). Vizzotto et al. (2007) reported that the total phenolic content had the most consistent and highest correlation with antioxidant activity indicating that it is more important in determining the antioxidant activity of peaches and plums than are the anthocyanin or carotenoid contents. Fruits are generally characterized by their low content of calories and a high amount of antioxidant substances which are able to prevent a wide range of pathogenic disorders, cardio-vascular diseases and degenerative illnesses related to the aging processes, which benefit human health (Ghasemi et al., 2011). Regarding the annual variation of antioxidant compounds content, results showed changes in the contents of flavonoids and RAC, whereas vitamin C and total phenolics showed similar results among years of study (Table 5.2). Manach et al. (2004) reported that pedoclimatic and agronomic factors have a major effect on polyphenol content. Exposure to light has a considerable effect on most flavonoids and the degree of ripeness considerably affects the concentrations and proportions of the various polyphenols (Manach et al., 2004). Polyphenol content determines also the susceptibility to flesh browning after mechanical wounding (mediated also by the polyphenol oxidase, and peroxidase activities; Jiménez-Atienzar et al., 2007). Results permit the preselection of six genotypes with enhanced concentrations of antioxidant compounds (10, 54, 65, 73, 91 and 120).

Regarding heritability of antioxidant compounds (data not shown), values are relatively low ($H^2 \leq 0.32$). Mratinić et al. (2007) reported that the values of heritability coefficients for antioxidant compounds studied were relatively higher ($H^2 = 0.88$-0.93) than those observed in our progeny (0.01-0.32). Much lower values for peach fruit weight ($H^2 = 0.32$) and dimensions ($H^2 = 0.31$) were obtained by De Souza et al. (1998). These differences are not unexpected if we know that heritability coefficient value is a function of variability of a specific character in the studied population as well as a function of ecological conditions (Mratinić et al., 2007). In addition, most of the mentioned authors use heritability coefficients in a narrow sense, which represent the relation between additive and phenotypic variance. However, in the present work, heritability coefficients have been used in a broader sense and they represent the relation between total genetic and phenotypic variance.

5.3.3. Total sugars content

The studied population exhibited wide phenotypic variations in sugar contents among genotypes (Table 5.3). Total sugars (the sum of sucrose, glucose, fructose and sorbitol contents) in peeled fruit ranged from 46.5 to 90.3 g/kg FW with an average of 70.3 g/kg FW. Quilot et al. (2004a) reported that for total sugars content, variation among trees, among fruits of the same tree, and among years are not negligible in comparison with the variation among genotypes. Cantín et al. (2010b) studying 205 peach genotypes from fifteen different progenies reported an average content of total sugars of 72.1 g/kg FW. Sucrose was the major sugar present in the evaluated genotypes (75.4% of total sugar), followed by fructose, glucose and sorbitol. Sorbitol content varied greatly among genotypes, ranging from 1.0 to 7.5 g/kg FW. Consequently, the percentage of sorbitol in the sugar composition was significantly different among genotypes (1-4.2%). Colaric et al. (2005) reported that sorbitol was the attribute most related to peach aroma and taste among carbohydrates and organic acids. Results showed a similar distribution of these sugar compounds throughout years with slight variability of total sugar content among years (Table 5.2). Glucose showed significant differences among the three years of study, and fructose and total sugars showed a slight superiority in 2009. Brooks et al. (1993) also reported a year-to-year variation in sugar and acid content in fruit of four clingstone peach seedling populations. Year-to-year variation in the percentage of sugar may be explained by the differences in climate and crop load between the years (Brooks et al., 1993), and also by differences in maturity at harvest. The transgressive segregation that was found for

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all the sugars also indicates that it is possible to select for high sugar content in most segregating peach seedling populations (Cantín et al., 2009b).

Table 5.3. Sugar content (g kg-1 FW) in the 'Babygold 9' x 'VAC-9510' progeny. For the progeny (n = 130 genotypes), data are mean of three years of study (2009-2011).

Sugar content	Range	Mean	SE
Sucrose	29.9-70.6	53.0	0.6
Glucose	4.8-9.6	6.7	0.1
Fructose	5.8-10.8	7.9	0.1
Sorbitol	1.0-7.5	2.6	0.1
Total sugars	46.5-90.3	70.3	0.7
% Sucrose	35.9-80.8	75.4	0.8
% Glucose	3.4-7.5	9.5	0.1
% Fructose	5.4-12.6	11.2	0.1
% Sorbitol	1.0-4.2	3.7	0.1

Units: sucrose, glucose, fructose, sorbitol and total sugars (g kg^{-1} FW).

5.3.4. Quantitative variation for chilling injury symptoms

The F1 progeny showed a high variability for all the evaluated CI symptoms. The variation of the symptoms was studied using the mean of 2-year data**.** Continuous distribution was shown for mealiness, graininess, browning, bleeding, off flavor and leatheriness, suggesting polygenic control of these symptoms as was reported in other non-related peach progeny populations (Peace et al., 2006; Cantín et al., 2010a). Mealiness, bleeding and browning were the major CI symptoms observed in this progeny. As expected, the duration of storage (2 or 4 weeks at 5 ºC) increased the severity of CI symptoms.

After 2 weeks of cold storage, the main CI symptoms observed were mealiness and bleeding (Figure 1). Although 15% of the progeny showed bleeding, its manifestation was less severe compared to other studied peach progenies (Peace et al., 2006; Ogundiwin et al., 2007). However, the flesh red color observed in the fruit flesh could be due to the characteristic pigmentation of fruit flesh in this progeny and it may have hindered the evaluation of CI-related red coloration. On the other hand, Lurie and Crisosto (2005) reported that flesh bleeding could be associated with fruit senescence and not with CI disorders which could be an explanation to the low impact of storage duration on this CI symptom in this study. For mealiness, this progeny showed less susceptibility to this CI symptom (41.2%) when compared to FMF progeny (Cantín et al., 2010a), and similar results were observed for flesh mealiness when other authors analyzed only within a CNMF progeny (Peace et al., 2006). It should be noted that the population evaluated in this study was entirely CNMF.

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After 4 weeks of cold storage, a considerable higher proportion of fruit was significantly affected by CI symptoms (with the exception of bleeding), showing that these disorders are triggered by the cold storage duration, as previously reported (Lurie and Crisosto, 2005). The major CI symptom observed after 4 weeks of storage was flesh browning. On the other hand, it is worth noting that browning scoring might be underestimated in the population since the visual scoring of this trait in the area surrounding the stone is more difficult to accomplish in the clingstone individuals due to the adhesion of the flesh tissue to the stone. No significant leatheriness was found in the fruit after both durations of cold storage, maybe due to the low susceptibility of these genotypes to this CI symptom (data not showed). Similar results were found by Martínez-García et al. (2012) who reported that flesh leatheriness occurred sporadically at low incidence and with low heritability, indicating that this trait is random rather than genetic. Since flesh leatheriness may have occurred in fruit that were harvested slightly immature before entering cold storage (Brovelli et al. 1998), the low incidence of this trait found in this work may result from our care to harvest all fruit at full commercial maturity. A decrease in bleeding was scored after 4 weeks of cold storage, which could be explained by the oxidation of the red pigments, and the difficulty of accurately scoring bleeding in this population (at 2 weeks) due to its natural red pigmentation. Similar results were found by other authors for bleeding when analyzed only within the FMF progeny and for mealiness when analyzed only within the CNMF progeny of peach populations (Peace et al., 2006). These authors reported that mealiness was almost non-existent in CNMF progeny, while bleeding incidence was higher in CNMF progeny (Peace et al., 2006; Ogundiwin et al., 2007). Similarly, Martínez-García et al. (2012) reported that flesh bleeding occurs primarily in non-melting flesh fruit, and particularly when the fruit is white-fleshed. Interestingly, the results of this work showed at least sixteen genotypes (14, 33, 52, 58, 68, 76, 78, 80, 93, 96, 113, 114, 118,120, 128, and 129) with very low susceptibility to CI symptoms.
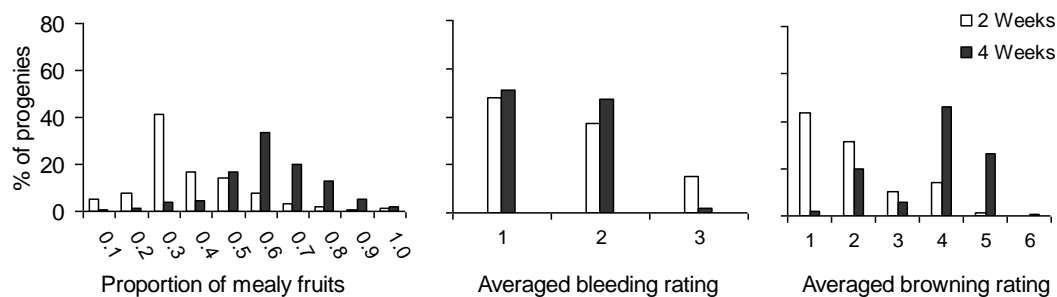
Figure 5.1. Distribution of internal breakdown browning in the 'Babygold 9' x 'VAC-9510' progeny averaged over 2 years of study after storage at 5 ºC for 2 and 4 weeks and then ripened at 20 ºC during 2 or 3 days. Mealiness, was scored as the proportion of fruit affected with these symptoms in the sample (0-1). Bleeding scored on a scale of 1 (no bleeding) to 3 (more than 50% of the flesh with bleeding). Browning scored on a scale of 1 (no browning) to 6 (severe browning).

Genotype was the main factor contributing to phenotypic variation for all the CI symptoms measured (Table 5.4), showing a contribution between 32.2% and 95.1%. These results agree with Peace et al. (2006) and Martínez-García et al. (2012) who reported that susceptibility of stone fruits to CI is highly influenced by the genetic background of the cultivar. Off-flavor and bleeding showed the higher proportion of phenotypic variance attributed to year and storage duration which agrees with reported variations between years in CI symptoms (Campos-Vargas et al., 2006; Martínez-García et al., 2012). Genotype explained 95.1% of phenotypic variation of bleeding and 32.2% of mealiness. Cantín et al. (2010a) reported that genotype was the main factor contributing to phenotypic variation for all the CI symptoms measured in the cross 'Venus' x 'Big Top', showing a contribution between 29% and 65% in total variability.

Table 5.4. Contribution (%) of factors (genotype, year and storage duration) to phenotypic variance that affect chilling injury symptoms, observed for 2 years in the 'Babygold 9'×'VAC 9510' progeny.

CI symptoms	Genotype[a]	Year	Storage duration
Mealiness	32.2	21.5	21.7
Graininess	39.6	27.4	27.3
Off flavor	53.4	35.8	35.0
Leatheriness	57.6	39.5	39.5
Bleeding	95.1	59.5	61.0
Browning	40.8	29.4	27.8
CI index	42.4	31.8	30.5

[a] This proportion of phenotypic variance attributed to Genotype is the broad sense heritability (Hb).

5.3.5. Correlations between CI symptoms and agronomic and biochemical traits

As expected, mealiness and graininess showed a significant correlation (r=0.433, $P \leq 0.01$), since graininess is the sensorial feeling of visual mealiness. Browning showed

significant correlations with mealiness, graininess and bleeding and CI index ($r=0.288$; $r=0.379$, $r=0.306$, $P \leq 0.01$ and $r=0.235$ $P \leq 0.05$, respectively), which means that all CI symptoms are related in the process of fruit damaging by cold storage (Table 5.5).

Table 5.5. Correlation coefficients between chilling injury (CI) symptoms and agronomic and biochemical traits observed for 2 years in the 'Babygold 9' × 'VAC- 9510' progeny.

CI symptoms	Total phenolics	Flavonoids	RAC	RI	SSC
Mealiness	0.383**	0.255**	0.263**	0.231*	0.220*
Browning	0.276**	0.346**	0.218*	NS	NS
CI index	NS	0.221*	NS	0.246**	NS

NS: Not significant; * Correlation is significant at the $P \leq 0.05$ level;** Correlation is significant at the $P \leq 0.01$ level.

Mealiness and browning showed significant correlations with total phenolics, flavonoids and RAC, implying that both symptoms are influenced by the phenolic and antioxidant content of the fruit. These results also suggest the role of antioxidant compounds content in the development of browning in fruit after cold storage. The significant positive correlation of flavonoids with CI index suggests that these phenolic compounds could be implicated in the development of internal browning of fruits. However, no significant correlation was found between CI and total phenolics or RAC, which could be due to the wide variation of these traits in the studied population. The significant correlation between CI and RI could be explained since the severity of CI depends on the ripening stage at harvest (Valero et al., 1997). Higher incidence of injury was reported for cultivars picked at more advanced ripening stage. González-Buesa et al (2011) found positive correlations between browning and RI studying CNMF genotypes. However, no significant correlation was found in our progeny. Manach et al. (2004) reported that storage may also affect the content of polyphenols that are easily oxidized. Oxidation reactions after cold storage of fruits result in the formation of more or less polymerized substances, which lead to changes in the quality of fruits, particularly in color and organoleptic characteristics and such changes may be harmful (browning of fruit) to consumer acceptability.

Nowadays, many studies focus in increasing the phenolic compounds in fruits. The development of new varieties with higher content of phenolics is of interest for the improvement of the nutritional quality of peaches. However, the oxidation of phenolic compounds causes browning of the fruit and reduces its acceptability. The correlations observed between antioxidant traits and CI symptoms suggests the possible role of antioxidant compounds in the development of mealiness and internal flesh browning of peaches after a long cold storage period. These correlations could be of interest for the

breeding programs in order to develop peach varieties with higher concentrations of phenolics and reduced chilling injury susceptibility. It is possible to find varieties with a high concentration of phenolics and relatively low or intermediate degree of internal browning as we found in 16 peach genotypes in our progeny. Further research has to be done in order to elucidate the role of the antioxidant compounds in the development of CI symptoms in peach fruit.

CAPÍTULO 6

Genetic control and location of QTLs involved in organoleptical traits in a nectarine [Prunus persica (L.) Batsch] progeny

Genetic control and location of QTLs involved in organoleptical traits in a nectarine [*Prunus persica* (L.) Batsch] progeny

Abstract

In peach fruits, bioactive compounds play a significant role in fruit quality. Phenolic compounds serve as a major source of potential antioxidants which are known to have impact in human well being preventing degenerative diseases. This study was conducted in an F1 population derived from the cross 'Venus' x 'Big Top' nectarines (75 seedlings) in order to investigate the genetic control and location of quantitative trait loci (QTLs) involved in the organoleptical fruit quality traits. Biochemical analyses have been performed to quantify soluble solids content (SSC), firmness (N), titratable acidity (TA), pH, ripening index (RI), L-ascorbic acid (vitamin C), total phenolics, flavonoids, anthocyanins and relative antioxidant capacity (RAC). All the evaluated traits in the progeny showed continuous distribution which is typical of quantitative or polygenic inheritance. A genetic linkage map was constructed using SNP and SSR markers and allowed the detection of significant QTLs for SSC and the main antioxidant compounds into the genomic regions corresponding to the linkage group 4 (LG4). QTLs for SSC, total phenolics and flavonoids were stable across two years of study, the QTL for RAC was stable across three years whereas the QTL for vitamin C was identified only for one year. The observed QTLs explained 30.4%, 19.5%, 26.5% and 21.2% of the phenotypic variation for SSC, total phenolics, RAC and flavonoids, respectively. This study will contribute to the knowledgment in breeding programs in order to apply marker assisted selection (MAS) to select peach cultivars with healthy attributes for the consumer.

6.1. Introduction

The external quality of fruits is determined by shape, colour and size, while the internal quality is determined by the texture, sugars, organic acids and antioxidant compounds contents, which contribute significantly to the taste and aroma of the fruit (Hudina et al., 2012). The last decade has seen the proliferation of an enormous number of scientific studies focused on the activity of antioxidant compounds present in our diet and able to prevent the occurrence of degenerative diseases (Russo et al., 2012).

Biochemical and genetic studies on the mechanisms of action of phytochemicals provide a functional explanation of how and why a diet rich in fruits and vegetables is considered healthy (Russo et al., 2012). It is now believed that polyphenols may exert their beneficial action through the modulation of gene expression and the activity of a wide

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range of enzymes and cell receptors (Chagné et al., 2012, and references therein). However, the health effects of dietary antioxidant compounds depend on the total amount consumed and on their bioavailability. In adition, the content in antioxidant compounds can vary according to the location within the fruit (skin vs flesh), the stage of fruit maturity and even the location of the fruit within the tree (Chagné et al., 2012).

Dissection of the genetic components underlying complex agricultural traits in plants has so far used mainly experimental bi-parental crosses and a limited number of genetic markers (Verde et al., 2012). Over the last two decades, availability of genetic knowledge of peach as the model for *Prunus* and the *Rosaceae* has accelerated with the development of molecular markers, linkage and physical maps, comparative genomics studies, databases, and the very recent release of the full genome sequence of a dihaploid peach genotype (Arús et al., 2012). Illumina's Infinium BeadArray Technology platform is an extremely high-throughput SNP genotyping system that allows the detection of up to 2.5 million SNPs per single DNA sample (Verde et al., 2012, and reference therein).

One way to assess the genetic determinism of traits variation without a priori knowledge is the quantitative trait loci (QTL) mapping. QTL detection is considered as a way to investigate the number of genes that control quantitative traits, and the magnitude and the distribution of their effects (Bost et al., 2001). QTL mapping makes use of segregating populations and gives global insights into the genetic architecture of the target phenotype, i.e. the number, position and effects of genomic regions (Huang et al., 2012, and reference therein). Among all available mapping methods, the multiple-QTL approach (Multiple QTL Model; MQM) is particularly suitable for complex trait analysis since it uses simultaneously multiple marker intervals with possible inclusion of epistasis terms in QTL mapping model (Huang et al., 2012, and reference therein).

In peach, several Mendelian characters involved in fruit quality have been already studied and mapped (see Arús et al., 2012, for a recent review), such as peach/nectarine, polycarpel and flesh color (Bliss et al., 2002), melting/non melting flesh (Warburton et al., 1996), and freestone/clingstone (Dettori et al., 2001). Moreover, several authors (Dirlewanger et al., 1998; Etienne et al., 2002; Quilot et al., 2004b) have localized QTLs involved in the control of physico-chemical components of different fruit quality traits, such as sugars and organic acid contents on linkage groups 4, 5 and 6. Regarding antioxidant compounds, Dirlewanger et al. (2006) analyzed the genetic control of fruit phenolics in the peach F_2 population ('Ferjalou-Jalousia'® x 'Fantasia') and detected QTLs involved in phenolic compounds on LGs 1, 2, 4 and 6.

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In the present study, we have applied the QTL analysis with R/qtl software using multiple-QTL-Model (MQM) in the R platform in order to find QTLs involved in the control of several organoleptical traits in peach fruit. These results contribute to define the peach antioxidant compounds map that can be useful for breeding and Marker Assisted Selection (MAS) purposes. The aim of the present research was to analyse the genetic control of the main organoleptical fruit quality traits evaluated for four years in an F1 population derived from the cross of 'Venus' x 'Big Top' nectarines.

6.2. Materials and methods

6.2.1. Plant material

The progeny assayed was a segregant F1 population of 75 seedlings obtained from a controlled cross, between *Prunus persica* cvs. 'Venus' (female parent) and 'Big Top' (male parent). 'Venus' is a freestone, melting and yellow flesh nectarine cultivar, whereas 'Big Top' is a clingstone, melting and yellow flesh nectarine cultivar. The segregant population is entirely melting flesh, either cling- or freestone. The resulting seedlings were budded on the same rootstock (GF 677) and established (one tree per genotype) at the Experimental Station of Aula Dei-CSIC (northern Spain, Zaragoza) in 2002. Trees were grown under standard conditions of irrigation, fertilization and pest and disease control. Fruits were harvested over four years at commercial maturity (2007-2010).

6.2.2. Quality parameters evaluated

During the years 2007-2010, agronomic and biochemical fruit quality traits were measured individually in each seedling tree. Annual yield, fruit weight, firmness, soluble solids content (SSC), titratable acidity, pH, vitamin C, total phenolics, flavonoids, anthocyanins and RAC were evaluated in each independent seedling as reported by Abidi et al. (2011) (see Chapter 3).

6.2.3. Phytochemical extraction and analysis

For all analyses, samples of 5 grams of fruit flesh were used, as flesh is usually consumed in peaches. All samples were frozen in liquid nitrogen and kept at -20ºC until analysis. For vitamin C analysis, samples were kept in 5 mL of 5 % metaphosphoric acid for preservation of ascorbic acid, frozen in liquid nitrogen and stored at -20 ºC until analyses. Then, samples were homogenized, centrifuged and supernatant was recovered as described by Cantín et al. (2009b) and Abidi et al. (2011). Vitamin C, total phenolics, flavonoids, anthocyanins and RAC were evaluated with colorimetric methods and

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measured using a spectrophotometer (Beckman Coulter DU 800) as described by Abidi et al. (2011).

6.2.4. DNA extraction, SSRs and SNPs analysis

DNA was extracted from young leaves of 'Venus', 'Big Top' and all the progeny (75 genotypes) by using the DNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA), following manufacturer's instructions. For SSR markers, two markers were assayed in LG4. For the PCR reactions, 10 ng of genomic DNA was amplified in a final volume of 15 µl containing 1X biotools buffer, 2.0 mM MgCl$_2$, 0.20 mM of dNTPs, 0.15 µM of each primer pair and 0.5 U Taq DNA polymerase (Biotools, Madrid, Spain). The PCR amplification conditions were as follows: preliminary denaturation (3 min at 94 ºC), followed by 35 cycles consisting of denaturation (1 min at 94 ºC), 45 s at the corresponding annealing temperature and extension (1 min at 72 ºC), and a final extension after the last cycle (7 min at 72 ºC). After amplification, acrylamide gels were prepared and 0.5 µl of the PCR product for each genotype was loaded either in acrylamide or on LI-Cor automatic sequencer and data were collected. Fragment sizes were estimated with the 100-bp ladder-DNA-Brand sizing markers.

For SNPs marker analysis, concentration and quality of DNA was checked using PicoGreen. Samples were genotyped using the *RosBREED_Peach_10k_11494376_A* chip from Illumina which includes more than 9000 SNPs peach markers (GDR, 2011; Verde et al., 2012) following the single base extension assay (Steemers, et al., 2006) and manufacturer conditions included in the Illumina® Infinium® HD Assay Ultra protocol.

6.2.5. Data analysis, genotyping and map construction

Individuals that showed the same genotype as the female parent 'Venus' in all the markers were identified as self-pollinated seedlings. All polymorphic, non skewed and non repetitive markers were selected. For map construction, pseudo-testcross strategy was used (Grattaglia and Sederoff, 1994) usingJoinMap® 4.0 software (Van Ooijen, 2006). Maximum Likelihood (ML) algorithm, cross pollinated population and Kosambi mapping function were the options selected to construct the map.

6.2.6. QTL analysis

QTL analysis was performed with R/qtl software using multiple-QTL-Model (MQM) in the R platform (Broman et al., 2003). The likelihood value of the presence of a QTL was expressed as a LOD (log of odds) score, which is the 10-base logarithm of the

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quotient of the likelihood of the existence of a segregating QTL, and the likelihood for the situation when a locus with zero genetic effect would segregate (i.e. there is no segregating QTL). When the LOD score exceeds the predefined significance threshold somewhere on a LG, a segregating QTL is declared. LOD significance threshold were determined with α=0.05 by permutation test (1,000 linkage group-based), with which the significance threshold can be determined based on the actual data rather than on assumed normally distributed data. QTL mapping were performed for each year and when the QTL was detected for more than one year, the mean was used for the final QTL mapping. The inner confidence intervals were calculated with the option BAYESINT in R/qtl software (Broman and Saunak, 2009) and the outer with the LOD threshold.

6.3. Results and discussion

6.3.1. Map and QTLs for organoleptical fruit traits

A complete genetic map of the 'Venus' x 'Big Top' F1 progeny was constructed using SNP and SSR markers. The complete map for the population (data not showed) includes in the 'Venus' map 104 markers in 9 LGs covering 260 cM, and in the 'Big Top' map 123 markers in 10 LGs covering 514 cM of the total genome. The map presented in this research correspond only to LG4 since all the QTLs affecting the main organoleptical traits discussed later were located in this linkage group. The maternal and paternal maps obtained for LG4 are showed in Figure 6.1. The LG4 of the parental 'Venus' covered a total length of 85.7 cM with an average distance of 3.29 cM/marker. In total, twenty six markers have been mapped and consisted of twenty four SNP markers and two SSR markers (UDP98-024 and CH15) (Figure 6.1). The LG4 of the parental 'Big Top' with covered a total length of 52.6 cM with an average distance of 5.26 cM/marker (Figure 6.1).
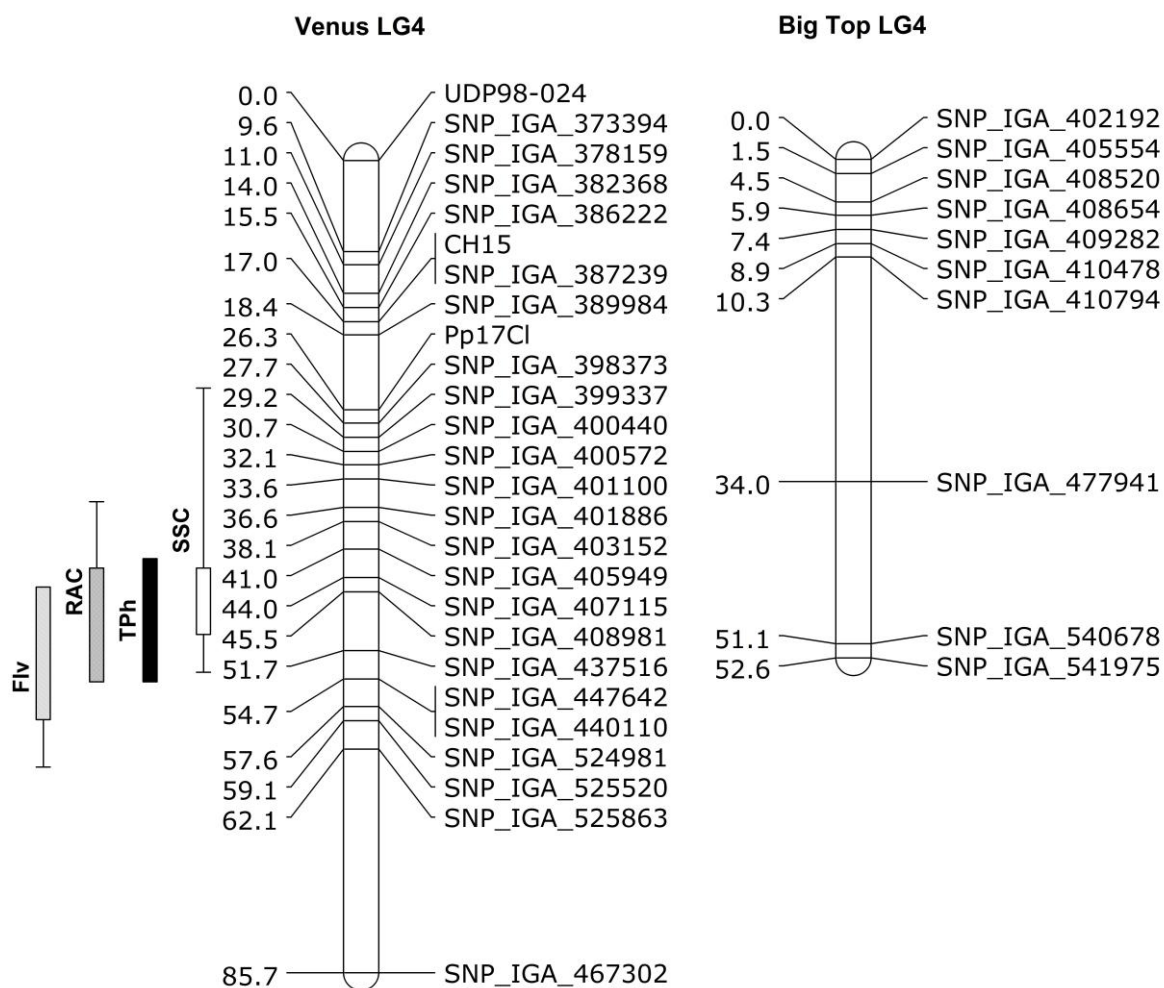
Figure 6.1. Maternal ('Venus') and paternal ('Big Top') linkage group 4 (LG4) map of the F1 progeny showing the position of SNP and SSR markers (right side) and the distances for all the markers (left side). Barrs on the left side represented the position for the QTLs found with the interval of confidence (1.5-LOD). Abbreviations: Flv, Flavonoids; RAC, Relative antioxidant capacity; TPh, Total phenolics; SSC, Soluble Solids Content.

Linkage mapping and QTL analysis were used to determine the location, number and effect of genomic sites contributing to the phenotypic variation in the 'Venus' x 'Big Top' population for organoleptical traits. The analysis of the genetic control permitted the identification of four QTLs in the maternal map for the studied traits (Figure 6.1, Table 6.1). LOD plots for SSC, total phenolics, flavonoids and RAC are represented in Figure 6.2.

Table 6.1. Traits, nearest marker, QTL position, Threshold LOD, maximum LOD score and percentage variance explained for the QTLs identified on LG4 by MQM mapping in the F1 population of 'Venus' x 'Big Top'.

Traits	Nearest marker	QTLs			Variance explained (%)
		Position	Threshold LOD	Max. LOD score	
SSC	SNP_IGA_407115	44.03	1.96	8.14	30.4
Total phenolics	SNP_IGA_408981	45.50	2.08	3.16	19.2
RAC	SNP_IGA_408981	50.00	2.14	6.09	26.5
Flavonoids	SNP_IGA_437516	51.00	1.99	4.35	21.2

Abbreviations: SSC, Soluble Solids Content; RAC: Relative Antioxidant Capacity

The QTL for each one of the following traits: SSC, total phenolics, RAC and flavonoids in the map of the maternal parental 'Venus' (Figure 6.1) explained 30.4%, 19.2%, 26.5% and 21.2% of the total variability, respectively (Table 6.1). The marker SNP_IGA_407115 linked to the QTL for SSC, the marker SNP_IGA_408981 was the nearest one linked to both QTLs for total phenolics content and RAC, and the SNP_IGA_437516 marker was linked near the QTL for flavonoids content. The analysis in the paternal map 'Big Top' did not show any QTL for antioxidant traits in LG4.

Most of the QTLs found were consistent through 2 or 3 years of study (2008-2010) but others showed low stability among years showing that the expression of these genes would be more influenced by the environmental or experimental conditions. The QTLs found for SSC, total phenolics and flavonoids contents were stable across two years of study (2009-2010, 2008-2009 and 2008 y 2010, respectively) and the QTL for RAC was consistent over 3 years of study (2008-2010) whereas the QTL for vitamin C were observed only for one year (data not showed, 2008).

Few QTLs associated with organoleptic fruit quality have been mapped (Abbott et al., 1998) and genes controlling organoleptic fruit quality often remain unknown (Etienne et al., 2002; Quilot et al., 2004b). Moreover, several authors pointed out that QTLs controlling these traits (antioxidant compounds) often show low stability due to their high sensibility to environmental factors (Quilot et al., 2004b, and reference therein).
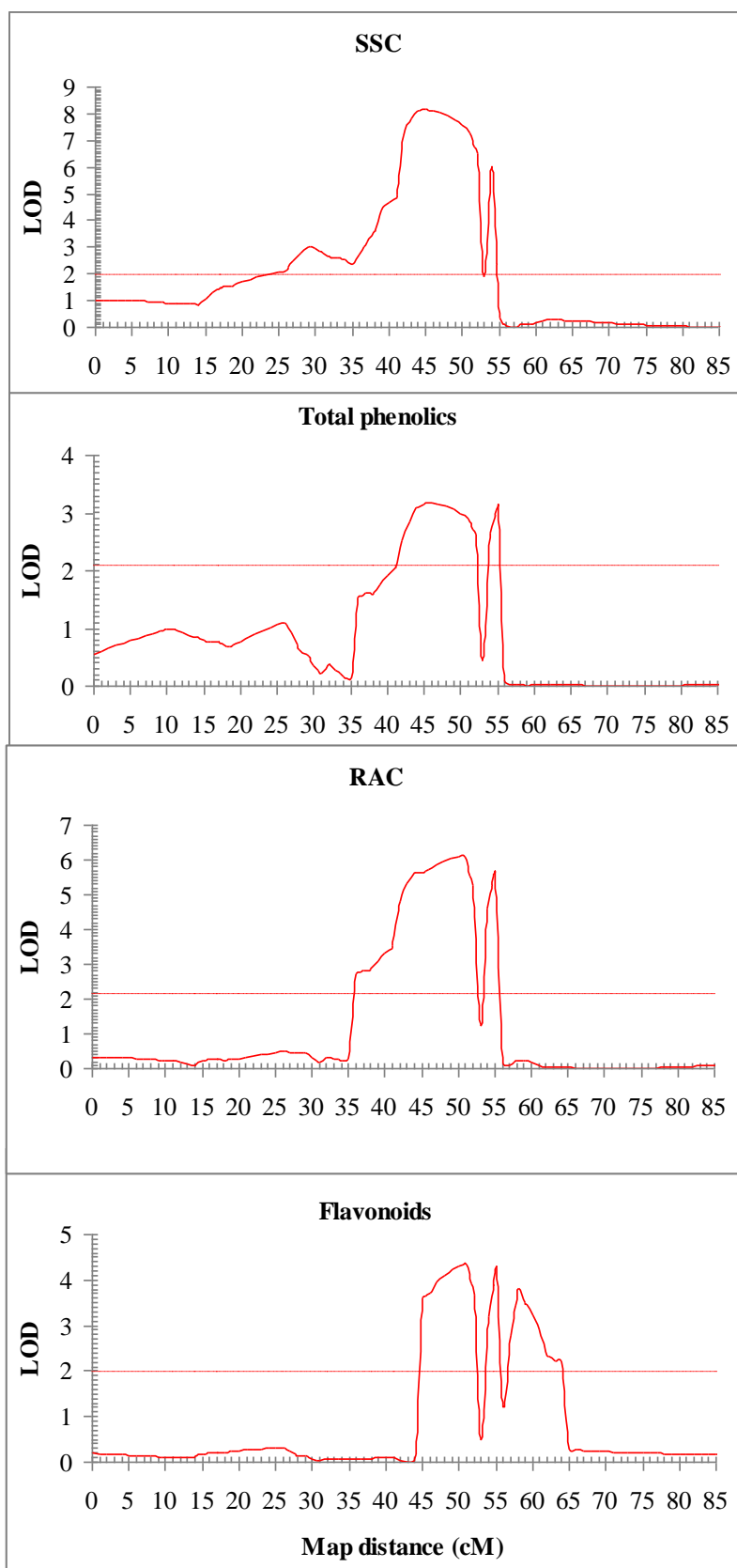
Figure 6.2. LOD plots for Soluble Solids Content (SSC), Total phenolics, Relative Antioxidant Capacity (RAC), and Flavonoids obtained on LG 4 of 'Venus'.

Other authors working with the same population (Cantín et al., 2010a) detected QTLs for chilling injury (CI) symptoms on LG4. These QTLs were associated with several agronomic and quality traits and accounted between the 26% and the 92% of the observed variation calculated by interval mapping. Significant QTLs for SSC, pH, TA and firmness were detected on the LG4 in this population as other authors have found in other populations. Our results revealed that the QTL controlling SSC content was, stable for two years. The soluble solids content (SSC) is a major agronomic trait studied in peach and is one of the most important traits from a commercial point of view. Nevertheless, other authors reported QTLs for SSC and for soluble sugars on LG6 in the 'Ferjalou-Jalousia' x 'Fantasia' F2 peach population (Dirlewanger et al., 2006) and on LGs 1 and 2 in a interspecific population derived from *Prunus persica* and *P. ferganensis* (Quarta et al., 2000; Arús et al., 2012).

Regarding antioxidant compounds, Dirlewanger et al. (2006) studied the genetic control of polyphenols only during one year in the 'Ferjalou-Jalousia' x 'Fantasia' F_2 peach population. QTLs controlling total soluble phenols, insoluble and soluble proanthocyanidins were detected on LG6. In contrast to the relatively abundant information on quantification of phenolic compounds in peach fruits, there is no many previous work that focuses on QTLs controlling these important traits inside the *Rosaceae* family. Recently there is available information for the genetic control of these compunds only in apples (Chagné et al., 2012).

Our SNPs and SSRs mapping and location of QTLs for antioxidant compounds in fruit from the 'Venus' x 'Big Top' segregating population has added information to the body of knowledge concerning the genetic control of the antioxidant content in peach. QTLs were stable across two years for SSC, total phenolics and flavonoids, and across three years for RAC. The present work confirmed presumptions about the complex genetic architecture of antioxidant control in peach fruits as well as the influence of the environmental conditions. To our knowledge, this study presents the first QTL analysis on nectarine fruits during more than three years of study dealing with genetic control of antioxidant compounds and basic biochemical fruit quality traits. Our results supported the QTLs positions related to antioxidant compounds and soluble solids content found in the same population (Cantín et al., 2010a) and in other unrelated peach progeny populations (Quilot et al., 2004b; Dirlewanger et al., 2006; Arús et al., 2012). The advantages of these results are, the broad coverage of the linkage group 4, and the consistency of the QTLs

over several years. These results will contribute to a better understanding of the genetic control of the important nutritional quality traits of peach and nectarine fruit.

CAPÍTULO 7

Discusión General

Discusión General

El melocotón y la nectarina son frutas muy saludables e inherentes a la dieta mediterránea, bajas en calorías, contenido razonable en vitamina C, mucha fibra y buen aporte en carotenos. El melocotonero es en producción la especie más importante de fruta dulce en España, siendo el segundo país productor de la UE y el más competitivo en base a la calidad ofrecida, las producciones y los menores costes con respecto a Italia, el principal país competidor. Esto es debido sobre todo a la mejora de la tecnología de la producción y a la reconversión varietal que ha hecho posible la introducción de nuevas variedades con mejor presentación y mejor calidad. A pesar de ello, la producción de la UE se encuentra, al límite de la sobreproducción, y el consumo está estancado debido principalmente a problemas de calidad y a la irregularidad de la fruta en destino. En este sentido, la crisis de los precios es recurrente y pone en cuestión la viabilidad futura de este sector en España (Iglesias, 2010).

En este contexto de crecimiento progresivo de las producciones en España, se considera clave para el futuro del sector el incremento tanto del consumo nacional, como de las exportaciones hacia nuevos mercados, como los países del Este y de Oriente Medio. En este sentido, se considera un objetivo esencial la satisfacción del consumidor para aumentar la aceptabilidad del producto final. Este objetivo no se alcanzará sin una tipificación e identificación de la fruta en destino, especialmente en lo referido al sabor y calidad del fruto.

En España, el consumo actual de melocotón se sitúa alrededor de 4 kg/habitante y año y se encuentra estabilizado, habiendo disminuido un 46% desde 1989 (Iglesias et al., 2010). Los consumidores señalan como causas principales del bajo consumo la falta de sabor, el estado inadecuado de madurez (frutos inmaduros), la diversidad de calidades y la falta de identificación en los puntos de venta respecto al gusto o tipo del fruto (dulce o ácido). La satisfacción del consumidor va a ser un factor clave en el futuro para incrementar el consumo de fruta, y para ello es importante conocer las características que más influyen en la aceptación del producto final por parte de los consumidores. En muchos programas de mejora ya se han estudiado algunas relaciones entre los diferentes atributos de calidad de los frutos de melocotón. También es cada vez más frecuente para la evaluación de la calidad organoléptica de melocotón incluir las determinaciones químicas (contenido en azúcares, ácidos y compuestos fenólicos) y sensoriales (sabor y astringencia) (Bassi y Selli, 1990; Tavarini et al., 2008; Cantín et al., 2009a; Rossato et al., 2009).

Por tanto, la renovación varietal iniciada en la última década deberá dirigirse hacia variedades con buen comportamiento agronómico, de gran calidad y ricas en compuestos biosaludables. En los últimos años, ha habido un incremento significativo de la producción y el consumo de melocotón y nectarinas por su sabor dulce y aromático y comodidad de consumo. Con la diversidad varietal disponible, es recomendable establecer los parámetros de cosecha (firmeza, contenido de azúcares, color, etc.) en los principales grupos y las épocas de recolección recomendadas, con el objetivo de evitar recolecciones anticipadas. Adaptar la oferta varietal a las preferencias del consumidor será clave para el futuro.

La variabilidad fenotípica existente en las poblaciones de mejora evaluadas en este trabajo en la Estación Experimental de Aula Dei del CSIC (Zaragoza), en las condiciones de cultivo del Valle Medio del Ebro, ha revelado el potencial de las mismas para seleccionar genotipos de tipo melocotón y nectarina con un buen comportamiento agronómico y buenas características organolépticas del fruto.

7.1. Evaluación de caracteres agronómicos y de calidad de fruta para la selección de nuevos cultivares.

Los resultados obtenidos en este estudio en la evaluación de las poblaciones de mejora 'Venus' x 'Big Top' y 'Babygold 9' x 'VAC-9510' mostraron una gran variabilidad fenotípica entre genotipos para los diferentes caracteres agronómicos evaluados. La fecha de cosecha para la población 'Venus' x 'Big Top' varió desde principios de julio hasta mitad de agosto y en la población 'Babygold 9' x 'VAC-9510' desde principio de julio hasta finales de agosto. La variabilidad observada en ambas poblaciones viene explicada por las distintas fechas de floración y de maduración de los cultivares utilizados en los cruzamientos (Iglesias y Echeverría, 2009; Cantín et al., 2010a). A principios de julio, 'Big Top' es la variedad de referencia indiscutible por sus excelentes características pomológicas: color, calibre, consistencia y sabor dulce (Iglesias et al., 2010). A finales de julio 'Venus' sigue siendo la variedad de referencia en su época de maduración, siendo una de las más rentables, por su elevado potencial productivo, rusticidad, facilidad de conducción y calibre, pero con frutos bicolores y de sabor marcadamente ácido. En cuanto a 'Babygold 9' es una variedad de media estación, con la fecha de cosecha hacia mitades de julio, de gran tamaño, con piel de color amarillo crema y chapa moteada de color rojo en la parte soleada, y pulpa amarilla y dura. 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 (Quilot et al., 2004b; Eduardo et al., 2011). Esta variabilidad permite la preselección de genotipos con las fechas de maduración más apropiadas para cubrir las demandas del mercado, respecto al consumo de melocotones y nectarinas, aunque éstos sean los meses con mayor oferta.

En cuanto a otros caracteres, se observó una amplia variabilidad entre genotipos en la producción, peso medio del fruto, acidez, sólidos solubles, firmeza e índice de madurez (RI = SSC/TA) en las dos poblaciones estudiadas. Esta variabilidad viene explicada tanto por una componente genética como por la influencia de factores ambientales. Milatović et al. (2010) observó que la producción del melocotonero depende de factores, como la floración, el cuajado y el tamaño del fruto como de las bajas temperaturas y las precipitaciones. La población 'Babygold 9' x 'VAC-9510' mostró una superioridad en cuanto a la producción y al peso medio del fruto respecto a la población 'Venus' x 'Big Top', tal y como se indica en el trabajo de Cantín et al. (2010b). En ambas poblaciones se obtuvieron frutos ácidos y no ácidos, ya que los frutos maduros con pH superior a 4.0 son considerados como no ácidos (Monet, 1979). También se observó una variabilidad entre genotipos en cuanto a la acidez valorable (TA) con valores más bajos que el límite máximo (0.9%) para melocotones de acidez normal (Hilaire, 2003). 'Venus' es una variedad de nectarina ácida (TA = 0.7%), y 'Big Top' es una nectarina subácida (TA = 0.4%), lo que explica la segregación de este carácter en la progenie. En cuanto a los sólidos solubles, la población 'Venus' x 'Big Top' presentó contenidos más altos que la población 'Babygold 9' x 'VAC-9510' lo que se reflejó en una superioridad en el índice de madurez (RI), resultados que coinciden con el trabajo de Cantín et al. (2010). La relación entre SSC y TA tiene un papel importante en la aceptación de la fruta por parte del consumidor y en muchos casos está controlada por la interacción entre ambos parámetros, más que por los contenidos en SSC (Crisosto et al., 2004). En cuanto a la firmeza del fruto, las dos poblaciones presentaron valores inferiores al máximo establecido por el reglamento de comercialización del fruto para el melocotonero.

En los programas de mejora de nuevas variedades es muy importante conocer las correlaciones entre los distintos parámetros agronómicos y bioquímicos. Así se observaron correlaciones significativas entre caracteres pomológicos en relación con la calidad del fruto. En la población 'Venus' x 'Big Top' se observaron correlaciones positivas entre firmeza, SSC, pH y RI, tal y como también se describe en el trabajo de Cantín et al. (2010b). Estas correlaciones son de gran importancia ya que la selección de genotipos con alto valor en SSC vendrá asociado con frutos de mayor firmeza y por tanto con menor

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sensibilidad a los daños mecánicos durante el transporte (Crisosto et al., 2001). Los resultados indican que tanto el genotipo como las condiciones de pre- y postcosecha (poda, riego, fecha de recolección, conservación de fruta) tienen una gran influencia sobre la varianza fenotípica de los caracteres agronómicos y de la calidad organoléptica del fruto en melocotonero (Tavarini et al., 2008; Cantín et al., 2010b).

Algunos de los parámetros evaluados que influyen en la calidad del fruto, mostraron una variabilidad significativa entre los distintos años de estudio, lo que subraya la importancia de incluir en un programa de mejora el estudio de las poblaciones a lo largo de diferentes años. Así, el efecto del año, debido fundamentalmente a diferencias de temperatura (Ruiz y Egea, 2008; Sánchez-Pérez et al., 2007a), pudo observarse entre años para las fechas de floración y de cosecha en el presente estudio. Cabe mencionar que tanto el período de floración como de cosecha, se produjeron cada año más temprano en los tres años de estudio.

7.2. Compuestos bioquímicos que influyen en la calidad de la fruta

El contenido en compuestos antioxidantes ha mostrado gran variabilidad entre genotipos en las dos poblaciones de estudio. Los valores de antioxidantes obtenidos en ambas poblaciones estuvieron dentro del rango señalado en pulpa del melocotón (Gil et al., 2002; Proteggente et al., 2002; Tavarini et al., 2008; Cantín et al., 2009b). Es remarcable que la población 'Venus' x 'Big Top' mostrara contenidos mayores de flavonoides y fenoles totales comparados con la población 'Babygold 9' x 'VAC-9510. Esta variabilidad es de interés para considerar en los programas de selección de genotipos con mayor contenido en compuestos antioxidantes (Vauzour et al., 2008). Las antocianinas mostraron bajas concentraciones y poca variabilidad entre genotipos debido a la escasa variación del color de la fruta en la población 'Venus' x 'Big Top' comparado con el estudio de Cantín et al. (2009a). La capacidad antioxidante mostró gran variabilidad entre genotipos, lo que puede explicarse por las variaciones dentro de genotipos de la misma especie (Gil et al., 2002). Diversos autores indican que la RAC en melocotonero vario en relación a los factores ambientales y de cultivo, así como debido al efecto del patrón, la variedad, o el estado de madurez de la futa en la fecha de cosecha (Giorgi et al., 2005; Tavarini et al., 2011).

Los niveles de compuestos fenólicos en las poblaciones estudiadas, mostraron una menor dependencia del efecto del año, aunque hubo variaciones anuales en los contenidos de flavonoides y antocianinas, probablemente debido a la diferencia de las condiciones

climáticas, especialmente de luz y temperatura (Kataota et al., 1984; Tomás-Barberán y Espín, 2001). Hegedús et al. (2010) encontraron diferencias de 21 a 35 veces en fenoles totales y actividad antioxidante en genotipos de albaricoque cultivados en las mismas condiciones. Por otra parte, los factores ambientales a los que el genotipo está expuesto, pueden explicar las diferencias observadas en compuestos antioxidantes entre años y por lo tanto sólo la evaluación de varios años de cosecha puede llevar a decisiones exactas en la selección de nuevas combinaciones patrón-variedad (Giorgi et al., 2005; Tulipani et al., 2011). Las correlaciones significativas positivas encontradas de RAC vs. fenoles totales, flavonoides y vitamina C, muestran la implicación de estos caracteres bioquímicos de calidad en la capacidad antioxidante de la fruta, resultados ya observados en el estudio de otros genotipos por Cantín et al. (2009a).

El contenido en azúcares del fruto es un carácter importante de calidad en los programas de selección y está muy relacionado con el aroma y sabor de melocotones y nectarinas (Colaric et al., 2005). Las dos poblaciones de estudio presentaron una considerable variabilidad fenotípica en el contenido de los azúcares entre genotipos. La sacarosa fue el azúcar mayoritario, seguido de la fructosa, la glucosa y el sorbitol. Genotipos con alto contenido de sorbitol son interesantes en un programa de selección (Ledbetter et al., 2006), ya que algunos autores han señalado su implicación en el aroma y el sabor del melocotón (Colaric et al., 2005). Por otra parte, se encontró una variación en la relación glucosa/fructosa que podría ser de particular interés, ya que las concentraciones relativas de estos azúcares influyen sobre el dulzor, ya que la fructosa es 2,3 veces y 1,7 veces más endulzantes que la glucosa y sacarosa respectivamente (Kulp et al., 1991).

7.3. Los compuestos volátiles como responsables del aroma en melocotonero

Los parámetros de calidad y los compuestos aromáticos volátiles de melocotón y nectarina juegan un papel importante en la satisfacción del consumidor, influenciando en gran medida el consumo de fruta. El gusto, el aroma, la textura y el aspecto o apariencia, se consideran como los atributos de calidad más importantes en melocotón y nectarina. La mayor parte de los compuestos volátiles identificados y cuantificados en el extracto de frutas de nectarinas han sido ya referidos en otros trabajos (Zhang et al., 2010) y están dentro de los compuestos identificados en melocotonero (Sánchez et al., 2012). En la población 'Venus' x 'Big Top' se observó gran variabilidad de compuestos volátiles entre genotipos y también entre los parentales 'Venus' y 'Big Top'. El análisis de componentes principales confirmó el alto contenido en compuestos volátiles especialmente en lactonas,

cetonas y terpenos en el parental 'Venus' y otros individuos de la progenie (5, 10, 27, 35, 36, 41, 44, 46, 47, 48, 50, 57 y 61) y en aldehídos (nonanal, heptadienal, octanal, benzaldehide) en el parental 'Big Top'. El análisis permitió identificar y seleccionardos genotipos que han sido descritos previamente por su mayor contenido en compuestos antioxidantes (35 y 44) (Abidi et al., 2011). La potente técnica de microextracción en fase sólida y cromatografía de gas acoplada a espectrometría de masas (HS-SPME-GC-MS) utilizada en la identificación de COVs en esta tesis ha permitido estudiar el perfil aromático de los parentales 'Venus' y 'Big Top' y comparar este perfil con todos los individuos de la progenie. Todo ello permitirá seleccionar los individuos con perfiles aromáticos diferenciales en función de la demanda del consumidor.

7.4. Susceptibilidad a los daños por frío y efecto del contenido en compuestos bioactivos

El análisis de la calidad postcosecha en la población de estudio 'Babygold 9' x 'VAC-9510', mostró que todos los síntomas de daños por frío evaluados (pardeamiento, harinosidad, enrojecimiento de la pulpa, maduración anormal y problemas de sabor) presentaron una variación continua, confirmando el control genético cuantitativo de estos caracteres (Peace et al., 2006; Ogundiwin et al., 2008; Cantín et al., 2010b). Los síntomas mayoritarios que afectaron a la calidad postcosecha en esta población fueron el pardeamiento, el enrojecimiento y la harinosidad, al igual que se refiere en otros trabajos en melocotonero (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 dos a cuatro semanas de almacenamiento a 5ºC de temperatura. La importancia de la duración del periodo de almacenamiento en frío en el desarrollo y la severidad de este desorden postcosecha ha sido previamente descrita por otros autores (Ben-Arie y Lavee, 1971; Crisosto y Labavitch, 2002; Lill et al., 1989; Lurie y Crisosto, 2005).

Por otra parte, se observó una correlación positiva del pardeamiento con el contenido de fenoles totales, flavonoides y con la capacidad antioxidante. También la harinosidad mostró correlaciones significativas positivas con el índice de madurez (RI), los SSC, y el contenido de fenoles totales, flavonoides y RAC, mostrando la posible influencia de los compuestos fenólicos sobre el desarrollo de los daños por frío en fruta almacenada a bajas temperaturas. El índice de DF mostró correlaciones significativas y positivas con flavonoides y RI mostrando la influencia del estado de madurez del fruto sobre la

severidad de los daños por frío. Valero et al. (1997) observaron que la severidad de los daños por frío era mayor en frutos cosechados en un estado de madurez avanzado. Estas correlaciones son de interés en los programas de mejora que buscan desarrollar frutos con mayor contenido en sustancias bioactivas y menor susceptibilidad a los daños por frío. Manach et al. (2004) indicaron que el almacenamiento de la fruta puede también afectar al contenido en polifenoles, ya que son fácilmente oxidables y por lo tanto disminuir la calidad de la fruta, particularmente al afectar el color y calidad organoléptica, debido al pardeamiento de la pulpa e influyendo así en la aceptabilidad de la fruta.

7.5. Control genético de caracteres bioquímicos de calidad de fruta

La construcción de un mapa parcial del grupo de ligamiento 4 (GL4) del parental 'Venus' con marcadores tipo SNPs y SSRs en la población 'Venus' x 'Big Top', permitió el posicionamiento de 26 marcadores, cubriendo 85,7 cM y separados entre ellos por un promedio de 3,3 cM. La estrategia de mapeo *Pseudo-testcross* utilizando una población F1 permite la construcción de mapas de ligamiento con cada uno de los parentales estudiados 'Venus' y 'Big Top'. Este mapa es el primero que se obtiene con el chip *RosBREED_Peach_10k_11494376_A* de Illumina (Verde et al., 2012).

El análisis de QTLs permitió la localización de distintos caracteres relacionados con la calidad organoléptica del fruto. En particular, se localizaron regiones significativas de control para los SSC, la vitamina C, los fenoles totales, los flavonoides, y la capacidad antioxidante del fruto. Los QTLs para los SSC, fenoles totales y los flavonoides fueron estables para al menos dos años de cosecha. El QTL para la RAC fue estable durante tres años de estudio. Sin embargo, se identificó un QTL significativo correspondiente a la vitamina C pero sólo durante un año del estudio. Los QTLs observados en la poblacion estudiada explicaron 30.4%, 19.5%, 26.5% y 21.2% de la variabilidad fenotipica de los SSC, fenoles totales, RAC y flavonoides respectivamente. Cantín et al. (2010a) realizaron el mapeo del GL4 en la misma población 'Venus' x 'Big Top' con marcadores de tipo SSR y detectaron QTLs para varios caracteres agronómicos de calidad del fruto además de QTLs para varios síntomas de daños por frío que explicaron entre el 26 y el 92% de la variabilidad fenotípica.

El contenido en sólidos solubles es el carácter básico de calidad agronómica desde el punto de vista comercial. Nuestros resultados confirman los resultados encontrados por Cantín et al. (2010a) que identificaron un QTL para los SSC en el GL4 explicando el 82,5% de la variación observada en esta misma población. Otros autores (Dirlewanger et

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al., 1999; Etienne et al., 2002b; Quilot et al., 2004b) también mapearon QTLs para los SSC, la sacarosa y fructosa en el GL4. También Dirlewanger et al. (2006) identificaron QTLs para SSC en el GL6 en dos poblaciones de estudio BC2 y ('Ferjalou Jalousia'® x 'Fantasia') (JxF).

En cuando a los compuestos antioxidantes, Dirlewanger et al. (2006) analizaron el control genético de los polifenoles durante un año de estudio (2002) en la población (JxF) identificaron QTLs implicados en el control de las concentraciones de compuestos fenólicos en los GLs 1, 2, 4 y 6. También se detectaron otros QTLs que controlaban los fenoles totales y las pro-antocianidinas en el GL6 en una población BC1 de melocotón (Dirlewanger et al., 2006). Resultados de nuestro laboratorio y no publicados en la población en estudio indican que la región responsable del contenido en antioxidantes se localiza únicamente en el GL4. Otros caracteres agronómicos y de calidad organoléptica se localizan en otros grupos de ligamiento.

El presente análisis de QTLs para los compuestos antioxidantes en fruto de la población 'Venus' × 'Big Top' añade una información importante al control genético de caracteres de calidad de melocotonero. Por otro lado, este trabajo confirma la dificultad del estudio del control genético de estos compuestos en frutales debido a la alta variabilidad de los mismos. Desde nuestro conocimiento, este estudio es el primer trabajo de análisis de QTLs en nectarina realizado durante cuatro años consecutivos.

CAPÍTULO 8

Conclusiones / Conclusions

Conclusiones

1. Se encontró una amplia variabilidad fenotípica entre genotipos en las dos poblaciones de mejora estudiadas para todos los parámetros evaluados. El estudio permitió la preselección de ocho genotipos en la población 'Venus' x 'Big Top' y seis en la población 'Babygold 9' x 'VAC-9510', con respecto al comportamiento agronómico, la calidad organoléptica y calidad nutricional del fruto.

2. La población de nectarina 'Venus' x 'Big Top' presentó, respecto a la población de melocotón 'Babygold 9' x 'VAC-9510', genotipos superiores en cuanto a los parámetros agronómicos y bioquímicos relacionados con la calidad del fruto. Los genotipos con fruto tipo nectarina mostraron en general mayor concentración de flavonoides, fenoles totales, azúcares totales, sólidos solubles y mayor firmeza que los frutos del tipo melocotón.

3. Los flavonoides y los compuestos fenólicos totales fueron los principales compuestos antioxidantes encontrados en nectarinas y melocotones. La gran variabilidad fenotípica observada, y las altas correlaciones entre fenoles totales, flavonoides y capacidad antioxidante muestran la importancia de los compuestos fenólicos como fuente principal de antioxidantes en el fruto. De la misma manera, las correlaciones positivas observadas entre los azúcares totales y los compuestos bioactivos, apuntan la posibilidad de seleccionar individuos con mayor calidad organoléptica y nutricional.

4. El estudio de compuestos volátiles en la población de nectarina 'Venus' x 'Big Top' por HS-SPME-GC-MS permitió la identificación de 77 compuestos ditintos. El análisis de componentes principales confirmó la mayor influencia en el aroma de algunos compuestos volátiles (lactosas) para algunos genotipos que también se caracterizaron por su alto contenido en antioxidantes. La variabilidad fenotípica observada en la progenie es de interés en cuanto que expande el perfil aromático de los parentales. Esta metodología nos va a permitir identificar genotipos con nuevas combinaciones aromáticas y con un buen perfil antioxidante.

5. La evaluación de la calidad postcosecha respecto a la incidencia de daños por frio en la población 'Babygold 9' x 'VAC-9510' mostró una alta variabilidad fenotípica entre genotipos y una gran influencia del genotipo y del periodo de almacenamiento en la susceptibilidad y la severidad de dichos daños. Este estudio permitió preseleccionar 16 individuos con menor susceptibilidad a los daños por frío.

6. El estudio del control genético de los caracteres de calidad del fruto en la población 'Venus' x 'Big Top' permitió la identificación en el grupo de ligamiento 4 de varios QTLs significativos para el contenido en sólidos solubles, vitamina C, fenoles totales, flavonoides y capacidad antioxidante del fruto. De la misma manera, los QTLs se han encontrado significativos en la mayor parte de los parámetros al menos dos años de los cuatro que ha durado el estudio. Por lo que de deduce que no existe interacción genotipo x ambiente para el control genético de dichos genes.

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Conclusions

1. The two peach progenies evaluated showed a great phenotypic variability for all the studied traits. The study permit the pre-selection of eight genotypes in the population 'Venus' x 'Big Top' and six in the population 'Babygold 9' x 'VAC-9510', for their high agronomical, organoleptical, and nutritional properties.

2. The nectarine population 'Venus' x 'Big Top' showed superiority for all agronomical and biochemical fruit quality traits in comparison with the 'Babygold 9' x 'VAC-9510' population. The nectarine genotypes showed high concentrations of flavonoides, total phenolics, total sugars, soluble solids and firmness.

3. The flavonoids and the phenolic compounds were the major antioxidant compounds found in necatarine and peach fruits. The high variability observed among genotypes and the high correlations observed between total phenolics, flavonoides and antioxidant capacity showed the importance of these two compounds in the relative antioxidant capacity of peach fruits. The significant positive correlations observed between total sugars and bioactive compounds permit the preselection of genotypes with high organoleptic and nutritional quality.

4. The characterization and quantification of the fruit volatile compounds in the 'Venus' x 'Big Top' population with HS-SPME-GC-MS permit the identification of 77 volatiles compounds. The PCA confirmed the high content in volatile compounds especially in lactones for some genotypes which showed also high content n antioxidant compounds preselection of three genotypes which are preselected also for their high content in phenolic compounds. The technique used allowed to select three genotypes with high content in antioxidant and volatiles compounds.

5. The evolution of the postharvest fruit quality in the 'Babygold 9' x 'VAC-9510' population showed high phenotypic variability between genotypes and the higher influence of genotype and the storage duration in the susceptibility and the severity for the chilling injury. The study permit to preselect 16 genotypes with minor susceptibility to chilling injury among the 130 individuals analyzed.

6. The study of the genetic control of the antioxidant fruit quality traits in the population 'Venus' x 'Big Top' allowed the identification, on linkage group 4 (LG 4), several significant QTLs for soluble solids content, vitamin C, total phenolics and flavonoids

content, and antioxidant capacity of fruit. Similarly, the QTLs were significant across two years or three years among the four years of study. These results demonstrated that there is no significant genotype x environment interaction for the genetic control of the genes controlling antioxidant compounds.

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CAPÍTULO 10

Anexos

Article

Evaluation of Antioxidant Compounds and Total Sugar Content in a Nectarine [*Prunus persica* (L.) Batsch] Progeny

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Abstract: Epidemiological studies suggest that consumption of fruit rich in phenolic compounds is associated with health-protective effects due to their antioxidant properties. For these reasons quality evaluation has become an important issue in fruit industry and in breeding programs. Phytochemical traits such as total phenolics, flavonoids, anthocyanins, L-ascorbic acid, sugar content and relative antioxidant capacity (RAC) were analyzed over four years in flesh fruit of an F1 population "Venus" × "Big Top" nectarines. Other traits such as harvesting date, yield, fruit weight, firmness, soluble solids concentration (SSC), pH, titratable acidity (TA) and ripening index (RI) were also determined in the progeny. Results showed high variability among genotypes for all analyzed traits. Total phenolics and flavonoids showed significant positive correlations with RAC implying that both are important antioxidant bioactive compounds in peaches. We found genotypes with enhanced antioxidant capacity and a better performance than progenitors, and in consequence the best marketability.

Keywords: antioxidant capacity; flavonoids; total phenolics; vitamin C

1. Introduction

Peach [*Prunus persica* (L.) Batsch] production has an important place in the world (18.6 million tons in 2009) with a cultivated area of around 1.6 million ha [1]. Peaches and nectarines are, after apples, the second most important fruit crop in the European Union (EU), with a production of 4.1 million tons in 2009 and a cultivated area of around 245,191 ha [1]. Spain is the third largest producer in the world, after China and Italy, and the second in Europe, with a production of 1.2 million tons in 2009 and a cultivated area of 72,000 ha [1].

Peaches are a popular summer fruit and there has been an increasing interest in their nutritional value [2]. Many epidemiological studies suggest that increased fruit consumption decreases the risk of several degenerative diseases including atherosclerosis, heart and brain disorders, and different types of cancer [3] which are still responsible for the highest mortality rate in Western countries [4]. In particular, the consumption of peaches can suppress reactive oxygen species (ROS) in human plasma and provide protection from chronic diseases [5]. Fruits have recently been accepted as a functional food, because of its low caloric content along with its high level of antioxidant and nutritional compounds, such as vitamins, phenols, minerals or fiber that could play an important role in preventing oxidative stress [6]. The phenolic compounds (anthocyanins, flavonoids, *etc.*) give fruits both desirable qualities like color and antioxidant properties and undesirable qualities like astringency and bitterness [7]. Slimestad *et al.* [8] reported that there is a correlation between taste (astringency, bitterness) and content of phenolic compounds which have an important role in the natural defense mechanisms and health benefits of fruits. Moreover, Koh *et al.* [9] reported that flavonoids are particularly interesting as they are potent *in vitro* antioxidants and are thought to play key roles in many of the processes underlying vascular dysfunction.

In recent years, there has been an increased interest in breeding programs identifying and quantifying phenolic substances in fruit in order to evaluate their potential health-promoting properties, as well as their visual appearance (pigmentation and browning) and taste (astringency) [6,10]. Peaches could be of great interest as an important antioxidant source and intake of these fruits may provide health-promoting advantages [11].

Apart from the antioxidant evaluation, peach breeding programs have stressed the importance of taste in the selection of new cultivars [12]. Orazem *et al.* [6] reported that the edible quality of peaches depends to a great extent on their sweetness, and that the amounts of sucrose, sorbitol and malic acid correlate positively with the taste and aroma of peach fruit. Sweetness and acidity are the most important factors affecting consumer acceptability of stone fruits and these factors are strictly correlated [11]. Sucrose, glucose and fructose are the main sugars in peaches [13,14], and in ripe fruit, these sugars comprise about 60% of the soluble solids concentration (SSC). The relative concentrations of these sugars also influence sweetness, as fructose is 2.3 times and 1.7 times sweeter than glucose and sucrose, respectively [15].

The main objective of this work was to evaluate, in a nectarine segregating F1 population derived from "Venus" × "Big Top" over a 4-year study, the existing phenotypic diversity of antioxidant compounds and total sugar content among genotypes, and to study the relationships among agronomic and biochemical fruit quality traits. The correlations between biochemical and agronomic traits will be very useful in peach breeding programs. The ultimate objective of this study was to select superior

genotypes with enhanced antioxidant capacity in fruits that will benefit consumers with health-promoting properties.

2. Results and Discussion

2.1. Agronomical and Basic Fruit Quality Traits

Traits were evaluated in both parents and each seedling separately over four years (2007–2010) of study (Table 1). Mean values of fruit weight, firmness, soluble solids content (SSC), pH, titratable acidity (TA) and the ripening index ratio (RI = SSC/TA) were calculated for parents ("Venus" and "Big Top") as well as for the 75 individual seedling progeny. Results showed high variability among genotypes for the different agronomic and fruit quality traits evaluated. Means for all analyzed traits were inside the interval values obtained for the parents and exhibited continuous variation, which is typical of quantitative or polygenic inheritance. The fruit weight varied greatly among genotypes (190.2 ± 3.8 g) as a consequence of the variability in tree production and fruits number for each seedling. Fruit weight is a major quantitative inherited factor determining yield, fruit quality and consumer acceptability [16]. The mean value for yield in the progeny for 2007–2010 was 6.9 ± 0.3 kg per tree with high variability among genotypes, which may occur due to year and genotype (data not shown). These values were similar to those obtained by Cantín *et al.* [17] in the same population during three years. Milatović *et al.* [18] reported that yield of the peach tree depends on different factors, such as density of flower buds and flowers, fruit set, fruit size, winter and late spring freeze damage, precipitation amount, and orchard management. The variation for agronomic and basic biochemical fruit quality traits among years of study showed lower yield for years 2008 and 2010 (5.9 and 5.4 kg, respectively) compared with the mean value of four years (6.9 ± 0.3 kg) and consequently higher fruit weight for these two years (209.0 and 214.2 g, respectively) than the average weight (190.2 ± 3.8 g). This variability in annual yield was mainly due to rains damage which occurred in the full blooming of the population limiting the number of available fruits. The pH, fruit firmness and SSC showed low variability among years (data not shown).

Table 1. Agronomical and basic fruit quality traits in the "Venus" × "Big Top" population. For progenitors data are means ± SE of two years (2009–2010). For the progeny (*n* = 75 genotypes), data are means ± SE of four years of study (2007–2010).

	Progenitors		Progeny [a]		
Traits	**"Venus"**	**"Big Top"**	**Min**	**Max**	**Mean ± SE**
Fruit weight	178.0 ± 58.3	204.0 ± 39.3	109.2	261.8	190.2 ± 3.8
Firmness	36.1 ± 0.1	49.2 ± 6.9	24.2	50.7	39.2 ± 0.6
SSC	13.9 ± 0.1	14.4 ± 0.1	11.2	17.5	13.9 ± 0.2
pH	3.4 ± 0.1	3.9 ± 0.1	3.2	4.0	3.6 ± 0.1
TA	0.7 ± 0.1	0.4 ± 0.1	0.5	1.1	0.7 ± 0.1
RI	20.3 ± 0.3	35.2 ± 0.3	13.8	35.8	23.8 ± 0.7

[a] Data from 2007 were partially presented in Cantín *et al.* 2010 [17]. Units and abbreviations: Fruit weight (g); Firmness (N); N = Newtons; SSC = Soluble solids content (°Brix); TA = Titratable acidity (g malic acid/100 g FW); RI = Ripening index (SSC/TA).

Regarding flesh fruit firmness, it ranged from 24.2 to 50.7 N with higher variability among seedlings. The two progenitors and some genotypes of the progeny showed firmness values higher than 35 N, which has been defined as the threshold between mature and immature fruit [19]. Our analysis revealed a mean firmness of 39.2 N (4.08 kg/cm^2) which is lower than the maximum level of fruit firmness for marketing fresh peaches and nectarines, set by the EU at a 6.5 kg/cm^2 (=63.7 N), using a 8 mm diameter probe (Commission Regulation EC, No.1861/2004 of 28 October 2004).

Regarding SSC, the population showed values from 11.2 to 17.5 °Brix with a mean of 13.9 ± 0.2 °Brix, which is greater than the minimum (8 °Brix) established by the EU to market peaches and nectarines (R-CE No.1861/2004). Kader [20] considered mean values of SSC over 10 °Brix as the minimum value for consumer acceptance for yellow-flesh nectarines, which is the case in our progeny. The variability found in SSC among seedlings can be explained by the quantitative performance of this quality trait [21].

The pH values varied from 3.2 to 4.0 with a mean value of 3.6 ± 0.1, which are values of normal acidity fruits. The progeny showed acid and non-acid fruits, since fruit with a pH higher than 4.0 at maturity are considered as non-acid [22]. The progeny showed variability of TA among genotypes with a mean value of 0.7 ± 0.1 g malic acid per 100 g fresh weight (FW), which is lower than the maximum limit (0.9%) for normal acidity peaches [23]. "Venus" is an acid nectarine (TA = 0.7%), and "Big Top" is a non-acid nectarine (TA = 0.4%), which explains the segregation of this trait in the progeny.

The ripening index (RI = SSC/TA) ranged from 13.8 to 35.8 among genotypes, depending on their SSC and TA values. In peaches, the RI is a major organoleptic quality trait of the mature fruit and is commonly used as a quality index [24]. The relationship between TA and SSC has an important role in consumer acceptance of some apricot, peach, nectarine and plum cultivars. Crisosto *et al.* [25] reported that in the case of cultivars with TA > 0.9% and SSC < 12.0 °Brix, consumer acceptance was controlled by the interaction between TA and SSC rather than SSC alone. Our results showed only four genotypes with the mean value of TA > 0.9% and the mean value of SSC < 12.0 °Brix (3, 14, 58, 65).

2.2. Correlations between Agronomical and Basic Biochemical Traits

Significant correlations were found among pomological traits related to fruit quality. In the progeny, annual yield was positively correlated with fruit weight (*r* = 0.278, *P* ≤ 0.05), also a positive correlation was found for SSC and RI (*r* = 0.263, *P* ≤ 0.05). Firmness was significantly correlated with SSC (*r* = 0.367, *P* ≤ 0.01), pH (*r* = 0.385, *P* ≤ 0.01) and RI (*r* = 0.347, *P* ≤ 0.01). Similar low correlations were found for Cantín *et al.* when studied 1100 peach genotypes [17]. The positive correlation between firmness and SSC is important since the selection of genotypes with high SSC will aim first at higher firmness and second at lower susceptibility to mechanical damage during handling and packaging [26]. The ripening index showed a high positive correlation with pH (*r* = 0.930, *P* ≤ 0.01), and a negative correlation with titratable acidity (*r* = −0.315, *P* ≤ 0.01) indicating that in our progeny when most of the fruits are mature pH seems to increase and acidity to decrease. The pH showed a negative correlation with TA (*r* = −0.343, *P* ≤ 0.01) in this progeny, as other authors reported in different peach genotypes [10,16].

2.3. Antioxidant Compounds Content

The antioxidant compounds content in the "Venus" × "Big Top" progeny, showed a high variability among genotypes (Table 2). The ascorbic acid (AsA) content ranged from 2.1 to 7.2 mg of AsA/100 g of FW, with a mean value of 4.0 ± 0.1 mg of AsA/100 g of FW. The parents, "Venus" and "Big Top", differed for vitamin C content, and as a consequence the progeny showed high segregation among genotypes. Our results indicate that peach is a good source of vitamin C and highlight the fact that ascorbic acid content is an important part of the overall evaluation of peach fruit quality. 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 [27]. Cantín *et al.* [10] reported that total ascorbic acid content in 218 peach genotypes from different progenies varied greatly 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. Preliminary data obtained by these authors in this progeny but only tested during one year of study, showed lower values (2.6 mg of AsA/100 g of FW) when studying a short number of genotypes. Also Gil *et al.* [28] quantifying the total ascorbic acid contents of nectarine cultivars from California reported contents from 6 to 8 mg of AsA/100 g of FW in yellow-flesh nectarines and from 5 to 14 mg/100 g of FW in white-flesh nectarines.

Table 2. Content of antioxidant compounds in the "Venus" × "Big Top" population. For progenitors data are means ± SE of two years (2009–2010). For the progeny (*n* = 42–75 genotypes), data are means ± SE of four years of study (2007–2010).

	Progenitors		Progeny		
Compounds	**"Venus"**	**"Big Top"**	**Min**	**Max**	**Mean ± SE**
Vitamin C	3.0 ± 0.7	4.9 ± 0.7	2.1	7.2	4.0 ± 0.1
Total phenolics	22.1 ± 8.0	26.4 ± 9.8	22.5	49.2	32.6 ± 0.7
Flavonoids	7.6 ± 3.8	7.8 ± 4.6	5.9	33.8	12.5 ± 0.6
Anthocyanins	2.1 ± 0.1	5.9 ± 2.2	1.2	9.5	3.2 ± 0.2
RAC	386.1 ± 18.5	521.4 ± 47.4	292.4	835.8	464.2 ± 12.5

Units: Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of FW); Anthocyanins (mg C3GE/kg of FW); RAC; Relative Antioxidant Capacity (μg Trolox Equivalents/g of FW). Abbreviations: AsA = Ascorbic acid; C3GE = Cyanidin-3-glucoside equivalents; CE = Catechin equivalents; GAE = Gallic acid equivalents.

Total phenolics ranged from 22.5 to 49.2 mg of gallic acid equivalent (GAE) per 100 g of FW. The amount of total phenolics in our progeny fell within the range reported in the literature for peach fruits, namely 14–77 mg GAE/100 g of FW. Tavarini *et al.* [27] reported similar total phenolics amounts (14–50 mg GAE/100 g of FW) in peach cultivars and other similar results were reported by Gil *et al.* [28] in yellow-flesh nectarines (18 to 54 mg GAE/100 g of FW).

Regarding flavonoids, it ranged from 5.9 to 33.8 mg catechin equivalent (CE) per 100 g of FW in our progeny with an average of 12.5 ± 0.6 mg of CE/100 g of FW. These results, revealed flavonoids content similar to that obtained by Cantín *et al.* [10] in peach and nectarine progenies ranging from 1.8 to 30.9 mg of CE/100 g of FW, with an average of 8.8 mg of CE/100 g of FW. It is remarkable that the "Venus" × "BigTop" progeny showed higher total phenolics and flavonoids content when compared with the parents. This fact could be very interesting in the peach genotype selection process,

mainly selecting fruits rich in flavonoids, since the consumption of flavonoid-rich foods holds the potential to limit neurodegeneration preventing age-dependent loses in cognitive performance [29].

The anthocyanins content ranged from 1.2 to 9.5 mg cyanidin-3-glucoside equivalents (C3GE) per kg of FW, showing less variability among genotypes due to the similar flesh color of seedlings and lower concentrations compared to the study of Cantín *et al*. [10] who reported that in some progenies total anthocyanins greatly varied among genotypes (0.1–26.7 mg of C3GE/kg of FW) depending on the red pigmentation of the flesh.

The relative antioxidant capacity (RAC) ranged from 292.4 to 835.8 (µg Trolox Equivalents (TE) per g of FW) showing a high variability among genotypes (mean value was 462.2 ± 12.5 µg TE/g of FW). This could be explained by the fact that the antioxidant capacity of fruits varies in relation to the antioxidant molecules present in the different species but variations can also occur within the genotypes of a single species [28]. Cantín *et al.* [10] reported values of RAC (227.3 to 629.9 µg of TE/g of FW, with an average of 405 µg of TE/g of FW) in the same range of these results even slightly lower. In general, antioxidant compounds content presented here were higher than those found for these authors when less genotypes of the same progeny were analyzed.

To evaluate the influence of the different environmental conditions on the fruit antioxidant compounds content, data related to 2007, 2008, 2009 and 2010 were separately evaluated (Table 3).

Table 3. Annual amounts of antioxidant compounds in the "Venus" × "Big Top" progeny. Data are means ± SE of four years of study (2007–2010) (*n* = 42–75 genotypes).

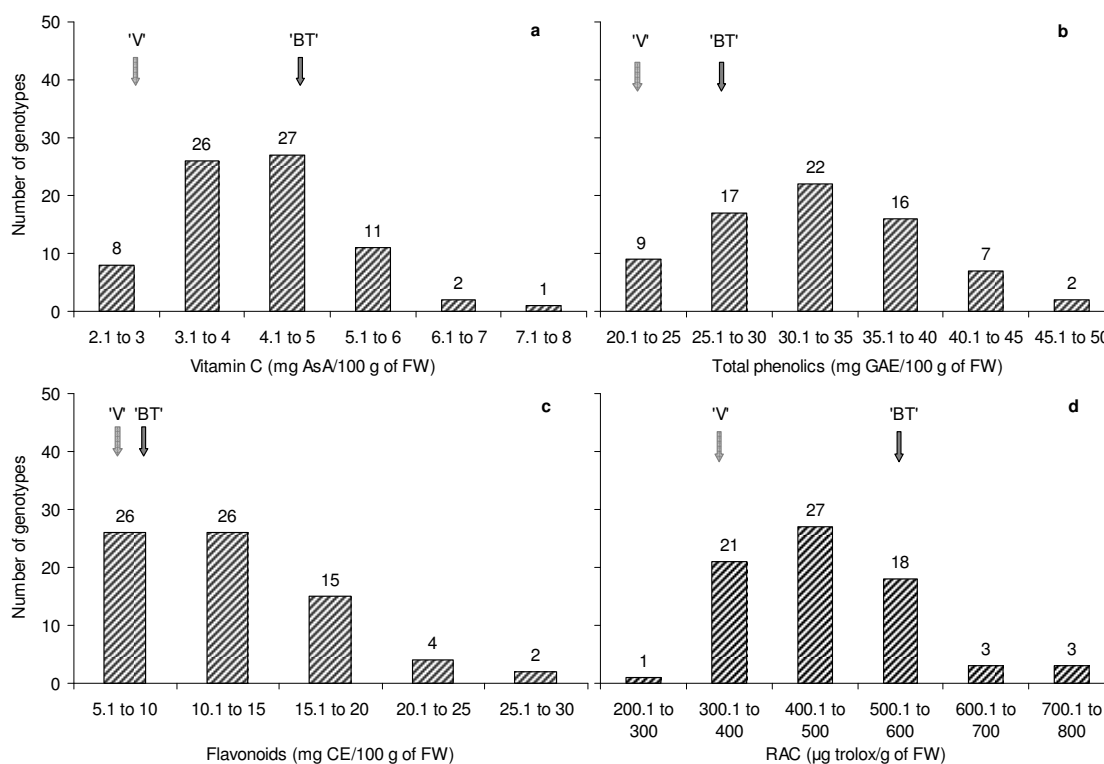| Compounds	2007	2008	2009	2010	Mean ± SE
Vitamin C	2.8 ± 0.1	3.9 ± 0.2	6.3 ± 0.2	3.2 ± 0.2	4.0 ± 0.1
Total phenolics	36.9 ± 1.7	44.2 ± 0.7	21.2 ± 0.6	23.3 ± 0.8	32.6 ± 0.7
Flavonoids	12.9 ± 1.0	21.7 ± 1.2	6.5 ± 0.4	8.1 ± 0.6	12.5 ± 0.6
Anthocyanins	2.2 ± 0.2	1.7 ± 0.1	4.0 ± 0.5	4.6 ± 0.4	3.2 ± 0.2
RAC	380.6 ± 14	617.0 ± 23	322.6 ± 11	444.8 ± 10	464.2 ± 12.5

Units: Vitamin C (mg AsA/100 g of FW); Total phenolics (mg GAE/100 g of FW); Flavonoids (mg CE/100 g of FW); Anthocyanins (mg C3GE/kg of FW); RAC; Relative Antioxidant Capacity (µg Trolox Equivalents/g of FW). Abbreviations: AsA = Ascorbic acid; C3GE = Cyanidin-3-glucoside equivalents; CE = Catechin equivalents; GAE = Gallic acid equivalents.

An interesting year-to-year and genotype-to-genotype variability differences in the antioxidant compounds were outlined. The vitamin C content showed higher values in 2009, whereas total phenolics, flavonoids and RAC showed comparable values among years. The anthocyanins content showed similar mean values in 2009 and 2010 but higher than those observed in the previous two years of the study, this could be due to the period of maturity of fruit and the harvest date which were more similar in those years. In 2008, the total phenolics, flavonoids content and the antioxidant capacity of the flesh fruit were notably higher than in the other years. These changes found in the antioxidant compounds content could be as a result of growing conditions, pre and postharvest conditions and genetic factors affecting the antioxidant compounds content. Harvesting date showed significant negative correlation with flavonoids ($r = -0.331$, $P \leq 0.01$) and sucrose content ($r = -0.310$, $P \leq 0.01$) indicating that harvesting time could present variability among years and consequently influence the antioxidant and total sugar content among genotypes.

The antioxidant content in the analyzed fruits should be attributed in part to the important role of the rootstock on fruit quality as Giorgi *et al.* reported [30]. Moreover, the environmental conditions to which the genotypes are subjected, and the annual climatic changes may partly explain the different accumulation patterns of antioxidant compounds. As a consequence, only the evaluation of several years of harvest may lead to an accurate assessment in the selection of new peach-rootstock combination [30,31].

All the antioxidant traits studied, except for flavonoids and anthocyanins (data not shown), showed a normal distribution (Figure 1) which is typical of quantitative characters.

Figure 1. Segregation of (**a**) vitamin C, (**b**) total phenolics, (**c**) flavonoids, and (**d**) antioxidant capacity (RAC), in the "Venus" × "Big Top" progeny. Data are means (*n* = 42–75 genotypes) of four years of study (2007–2010). Arrows show the values for the parents "Venus" ('V') and "Big Top" ('BT').

Segregation was also observed when comparing the progeny with its parents, and some genotypes showed even higher values than "Venus" and "Big Top". For vitamin C, at least ten genotypes (12, 27, 37, 38, 39, 40, 42, 43, 52 and 74) showed higher vitamin C contents. For total phenolics most of the progeny (5, 10, 18, 20, 23, 32, 35, 37, 40, 43, 44, 47, 61 and 74) showed higher contents compared with "Venus" and "Big Top". The same thing occurred in the flavonoids content and these genotypes (10, 23, 28, 35, 37, 40, 43, 44, 47, 61 and 74) showing higher contents compared with the progenitors. For the relative antioxidant activity, seventeen genotypes (9, 10, 18, 20, 21, 23, 24, 27, 32, 35, 36, 37, 43, 44, 47, 61 and 74) showed values higher than the progenitors. In general, at least nine genotypes (18, 27, 32, 35, 37, 43, 44, 47 and 74) can be highlighted due to their higher contents of antioxidant compounds.

2.4. Total Sugar Content

The sucrose, glucose, fructose and sorbitol contents in the "Venus" × "Big Top" progeny were analyzed separately (Table 4), as they play an important role in peach flavor quality [32].

Table 4. Sugar content (g per kg FW) in the "Venus" × "Big Top" population. For progenitors data are means ± SE of two years (2009–2010). For the progeny (*n* = 42–75 genotypes), data are means ± SE of four years of study (2007–2010).

| Sugar content	Progenitors		Progeny		
	"Venus"	"Big Top"	Min	Max	Mean ± SE
Sucrose	41.0 ± 5.7	85.1 ± 17.0	40.7	102.3	58.4 ± 1.2
Glucose	10.0 ± 0.4	8.9 ± 0.8	8.3	23.4	12.2 ± 0.3
Fructose	13.4 ± 0.5	10.9 ± 1.7	8.9	19.1	12.4 ± 0.2
Sorbitol	8.6 ± 3.8	6.5 ± 1.7	1.7	19.5	6.6 ± 0.5
Sucrose/glucose	4.0 ± 0.4	9.5 ± 2.1	3.2	7.6	4.9 ± 0.1
Glucose/fructose	0.7 ± 0.1	0.8 ± 0.1	0.8	1.2	0.9 ± 0.1
% Sorbitol	5.6 ± 1.9	2.9 ± 0.4	1.1	8.7	3.5 ± 0.2
Total sugars	73.0 ± 9.6	111.5 ± 14.1	67.4	138.9	89.7 ± 1.6

The studied population exhibited considerable phenotypic variation in sugar contents among genotypes. Mean values for all sugars were inside the interval values obtained for the parents, except for glucose that were higher, and these contents exhibited continuous variation, which is typical of quantitative or polygenic inheritance. Sucrose was the major sugar present in the evaluated genotypes, with 58.4 ± 1.2 g per kg FW, followed by fructose, glucose and sorbitol. The sorbitol content varied greatly among genotypes, ranging from 1.7 to 19.5 g per kg FW. Consequently, the percentage of sorbitol in the sugar composition was significantly different among genotypes, ranging from 1.1 to 8.7%. Colaric *et al.* [33] reported that sorbitol was the attribute most related to peach aroma and taste among carbohydrates and organic acids.

Wu *et al.* [34] reported that sucrose in peaches is dominant at maturity, followed by the reducing sugars (glucose and fructose) and then sorbitol. In our progeny the mean levels of glucose and fructose were quite similar (mean glucose/fructose ratio = 0.98) and about five times lower than the mean value for sucrose (mean sucrose/glucose ratio = 4.9). Some researchers reported glucose and fructose in comparable amounts [34]. However, a slight variation in glucose/fructose ratio (from 0.8 to 1.2) was detected in the studied seedlings. Identifying genotypes with low glucose/fructose ratio might be of particular interest, since the relative concentrations of these sugars influence sweetness, as fructose is 2.3 times and 1.7 times sweeter than glucose and sucrose, respectively [15]. In agreement, Robertson and Meredith [32] found that "high quality" peaches contained lower glucose/fructose ratios than "low-quality" peaches.

Total sugar content (the sum of sucrose, glucose, fructose and sorbitol) in peeled fruits ranged from 67.4 to 138.9 g per kg FW with an average of 89.7 ± 1.6 g per kg FW. Total sugar content is an important quality trait in fruit breeding programs, since it has been reported to be highly related to the aroma and taste of peaches and nectarines [33]. The "Venus" cultivar showed lower content than that observed by Colaric *et al.* [33]. Quilot *et al.* [21] reported that for total sugar content, variation among trees, among fruits of the same tree, and among years are not negligible in comparison with the variation among genotypes. Cantín *et al.* [14] studying 205 genotypes from different progenies reported that the average content of total sugars in the peeled fruit was 72.1 g per kg FW in peaches and 77.1 g per kg FW in nectarines.

The annual variation for sugars compounds and total sugar content (Figure 2) showed small variation among years except for 2008. This year high values of sucrose, glucose and fructose were obtained leading to high total sugar content in agreement with the high SSC found (15.5 °Brix).

Figure 2. Annual amounts of sugar compounds (g per kg of FW) in the "Venus" × "Big Top" progeny. Data are means ± SE of four years of study (2007–2010) (*n* = 42–75 genotypes).
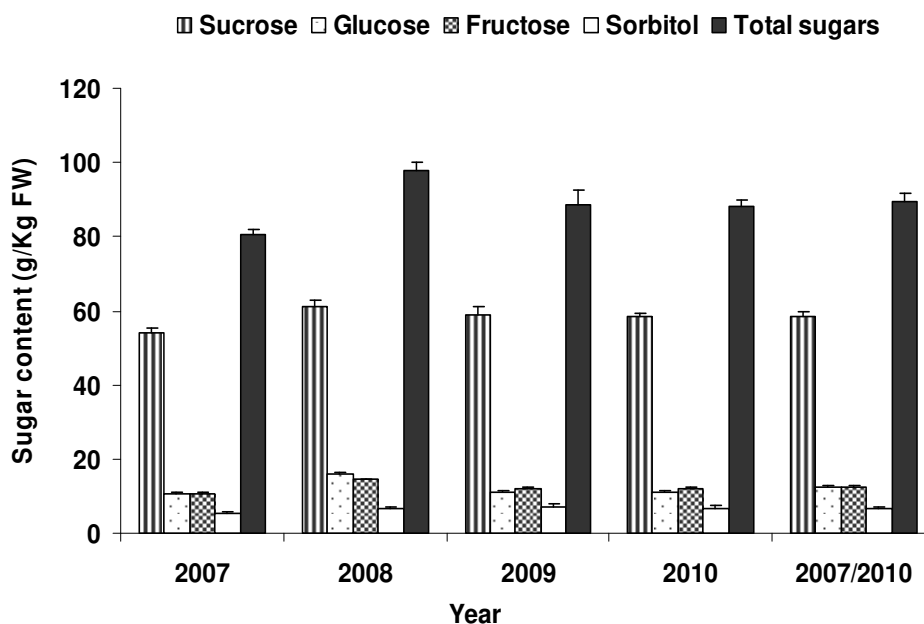
2.5. Correlations between Phytochemicals Traits

We found significant positive correlations ($P \leq 0.01$) of relative antioxidant capacity *versus* total phenolics (Table 5), flavonoids, and vitamin C ($r = 0.738$, $r = 0.851$ and $r = 0.455$, respectively), implying that they are important bioactive compounds for the antioxidant activity of peaches, in accordance with Cantín *et al.* [10]. The DPPH assay for the RAC determination explains the correlation found with total phenolics. The high positive correlation found between total phenolics and flavonoids content ($r = 0.807$, $P \leq 0.01$) (Table 5), indicates that flavonoids are an important group of phenolic compounds in peaches and nectarines with high antioxidant activity. Moreover, total sugars showed positive significant correlations with total phenolics ($r = 0.398$), vitamin C ($r = 0.350$), and RAC

(r = 0.384) at $P \leq 0.05$. Pirie and Mullins [35] reported a good correlation in grapes between sugar content in berries and levels of phenolic substances, due to the role of sugars in the regulation of phenolic biosynthesis. Linear regression between RAC and total phenolics and flavonoids were also high (r = 0.738 and r = 0.851, respectively at $P \leq 0.01$, data not shown). Similarly, Gil *et al.* [28] reported a strong correlation (r = 0.93–0.96) between total phenolics and antioxidant activities in fresh nectarine and peach fruits. It is well established that a strong and positive relationship exists between total phenolics and total anthocyanins content and RAC, suggesting that breeders can select for higher phenolics.

Table 5. Correlation coefficients between some phytochemical traits in the "Venus" × "Big Top" progeny.

Traits	Flavonoids	Total phenolics	RAC	SSC	Total Sugars
Vitamin C	0.420 **	0.374 **	0.455 **	0.579 **	0.350 **
Flavonoids		0.807 **	0.851 **	0.482 **	ns
Total phenolics			0.738 **	0.524 **	0.398 **
RAC				0.597 **	0.384 **

** $P \leq 0.01$; ns, not significant. Abbreviations: RAC; Relative Antioxidant Capacity.

2.6. Principal Component Analysis for Agronomical and Biochemical Traits

A Principal Component Analysis (PCA) was performed on agronomical and biochemical data in the "Venus" × "Big Top" progeny (Figure 3). A four component model accounted for more than 70% of total variance, with the first two components, PC1 and PC2, explaining 21.9% and 19.4% of total variance, respectively. Progeny displayed a great variability (Figure 3a). PC1 discriminated between parental acid "Venus" and non-acid "Big Top". An examination of PC1 loadings (Figure 3b) suggested that this separation was mainly due to basic biochemical traits (TA, pH and RI). Genotypes on the positive side of PC1 were in general less acid, showed higher firmness and accumulated more sugars and less anthocyanins than individuals on the negative side. An examination of PC2 loadings (Figure 3b) suggested that separation on this component was mainly due to antioxidant traits (flavonoids, total phenolics and RAC) and in a less extent to sugar compound accumulation (glucose, fructose and sorbitol). Analysis confirmed the higher contents in total phenolics, flavonoids and RAC for some genotypes (individuals on the positive side of PC2, especially 18, 27, 32, 35, 37, 43, 44 and 47) than progenitors. The PCA shows a close relationship between flavonoids, total phenolics and RAC as well as between RI, pH and firmness. The results obtained in this progeny were coherent and reflected the known correlations between bioactive and agronomical traits as described in others studies [14,17].

Figure 3. Principal component analysis of agronomical and biochemical traits in the "Venus" × "Big Top" progeny. Analysis was performed using mean data of four years of study (2007–2010). PC1/PC2 scores plot (**a**) explaining 41.3% of the total variance. Symbols: (■) parents "Venus" ('V') and "Big Top" ('BT'), (●) progeny. PC1/PC2 loadings plot (**b**) generated from PCA analysis. Symbols: (■) agronomical and basic biochemical traits, (●) antioxidants, (▲) sugars.
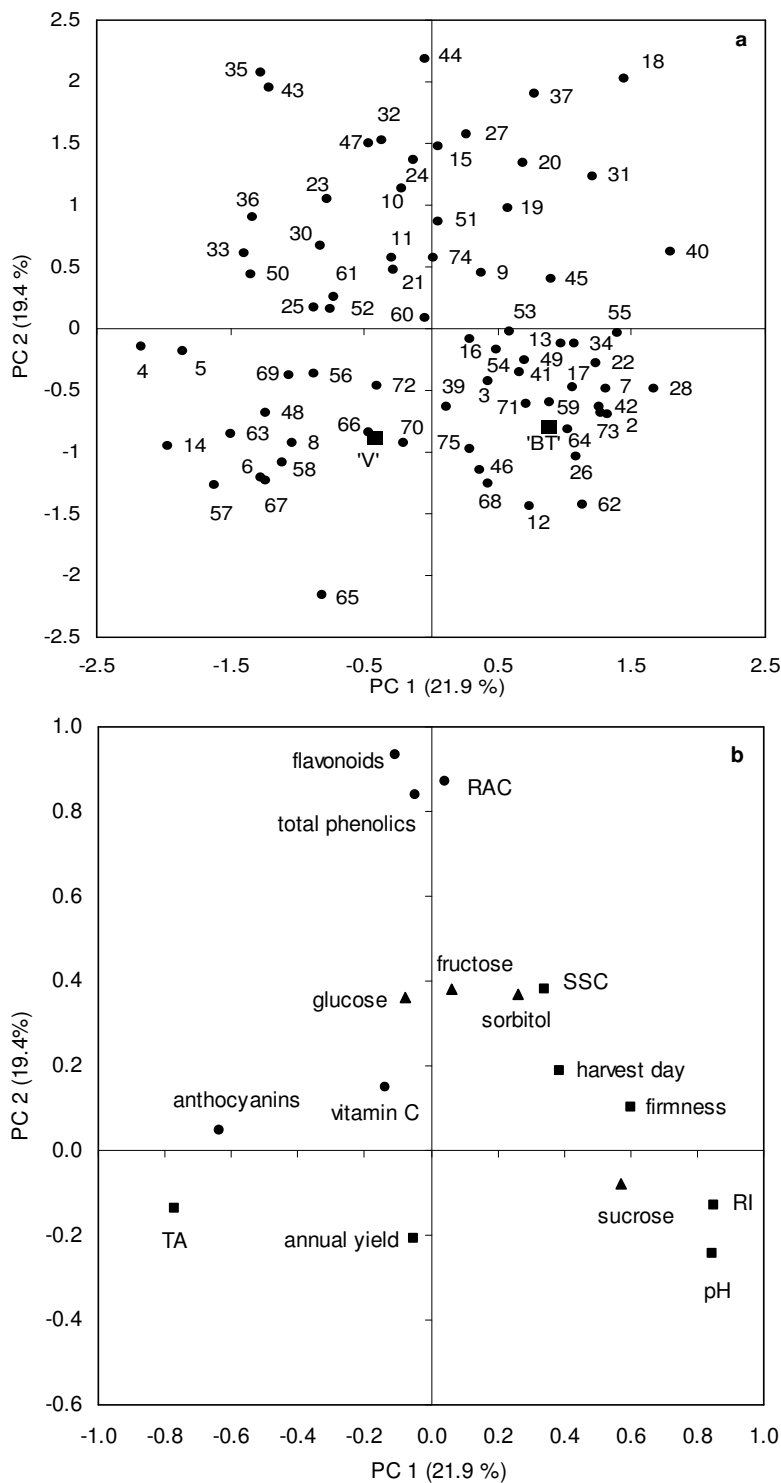
3. Experimental Section

3.1. Plant Material

The progeny assayed was a segregant F1 population of 75 seedlings obtained from a controlled cross, between *Prunus persica* cvs. "Venus" (female parent) and "Big Top" (male parent). "Venus" is a freestone, melting and yellow flesh nectarine cultivar, whereas "Big Top" is a clingstone, melting and yellow flesh nectarine cultivar. The segregant population is entirely melting flesh, either cling- or freestone. 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 × 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. Samples were harvested over four years (2007–2010).

3.2. Agronomical and Basic Fruit Quality Parameters

During the years 2007–2010, agronomic and fruit quality traits were measured individually in each seedling tree. Harvesting date and annual yield were evaluated in each independent seedling. Harvesting date ranged from first-July to mid-August, depending on the genotypes. Fruits were handpicked at commercial maturity and assessed by peel fruit color and flesh firmness. Fruits were considered ripe in the tree when their growth had stopped, exhibited orange-red ground color, began softening, and were easily detached. Yield (kg/tree) was measured and a representative fruit sample (20 fruits) was taken for fruit quality evaluations [10]. Fruit weight was 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 and data were expressed in Newtons. The 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 initial pH and titratable acidity (TA) were measured by automatic titration system with NaOH 0.1 N to pH 8.1 (862 Compact Titrosampler); data are given as g malic acid per 100 g FW, since this is the dominant organic acid in peach. The ripening index (RI) was calculated as the ratio between SSC and TA.

3.3. Phytochemical Extraction

For all analyses only fruit flesh was used, as it is usually consumed. Twenty representative fruits were peeled with a sharp knife, flesh was weighted, immediately frozen separately in liquid nitrogen, and stored at −20 °C until analysis. For vitamin C determination, samples at harvest were kept in 5 mL of 5% metaphosphoric acid for preservation of ascorbic acid, frozen in liquid nitrogen and stored at −20 °C until analysis. Samples were homogenized with a polytron in 5 mL 5% metaphosphoric acid and centrifuged at 20,000 g for 20 min at 4 °C, filtered with Miracloth and the supernatant was used for vitamin C analysis. For phenolic compounds, frozen fruit material (5 g) was homogenized in a polytron with 10 mL of extraction solution, consisting of 0.5 N HCl in methanol/Milli-Q water (80% v/v). The mixture was then centrifuged for 20 min at 4 °C and 20,000 g. Supernatant was

recovered and the volume measured. This hydroalcoholic extract was used for total phenolics, flavonoids, anthocyanins and antioxidant capacity assays. For the determination of sugars, the frozen fruit material (5 g) was homogenized in a Polytron with 10 mL of extraction solution consisting of ethanol/Milli-Q water (80% v/v). The mixture was centrifuged at 20,000 g for 20 min at 4 °C. The supernatant was recovered and processed to be assayed by high-performance liquid chromatography (HPLC) as described by Cantín *et al.* [14] with some modifications.

3.4. Antioxidant Compounds Analysis

Vitamin C, total phenolics, flavonoids, anthocyanins and relative antioxidant capacity were evaluated with colorimetric methods and measured using a spectrophotometer (Beckman Coulter DU 800) as described by Cantín *et al.* [10] and methods therein. In order to avoid interferences, other analysis could be recomended for specific determinations of anthocyanins, flavonoids and total phenolics [36,37]. Standard calibration curves were daily prepared. For vitamin C determinations, absorbance was measured at 525 nm and the amount of vitamin C was expressed as mg of ascorbic acid (AsA) per 100 g fresh weight (FW). For total phenolics content, the colorimetric method based on the chemical reduction of the Folin-Ciocalteu reagent was used. Absorbance was measured at 725 nm and the content was expressed in milligrams of gallic acid (3,4,5-Trihydroxy-benzoic acid) equivalents (GAE) per 100 g of FW. Total flavonoids content was determined measuring absorbance at 510 nm and the results were expressed as milligrams of catechin equivalents (CE) per 100 g of FW. The total anthocyanins content was evaluated measuring in the hydroalcoholic extract the absorbance at 535 and 700 nm. The anthocyanins concentration was calculated using the molar extinction absorptivity coefficient $\varepsilon = 25,965$/cm M and was expressed in mg of cyanidin 3-glucoside equivalents (C3GE) per kg of FW. The relative antioxidant capacity (RAC) was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). The absorbance was measured after 10 min of reaction at 515 nm and RAC was expressed as µg of Trolox equivalents per g of FW.

3.5. Total Sugars Analysis

To estimate the variation in sugar profile among genotypes, sugar composition and quantification were measured as described by Cantín *et al.* [14]. For the analysis, 250 µL of the homogenized extract was incubated at 80 °C for 20 min in 200 µL of 800 mL/L ethanol, with 5 g/L manitol added as an internal standard. Samples were purified using ion exchange resins (Bio-Rad Barcelona, Spain) [38]. Twenty µL was injected into the HPLC system (Aminex HPX-87C column, 300 mm × 7.8 mm; Bio-Rad, Barcelona, Spain) with a refractive index detector (Waters 2410). The solvent was deionized water running at a flow rate of 0.6 mL per min at 85 °C. Sugar quantification was performed with Millenium 3.2 software (Waters) using standards of analytical grade (Panreac Quimica SA, Barcelona, Spain). Sugar concentrations were expressed as g per kg FW.

3.6. Statistical Analysis

All traits were measured or scored for each genotype separately over the four year period, and means of four years were calculated. All statistical analyses were performed using SPSS 19.0 (SPSS

Inc., Chicago, IL). To obtain basic statistics for the entire plant material studied, minimum and maximum values, mean and mean standard error (SE), were calculated for each trait. Data for each genotype were averaged, and mean values were used as estimated genotypic values. Finally, correlations were calculated with raw data of the four years, according to Pearson's test at $P \leq 0.01$. Principal component analysis (PCA) was applied on the antioxidant compounds and basic agronomical traits in the studied population as an attempt to identify superior genotypes based on their antioxidant compound contents. The component matrix was evaluated and orthogonal factors were rotated using variance maximizing (Varimax).

4. Conclusions

To summarize, the progeny showed a great phenotypic variance for all the pomological and antioxidant studied traits. High variability was found in yield, fruit weight, firmness, SSC, TA, ascorbic acid, total phenolics, flavonoids, anthocyanins, antioxidant capacity, and total sugars which indicate that there is an important genetic potential to develop new nectarine cultivars with high fruit quality. On the other hand, the significant correlations found between some agronomical and quality attributes could be of interest for quality oriented fruit breeding programs. The study also emphasizes the usefulness of PCA in evaluating the fruit quality of new breeding releases and studying relationships among pomological traits.

Our results lead us to the conclusion that the antioxidant capacity of peach is characterized by huge levels of variations, much explained by the genotype, but harvest conditions and season may also be significant factors. Most of the progeny showed higher total phenolics and flavonoids content than parents. This fact could be of importance for selection of specific traits in the progeny. The phenotypic variation found in all studied traits will allow selecting superior genotypes with higher antioxidant content than the existing commercial varieties and this will naturally be beneficial for health.

Acknowledgments

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