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RESEARCH PAPER

## Genetic diversity, polyphenolic composition and fruit quality trait phenotypic analyses of a Chilean heritage blood-flesh peach (*Prunus persica* L.)

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### Abstract

**R. Corral, B. Carrasco, C. Ramirez, L. Marchant, A. Peña, J. I. Covarrubias, L. A. Meisel, I. Pacheco, E. R. Bascuñan-Ortiz, and H. Silva. 2022. Genetic diversity, polyphenolic composition and fruit quality trait phenotypic analyses of a Chilean heritage blood-flesh peach (*Prunus persica* L.). Int. J. Agric. Nat. Resour. 169-198.** This study reports the genetic diversity among Chilean heritage blood-flesh peaches and the characterization of phytochemicals and bioactive compounds present in these fruits. A genetic diversity analysis using 7,934 SNP markers was performed. The average observed heterozygosity ( $H_o=0.09$ ) was very low in the 75 Chilean blood-flesh peach trees, whereas 14 commercial peach varieties had significantly higher levels of heterozygosity ( $H_o=0.32$ ). Furthermore, the blood-flesh peach lines were genetically similar, and all of these lines were genetically different from the commercial varieties. A comparative analysis was carried out between the epicarp and mesocarp of the peach fruits. Fruit quality parameters were evaluated at harvest (weight, size, firmness and soluble solids), and concentrations of total polyphenols, anthocyanins, carotenoids, as were macro (P, K, Ca, Mg) and microelements (Fe, Zn, Mn, B, Cu). These analyses showed that blood-flesh peaches have high concentrations of anthocyanins (cyanidin-3-glucoside (C3G) and cyanidin-3-rutinoside (C3R)) when compared to commercial varieties with white or yellow mesocarps. A comparison was performed among Chinese, French and Chilean varieties, with similar values found for the antioxidant compounds. No significant differences in the micro- and macroelement contents were detected in these blood-flesh fruits compared to commercial varieties.

**Keywords:** Bioactive compounds, durazno betarraga, fruit development, Rosaceae.

## Introduction

Biodiversity in agriculture is essential to produce nutritious food sustainably and safeguard food security. The effects that the COVID-19 pandemic had on global food security demonstrated the fragility of international food chains and the need to strengthen informal food chains and agrobiodiversity (Zimmerer & de Haan, 2020; Markandya et al., 2021). Production must address the amount of food or calories and high-value nutrients, such as vitamins, minerals, and other micro/macro elements. As stated in the 'EAT-Lancet Commission on Healthy Diets from Sustainable Food Systems' 2019 report (Willett et al., 2019), although food production systems could secure human health and maintain environmental stability, both are being threatened. To ensure global food security from sustainable food systems by 2050, a "Great Food Transformation" must be rapidly adopted (Willett et al., 2019). This transformation focuses on healthier, plant-based foods, reducing red meat and sugar consumption by over 50% and increasing the consumption of fruit, vegetables and nuts by over 100% (Willett et al., 2019).

The increase in consumer demand for local and nutritious food products is a force that drives the conservation and use of local heritage and traditional varieties. These local heritage varieties correspond mainly to plant species cultivated in a specific region for more than a century that have adapted to particular agro-environmental conditions (ODEPA, 2014).

Chile's remarkable climatic diversity gives this country a clear advantage in producing fresh fruits, vegetables, and other food products, including well-known components of the "Mediterranean diet." Furthermore, Chilean natural resources consist of not solely fertile arable land with underground and fluvial water resources but also the genetic diversity of the local heritages (traditional varieties) of plants that have adapted over time to the diverse microclimates that make up the Chilean landscape. These local heritages are a valuable

source of genetic diversity that may be used to understand the genetic and biochemical basis of how plants produce secondary metabolites with bioactive properties that are beneficial to human health (Berni et al., 2018).

The limited genetic diversity of commercial fruit and vegetable varieties makes it challenging to obtain new varieties with good taste, desirable texture and healthy levels of bioactive compounds. In contrast, due to years of adaptation to different agroclimatic and geographical landscapes, non-domesticated wild relatives and local heritages have a higher genetic diversity and are a source of unique genetic characteristics. Furthermore, as mentioned in a recent report by the FAO, titled "The state of the World's Biodiversity for Food and Agriculture" (FAO, 2019), crop wild relatives and traditional heritages are essential resources for future resilience breeding programs in a global climate crisis scenario. Obtaining information on the genetic diversity and genomic structure of local Chilean heritages and associating this information with biochemical and metabolic profiles will provide useful information for future breeding programs toward healthier, nutritious and sustainable food production.

Peach [*Prunus persica* (L.) Batsch] belongs to the Rosaceae family and has a chromosome number of eight and a genome size of approximately 230 Mb/haploid (Verde et al., 2013). The fruit species originated in China more than 4,000 years ago (Faust & Timón, 1995; Wang & Zhuang, 2001). Over the years, travelers carried peach seeds from China to Persia and eventually brought and spread them into Europe. Over a long period of cultivation, naturally pollinated seedlings were selected, adapting well to the local climate and soil from which they were selected, resulting in local heritages or commercial varieties of this fruit tree species. Peaches were chosen for their fruit qualities or ornamental characteristics and propagated by seeds or grafts, resulting in the production of various cultivars (Zhang et al., 2015). Peaches were introduced to Chile by European

explorers and were easily propagated in central Chile due to the rich soil and warm climate (Gay, 1865). Many traditional peach varieties were spread and cultivated during the XVIII and XIX centuries (Lacoste et al., 2011).

Peaches are classified into three groups based on the color of the mesocarp, which can be white, yellow, or red (Wang & Zhuang, 2001). There are several peach cultivars with red mesocarps, known as blood-flesh peaches; however, they are very scarce in some countries and unknown in most. A large number of blood-flesh peach cultivars have been cultivated in China for hundreds of years, including the peach heritage “Dahongpao” (Zhou et al., 2015; Yan et al., 2017) and “Wu Yue Xian”, which was introduced in France in 1980 (Shen et al., 2013). In France, there are old blood flesh varieties, especially in the Auvergne-Rhône-Alpes region, where they are referred to as “peche de vigne”, “peche sanguine” or “peche sanguine vineuse”, among other names. In New Zealand, they are known as “black boy” peaches, while in the USA, they are “Indian blood,” and in Canada, they are “Harrow blood” (Thornton, 2015; Chaparro, 1995). Although blood-flesh peaches have not been previously described in Latin America, we recently reported a traditional Chilean blood-fleshed peach grown in some rural areas of central Chile for many generations (Marchant et al., 2022). Locally, this blood-fleshed peach is referred to as “durazno-betarraga”, “durazno vino” or “durazno conchovino,” a traditional variety or heritage used just for family consumption as fresh or dried fruits and jams. This Chilean heritage blood-flesh peach corresponds to a late variety, freestone peach that is self-fertile, propagated from seeds and has significant antioxidant potential.

Red mesocarp varieties are attractive due to the pigmentation caused by the accumulated anthocyanins. These compounds play important roles in plant cycles, including protecting them from biotic and abiotic stresses and attracting animals to

distribute seeds or pollen for reproduction (Kong et al., 2003). These phytonutrients are synthesized by the phenylpropanoid pathway and have been characterized in different plant species (Pandey et al., 2014). Anthocyanins are present in relatively high amounts in fresh fruit, and their colors make them more attractive to the consumer. Additionally, anthocyanins have strong antioxidant properties (Dragsted et al., 2006; Shin et al., 2006; Butelli et al., 2008; Williams et al., 2008). Previous studies in blood-flesh cultivars have reported high contents of total anthocyanins and polyphenols using spectrophotometric methods (Chaparro et al., 1995; Cevallos-Casals et al., 2006; Vizzotto et al., 2007). Quantitative analyses of individual phenolic compounds have been carried out recently in French blood-flesh peach cultivars (Aubert & Chalot, 2020) and Chinese cultivars (Zhao et al., 2015; Yan et al., 2017). In this study, we genetically and phenotypically describe the Chilean blood-flesh heritage variety. This study was performed by analyzing genetic variability using the RosBREED Peach v2 SNP chip. Additionally, quantitative and qualitative analyses of polyphenol composition and carotenoids and micro- and macroelement analyses were performed on the epicarp and mesocarp of this old Chilean heritage variety.

## Materials and Methods

### *Plant material*

During the 2017 and 2018 seasons, nine Chilean heritage peach trees located in Putu (latitude 35° 12' S and longitude 72° 17' W, one farm) and Las Corrientes (latitude 35° 30' S and longitude 72° 32' W, two farms), Constitución, in the Maule Region of Chile, were studied. Three fruits from each tree were sampled. These trees were selected based on morphological characteristics and the pigmentation of the mesocarp and epicarp. Ripe fruits were selected for uniformity based on color, size, weight, soluble solids (SS), and absence of surface damage (Figure 1). Three commercial varieties of peaches were used for comparative analyses:

two yellow mesocarp varieties (Romea and Alice Col) and a white mesocarp variety (White Flesh). These varieties were obtained from the German Greve Silva Experimental Station, Universidad de Chile, courtesy of Professor Rodrigo Infante. For the genetic diversity analyses, these same nine peach trees were used and compared to the previously genetically characterized commercial cultivar, Dr. Davis. The study presented in this manuscript will show the results from the 2018 season because no significant difference was found between the two seasons.



**Figure 1:** “Durazno betarraga”, a Chilean heritage blood-flesh peach (*Prunus persica L.*). a: Tree with fruits at the physiologically mature stage. b: Fruit mesocarp at the ripened stage.

### *DNA extraction and genotyping*

Young leaves were collected from 75 Chilean blood-flesh peaches (heritages), including the nine heritages mentioned in the Plant material section, between Putu (35°12'51''S/72°17'01''W) and Constitución (35°20'00''S/72°25'00''), Maule Region. Additionally, samples were collected from 14 commercial varieties (Dr. Davis, Summer Fire, Rich Lady 1, Rich Lady 2, Rich Lady 3, Venus, August Red 1, August Red 2, August Red 3, Sweet Ice, Super August, Rich May 1, Rich May 2 and Rich May 3) from different nurseries located between Codegua (34°02'00''S/70°40'00''W) and San Fernando (34°35'02''S/70°59'21''W). The samples were frozen in liquid nitrogen and stored at -80 °C until DNA extraction. DNA was isolated using a modified CTAB protocol (Carrasco et al., 2014; Meisel et al., 2005). At least 100 ng  $\mu\text{l}^{-1}$  of double-stranded DNA was measured with a Qubit 3.0 Fluorimeter (Thermo Fisher Scientific).

DNA from the 75 Chilean blood-flesh peaches and the commercial varieties were sent to Eurofins BioDiagnostics, EEUU (<https://www.eurofinsus.com/biodiagnostics/>). The DNA samples were genotyped using the International Peach SNP Consortium 16K SNP peach v.2 array on the Illumina Infinium platform. The genetic information obtained as genotypes was stored as a VCF file for posterior analysis with Tassel 5.2.52 (Glaubitz et al., 2014) and GenAlEx v. 6.5 (Peakall & Smouse, 2006).

### *Genetic analysis*

Nucleotide heterozygosity was explored using Tassel v5.2.52 software (Bradbury et al., 2007). The software Genalex v. 6.5 (Peakall & Smouse, 2006) was used to carry out a principal coordinate analysis (PCo) and to partition the genetic variability within and among populations ( $\phi_{PT}$  statistic) according to the analysis of molecular variance (AMOVA; Excoffier et al., 1992). The

significance of  $\phi_{PT}$  values was estimated by using 1,000 permutations.

#### *Determination of fruit morphological and harvesting parameters.*

**Size:** Obtained through the measurement of the equatorial and edge diameters of the fruit using a digital caliper (Bull Tools, China), expressing the results in centimeters with their corresponding standard deviations.

**Weight:** Determined using an electronic precision balance, and the results are expressed in grams with their corresponding standard deviations.

**Fruit firmness:** Three fruits from each tree were measured with an FTA electronic penetrometer using a 7.9 mm plunger. Equatorial area measurements were made on both sides of the fruit after epidermis removal. The results are expressed in pounds with their corresponding standard deviations.

**Soluble solids:** The measurement was carried out using a refractometer according to the method described by AOAC (2007), and the results are expressed in Brix with their corresponding standard deviations.

**Fruit harvest:** Three fruits were harvested with a fruit firmness between 12-14 lb and soluble solids content between 8-12% (Gil 2001) using an electronic penetrometer and a refractometer, respectively.

#### *Determination of individual phenolic compounds*

Anthocyanin profiling was performed using the method described by Peña et al. (2007), with the following modifications: five grams of tissue ground in liquid nitrogen was used for solid/liquid extraction with 20 mL of an 80% methanol solution for 30 min on ice with stirring and

subsequently centrifuged at  $12,300 \times g$  for 10 minutes. This solution was filtered, and 100  $\mu$ l was injected directly into the HPLC-DAD. The anthocyanin content of the sample was quantified using a calibration curve performed with cyanidin-3-rutinoside.

The contents of low-molecular-weight phenols (i.e., phenolic acids, flavonols and flavanols) in the mesocarp and epicarp were determined using the method described by Obreque et al. (2010) with the following modifications: 5 g of each tissue was taken and ground in liquid nitrogen; then, a second solid/liquid extraction with 20 mL of 80% methanol was performed for 10 minutes on ice with stirring and subsequently centrifuged at  $12,300 \times g$  for 30 minutes. The aqueous fraction was extracted with 25% of their initial volume of Milli-Q water and then extracted three more times with 20 mL of ethyl ether, followed by three extractions with 20 mL of ethyl acetate using decantation funnels. These extracts were dried on a rotary evaporator, rehydrated with 2 mL of a 1/1 v/v methanol/water solution, and finally filtered through a 0.22  $\mu$ m membrane filter. From this sample, 40  $\mu$ l was injected into the HPLC-DAD. Chromatographic separation was performed with a Nova Pak (Waters) C-18 column, 4  $\mu$ m 3.9 \* 300 mm with three mobile phases: 2% acetic acid (Merck) in water, methanol and water: acetonitrile: acetic acid (78: 20: 2) in gradient flow.

#### *Macro- and microelement determinations*

Macro- and microelement concentrations were determined in samples that were oven dried at 70 °C for 72 hours using 2 g dry weight of each sample. These samples were subsequently ground in a mortar, and 200 mg of each sample was used to perform the first phase of acid digestion with 6 mL of nitric acid, 4 mL of hydrogen peroxide, and 10 mL of distilled water overnight. The second stage of digestion was performed by bringing the solution up to 121 °C for 1 hour at a pressure of

0.1 MPa using an autoclave. The liquid resulting from mineralization was filtered and diluted to 20 mL with deionized water. Finally, quantification of the mineralized samples was performed using a microwave plasma atomic emission spectrometer (MP-AES 4200; Agilent Technologies) as described by Molina and Covarrubias (2019), for which standards of known concentrations were used. The blank contained 10% nitric acid. The first standard had macroelements (K, P, Ca and Mg) at 2 ppm and microelements (Fe, Zn, Mn, B and Cu) at 0.2 ppm. In the second standard, all the macroelements were at a concentration of four ppm, and the microelements were at 0.4 ppm; for the third standard, the macroelements were at 70 ppm, and the microelements were at 4 ppm. Finally, in the fourth standard, the macroelements were at 150 ppm, and the microelements were at 15 ppm.

#### *Carotenoid determination*

For carotenoid analyses, 1 g of tissue (mesocarp or epicarp) was ground in liquid nitrogen and macerated with 8 mL of hexane/acetone/ethanol (2:1:1; v/v) in a precooled mortar. The homogenate was transferred to a 15 mL Falcon tube, stirred with a vortexer for 2 min and incubated on ice in the dark for 2 min. The samples were subsequently centrifuged at  $12,300 \times g$  for 10 min at 4 °C. The supernatant containing the carotenoids was transferred to a new 15 mL Falcon tube. This solution was dried with a flow of N<sub>2</sub> and resuspended in 2 mL of acetone: methanol: water (6: 3: 1). It was then filtered and injected into the HPLC-DAD equipment. Chromatographic separation was performed with a Nova Pak C-18 column, 4 µm 3.9 \* 300 mm. The mobile phase used was acetone:methanol:water (60:30:10) in an isocratic flow of 1.5 mL min<sup>-1</sup>. All operations were carried out in dark and cold conditions to avoid photodegradation, isomerization and structural changes of the carotenoids (Stange et al., 2013). A lycopene calibration curve was used to calculate the concentrations of the carotenoids.

#### *Data analysis*

Variance analysis (ANOVA) and Tukey's test were carried out for parameters with normal distribution using the Past 4.11 software package (Hammer et al., 2001).

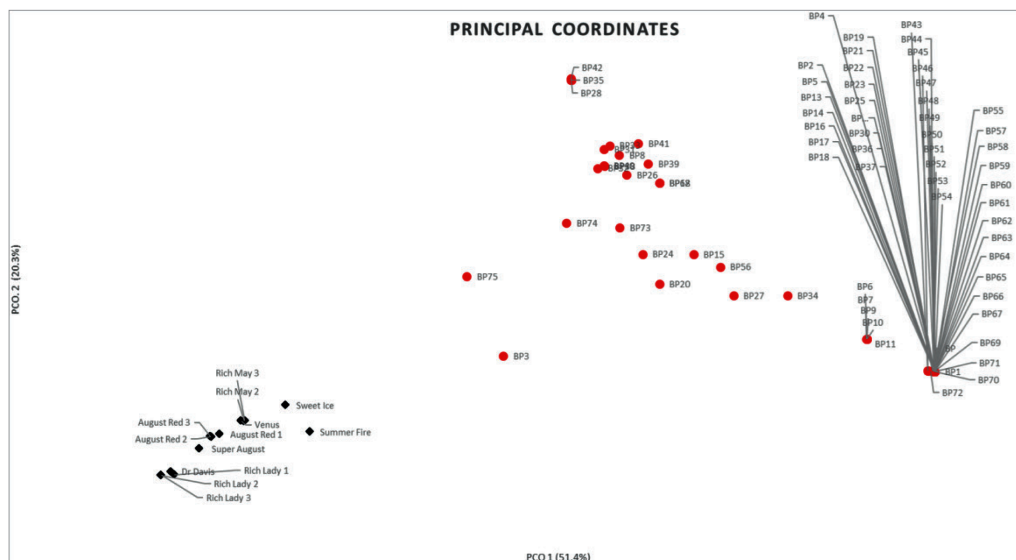
## **Results**

#### *Genotyping*

Chilean blood-flesh peach trees were genotyped using the Peach v.2 (16K) array. After filtering SNPs with frequencies less than 5% and missing data greater than 10%, 7,439 SNPs were used to determine the genetic diversity between the blood-flesh peach heritages and commercial peach varieties. The average observed heterozygosity was low for the 75 blood-flesh peach trees (Ho=0.09). In contrast, commercial peach varieties showed approximately three times superior heterozygosity levels (Ho=0.32).

AMOVA indicated significantly high genetic differences among the 75 Chilean blood-flesh and 14 commercial peach varieties ( $\phi_{PT}=0.56$ ;  $p<0.01$ ), which was consistent with the principal coordinate analysis (PCo). For instance, the first two components of PCo explained 71.7% of the genetic variability (Figure 2) and showed that the blood-flesh heritage peaches tended to form a genetically different group from the commercial varieties. Additionally, it is important to highlight that the Rich May, Rich Lady, and August Red varieties were sampled three times from different orchards to verify their genetic identity and the precision of the genetic analysis. The results showed high genetic similarity among replications of those three commercial varieties.

As illustrated in Figure 2, the local varieties of red-fleshed heritage peaches showed more significant genetic differentiation than the commercial varieties. However, 73% of heritages showed high genetic similarity, even though some were located at the same point in Figure 2. In contrast, 20 heritage lines



**Figure 2.** Principal coordinates analysis (PCo) based on 7,439 SNPs and 75 blood-flesh peach heritages (red circles, BP) and 14 commercial varieties (black diamonds).

showed some genetic differences. Based on these genetic results, nine red-fleshed peach heritage lines (13.3%) were used to estimate fruit quality parameters. These heritage lines were randomly selected.

#### *Evaluation of morphological and harvest parameters that define quality characteristics in the fruit*

The quality parameters of Chilean blood-flesh peaches harvested in February 2018 were determined. A summary of these determinations is presented in Table 1. Three fruits from each of the nine trees were used to determine the following quality parameters: equatorial diameter, edge diameter, weight, fruit firmness, and soluble solids, together with their respective standard deviations, obtained from three repetitions, corresponding to three fruits per tree. ANOVA results for these quality parameter analyses revealed that the blood-flesh peaches were smaller (size and weight) and less firm ( $p > 0.01$ ) than the yellow (Romea and Alice Col) and white (White Flesh) mesocarp commercial varieties. However, no significant differences ( $p > 0.05$ ) were observed in solid soluble contents.

#### *Content and analysis of the anthocyanin profiles present in the mesocarp and epicarp of fruit*

Anthocyanin contents were determined in the mesocarp and epicarp of the blood-flesh peaches from the 2018 season (Table 2). Only one type of anthocyanin was identified: cyanidin. This compound was linked to two different sugars: cyanidin-3-glucoside (C3G) and cyanidin-3-rutinoside (C3R), the former being the predominant sugar. The blood-flesh peach fruits presented high levels of C3G and C3R in the mesocarp, ranging from 394 to 1930  $\mu\text{g}$  of C3R equivalent/g FW of C3G and from 20 to 468  $\mu\text{g g}^{-1}$  FW of C3R. C3G and C3R were undetectable in the mesocarp of the control commercial varieties (Alice Col, Romea, and White Flesh). When the epicarp was evaluated, the range of C3G in the blood-flesh peaches was between 98 and 276  $\mu\text{g}$  of C3R equivalent  $\text{g}^{-1}$  FW and from 36 to 111  $\mu\text{g g}^{-1}$  FW of C3R. C3G was undetectable in the epicarp of the commercial variety Romea; lower levels were present in the White Flesh peaches than in the blood-flesh peaches ( $75.6 \pm 8.6$ ), but equivalent levels were detected in the Alice Col variety ( $209.0 \pm 12.3$ ). C3R was undetected in the epicarp of the Romea and White Flesh varieties,

but low levels ( $9.6 \pm 1.0$ ) were detected in Alice Col peaches.

The variance analysis of anthocyanin content in the blood-flesh peaches showed significant differences between the studied trees ( $p < 0.01$ )

only for cyanidin-3-glucoside (C3G) present in the mesocarp and epicarp. In this regard, tree 56 produced significantly higher C3G, followed by trees 53, 60, and 71. Moreover, the blood-flesh peaches showed higher levels of anthocyanin than commercial varieties.

**Table 1.** Quality parameters of blood-flesh peaches measured in the 2018 season.

| Group                | Tree I.D.*  | Equatorial diameter (mm) | Edge diameter(mm)     | Weight (g)              | Firmness (lb)        | Soluble solids (°Brix) <sup>NS</sup> |
|----------------------|-------------|--------------------------|-----------------------|-------------------------|----------------------|--------------------------------------|
| Blood-flesh peaches  | 44          | 45.8±3.8 <sup>b</sup>    | 46.1±4.3 <sup>b</sup> | 60.2±9.9 <sup>b</sup>   | 1.9±0.5 <sup>b</sup> | 16.6±1.8                             |
|                      | 52          | 54.0±4.3 <sup>b</sup>    | 54.5±3.9 <sup>b</sup> | 85.8±15.2 <sup>b</sup>  | 1.6±0.6 <sup>b</sup> | 12.9±1.7                             |
|                      | 53          | 54.5±5.6 <sup>b</sup>    | 51.3±4.8 <sup>b</sup> | 82.9±26.7 <sup>b</sup>  | 1.3±0.4 <sup>b</sup> | 11.5±1.8                             |
|                      | 56          | 44.2±2.5 <sup>b</sup>    | 45.7±2.2 <sup>b</sup> | 50.6±7.0 <sup>b</sup>   | 1.5±0.4 <sup>b</sup> | 15.9±1.9                             |
|                      | 58          | 47.5±2.5 <sup>b</sup>    | 48.6±2.7 <sup>b</sup> | 54.8±6.8 <sup>b</sup>   | 1.6±0.5 <sup>b</sup> | 14.4±1.9                             |
|                      | 59          | 48.6±2.5 <sup>b</sup>    | 51.9±2.8 <sup>b</sup> | 64.2±8.6 <sup>b</sup>   | 1.8±0.5 <sup>b</sup> | 17.6±0.9                             |
|                      | 60          | 44.6±2.8 <sup>b</sup>    | 47.8±1.9 <sup>b</sup> | 52.6±6.8 <sup>b</sup>   | 1.7±0.6 <sup>b</sup> | 15.6±1.4                             |
|                      | 68          | 52.8±3.3 <sup>b</sup>    | 58.4±3.6 <sup>b</sup> | 87.1±17.3 <sup>b</sup>  | 2.7±1.3 <sup>b</sup> | 12.5±2.0                             |
|                      | 71          | 47.4±1.6 <sup>b</sup>    | 47.4±2.2 <sup>b</sup> | 59.1±4.9 <sup>b</sup>   | 1.5±0.4 <sup>b</sup> | 11.5±1.8                             |
| Peach control fruits | Alice Col   | 69.5±4.0 <sup>a</sup>    | 66.7±4.9 <sup>a</sup> | 161.8±29.1 <sup>a</sup> | 6.0±4.5 <sup>a</sup> | 14.4±2.3                             |
|                      | Romea       | 70.3±2.4 <sup>a</sup>    | 58.8±2.9 <sup>a</sup> | 159.5±14.4 <sup>a</sup> | 4.8±1.1 <sup>a</sup> | 12.6±1.3                             |
|                      | White Flesh | 75.1±6.5 <sup>a</sup>    | 65.5±5.4 <sup>a</sup> | 205.2±46.0 <sup>a</sup> | 3.3±1.1 <sup>a</sup> | 11.4±2.1                             |

\*The number of analyzed fruits was three per tree; Peach control fruits correspond to commercial varieties (Alice Col: nectarine with yellowish flesh; White Flesh: nectarine variety with white mesocarp; Romea: cling peach variety). mm: millimeters; g: grams; lb: pounds; ±SD: represents the average of three samples. a, b = similar letters indicate no significant differences; different letters indicate significant differences according to ANOVA and Tukey's test. NS = no significant difference.

**Table 2.** Anthocyanin contents (cyanidin-3-glucoside, C3G, and cyanidin-3-rutinoside, C3R) in blood-flesh peaches.

| Group                | Tree I.D.*  | Mesocarp                  |                     | Epicarp                 |                     |
|----------------------|-------------|---------------------------|---------------------|-------------------------|---------------------|
|                      |             | C3G <sup>2</sup>          | C3R <sup>3</sup> NS | C3G <sup>2</sup>        | C3R <sup>3</sup> NS |
| Blood-flesh peach    | 44          | 588.4±92.1 <sup>c</sup>   | 92.1±125.1          | 216.7±21.8 <sup>b</sup> | 46.4 ± 2.7          |
|                      | 52          | 394.2±38.1 <sup>c</sup>   | 38.1±117.0          | 98.9±27.9 <sup>c</sup>  | 37.3 ± 9.7          |
|                      | 53          | 1083.5±102.5 <sup>b</sup> | 102.5±339.1         | 226.9±71.9 <sup>b</sup> | 79.5 ± 16.6         |
|                      | 56          | 1930.6±377.7 <sup>a</sup> | 377. 730.3          | 276.6±55.6 <sup>a</sup> | 93.8 ± 34.4         |
|                      | 58          | 930.1±226.1 <sup>b</sup>  | 226.1±273.6         | 106.6±10.5 <sup>c</sup> | 46.8 ± 0.8          |
|                      | 59          | 429.2±20.1 <sup>c</sup>   | 20.1±140.3          | 108.4±32.3 <sup>c</sup> | 40.7 ± 10.4         |
|                      | 60          | 971.8±307.7 <sup>b</sup>  | 307.7±294.8         | 204.7±6.1 <sup>b</sup>  | 74.3 ± 7.3          |
|                      | 68          | 1262.1±44.3 <sup>b</sup>  | 44.3±336            | 102.4±63.7 <sup>c</sup> | 36.1±19.7           |
|                      | 71          | 902.2±468.2 <sup>b</sup>  | 468.2±350.0         | 242.4±28.8 <sup>b</sup> | 111.3±14.0          |
| Peach control fruits | Alice Col   | -                         | -                   | 209.0±12.3              | 9.6±1,0             |
|                      | Romea       | -                         | -                   | -                       | -                   |
|                      | White Flesh | -                         | -                   | 75.6±8.6                | -                   |

\*The number of analyzed fruits was 3 per tree; <sup>2</sup>: the level of C3G is expressed in µg of C3R equivalent/g FW (FW: fresh weight); <sup>3</sup>: the level of C3R is expressed in µg of C3R/g FW. Peach control fruits correspond to commercial varieties (Alice Col: nectarine with yellowish flesh; White Flesh: nectarine variety with white mesocarp; Romea: cling peach variety). SD: represents the average of three samples. -: not detected. a, b, c = similar letters indicate no significant differences; different letters indicate significant differences according to ANOVA and Tukey's test. NS = no significant difference



### Identification and quantification of low-molecular-weight phenols

Low-molecular-weight phenolic compounds were identified and quantified (Tables 3 and 4). These analyses were performed in the epicarp (Table 3) and mesocarp (Table 4) in all fruits, and the results are expressed as the concentration of phenols ( $\mu\text{g g}^{-1}$  fresh weight) and their corresponding standard deviations. A total of 27 different phenolic compounds were detected in the blood-flesh peaches, with higher levels and diversity of phenolic compounds detected in the epicarp and the mesocarp of the blood-flesh peaches compared to the commercial varieties.

Ten different flavan 3-ols were found in blood-flesh peaches. Among them, procyanidins B2, B3 and B4 were present at high levels in the mesocarp,

ranging from 1.87 to 16.18  $\mu\text{g g}^{-1}$ , while these flavan 3-ols were not detected in any control peach varieties. Similar results were obtained for catechin in mesocarp, with levels ranging from 0.52 to 2.87  $\mu\text{g g}^{-1}$ .

Among flavonols, three different glycosylated flavonoids, quercetin and kaempferol (3-glucoside, 3-galactoside and 3-rutinoside), were detected in the blood-flesh mesocarp and epicarp when compared to control peaches. Quercetin and kaempferol glycosides have been previously reported in other peach cultivars, including French and Chinese blood-fleshed collections (Tomás-Berberán et al., 2001; Zhao et al., 2015; Aubert et al., 2020).

Nine hydroxycinnamic acids were identified exclusively in the mesocarp of the blood-fleshed

**Table 3.** Identification and quantification of low-molecular-weight phenolic compounds in blood-flesh peach epicarp ( $\mu\text{g/g}$  fresh weight).

| EPICARP<br>Phenolic compounds | Blood-flesh peach trees |             |             |             |            |            |             |            |           |             | Peach control fruits |           |  |
|-------------------------------|-------------------------|-------------|-------------|-------------|------------|------------|-------------|------------|-----------|-------------|----------------------|-----------|--|
|                               | 44                      | 52          | 53          | 56          | 58         | 59         | 60          | 68         | 71        | Alice Col   | White Flesh          | Romea     |  |
| Flavan 3-ols                  |                         |             |             |             |            |            |             |            |           |             |                      |           |  |
| protocatechic acid            | -                       | -           | 0.73±0.52   | -           | -          | 0.22±0.19  | -           | 0.41±0.41  | -         | 0.24±0.21   | -                    | -         |  |
| procyanidin dimer             | -                       | 0.33±0.57   | -           | -           | -          | -          | -           | 6.87±6.85  | 5.43±1.51 | 0.24±0.21   | -                    | -         |  |
| procyanidin B1                | -                       | -           | -           | -           | -          | -          | -           | -          | -         | -           | -                    | -         |  |
| procyanidin B2                | 0.8±0.8                 | -           | -           | -           | -          | -          | -           | 15.25±4.77 | 5.09±1.38 | -           | 7.47±3.62            | -         |  |
| procyanidin B3                | 1.16±1.16               | -           | -           | -           | -          | -          | 0.82±0.79   | 2.55±2.4   | 1.49±0.99 | -           | -                    | -         |  |
| procyanidin B4                | 4.57±2.96               | -           | -           | -           | -          | -          | -           | 10.49±7.89 | -         | 1.26±1.09   | -                    | 15.6±15.6 |  |
| procyanidin                   | 1.09±0.27               | -           | -           | -           | -          | -          | -           | 1.31±1.16  | 1.14±0.04 | -           | -                    | -         |  |
| procyanidin gallate           | 0.43±0.43               | -           | 2.66±0.95   | -           | -          | -          | 2.00±1.74   | 2.74±1.42  | 2.47±0.96 | 7.5±5.55    | 0.70±0.10            | 0.66±0.12 |  |
| catechin                      | 2.23±1.32               | -           | -           | -           | -          | -          | -           | 1.09±0.71  | 1.23±0.57 | -           | 0.31±0.29            | -         |  |
| epigallocatechin              | 0.88±0.88               | -           | -           | -           | -          | -          | -           | -          | -         | -           | -                    | -         |  |
| Flavonols                     |                         |             |             |             |            |            |             |            |           |             |                      |           |  |
| quercetin-3-glucoside         | 30.66±17.14             | -           | -           | 27.48±17.72 | 4.84±4.84  | 16.2±13.09 | -           | 15.57±8.17 | -         | 29.53±16.47 | -                    | 4.38±3.69 |  |
| quercetin-3-galactoside       | -                       | 2.28±0.79   | -           | -           | -          | 2.95±1.65  | -           | 4.97±4.52  | 4.21±0.50 | -           | -                    | -         |  |
| quercetin-3-rutinoside        | 15.84±7.81              | -           | 25.37±24.05 | -           | 10.83±7.44 | -          | 2.45±1.39   | -          | 8.24±3.30 | 18.95±12.27 | -                    | -         |  |
| kaempferol-3-glucoside        | 1.78±0.91               | -           | 2.86±0.93   | -           | 0.57±0.55  | 0.69±0.43  | 3.67±1.75   | 3.56±2.62  | 4.66±3.11 | 2.18±1.52   | 1.10±1.01            | -         |  |
| kaempferol-3-galactoside      | 2.94±1.47               | 1.04±0.62   | 3.79±2.44   | -           | 1.27±0.92  | 0.32±0.28  | 0.73±0.41   | 3.67±3.05  | 0.75±1.30 | 5.17±1.55   | -                    | 0.81±0.69 |  |
| kaempferol-3-rutinoside       | 0.30±0.30               | -           | -           | -           | -          | -          | 4.37±4.02   | 2.18±0.16  | 0.02±0.04 | 0.17±0.14   | -                    | -         |  |
| Hydroxycinnamic Acid          |                         |             |             |             |            |            |             |            |           |             |                      |           |  |
| trans-neochlorogenic acid     | 1.62±0.50               | 0.88±1.53   | -           | -           | -          | -          | -           | 1.2±0.64   | -         | -           | -                    | -         |  |
| cis-chlorogenic acid          | 62.95±61.62             | 20.69±11.44 | 12.26±9.65  | 3.60±2.30   | -          | 5.11±0.01  | 17.81±16.03 | -          | 4.22±2.75 | -           | 2.43±1.26            | 5.18±2.04 |  |
| trans-chlorogenic acid        | 67.3±4.45               | -           | 3.69±6.40   | -           | 4.85±2.85  | -          | -           | -          | 3.18±1.73 | -           | 3.66±1.15            | -         |  |
| cis-ferulic acid ester        | 0.12±0.12               | -           | -           | -           | -          | -          | -           | -          | 0.54±0.41 | -           | -                    | -         |  |
| trans-ferulic acid ester      | 0.21±0.13               | -           | -           | -           | -          | -          | -           | -          | -         | 0.20±0.06   | -                    | -         |  |
| cis-ferulic acid              | 0.09±0.09               | -           | -           | -           | -          | -          | -           | -          | 0.18±0.02 | -           | -                    | -         |  |
| cis-p-coumaric acid ester     | 0.09±0.09               | -           | -           | -           | -          | -          | -           | -          | -         | -           | -                    | -         |  |
| trans-p-coumaric acid ester   | 0.25±0.03               | -           | -           | -           | -          | -          | -           | 0.20±0.07  | -         | -           | -                    | -         |  |
| Stilbenes                     |                         |             |             |             |            |            |             |            |           |             |                      |           |  |
| trans-resveratrol             | 0.18±0.00               | 0.24±0.22   | -           | -           | -          | 0.11±0.09  | -           | 0.41±0.2   | 0.36±0.33 | 0.29±0.05   | -                    | -         |  |
| Tannins                       |                         |             |             |             |            |            |             |            |           |             |                      |           |  |
| elagitanine                   | 0.84±0.28               | 0.83±0.72   | 0.95±0.35   | -           | -          | 0.47±0.44  | -           | -          | 2.20±1.10 | 3.49±2.74   | -                    | 1.09±0.12 |  |

Peach control fruits correspond to commercial varieties (Alice Col: nectarine with yellowish flesh; White Flesh: nectarine variety with white mesocarp; Romea: cling peach variety). SD: represents the average of three samples. “-”: not detected

peaches. In contrast, only trans-chlorogenic acid was also detected in the commercial variety Alice Col. However, the trans-chlorogenic levels in the blood-flesh peach mesocarp were between 4 and 30 times higher than those in the commercial variety Alice Col. Cis- and trans-chlorogenic acids were also present in the epicarp in both blood-flesh and control peaches. The presence of chlorogenic acid in blood-flesh varieties has already been reported (Cavallos-Cassals et al., 2006; Zhao et al., 2015; Aubert et al., 2020), as has p-coumaroyl acid (Aubert et al., 2020).

We were able to identify cis- and trans-ferulic acid and trans-resveratrol in the mesocarp of blood-flesh peaches for the first time (Table 4).

It is important to mention that the phenolic compounds identified in the mesocarp and epicarp in the blood-flesh peaches and the commercial varieties fluctuated greatly. Some of the phenolic compounds were below the minimum required for HPLC detection. For instance, it was not possible to perform a variance analysis, and the data are shown to qualitatively compare the obtained data.

**Table 4.** Identification and quantification of low-molecular-weight phenolic compounds in blood-flesh peach mesocarp ( $\mu\text{g/g}$  fresh weight).

| MESOCARP<br>Phenolic compounds | Blood-flesh peach tree |            |            |             |             |           |            |             |             |           | Peach control fruit |           |  |
|--------------------------------|------------------------|------------|------------|-------------|-------------|-----------|------------|-------------|-------------|-----------|---------------------|-----------|--|
|                                | 44                     | 52         | 53         | 56          | 58          | 59        | 60         | 68          | 71          | Alice Col | White Flesh         | Romea     |  |
| <b>Flavan 3-ols</b>            |                        |            |            |             |             |           |            |             |             |           |                     |           |  |
| protocatechic acid             | 0.19±0.09              | -          | 0.05±0.00  | 0.14 ± 0.09 | 0.17 ± 0.00 | 0.10±0.09 | 0.18±0.18  | 0.58±0.33   | 0.20±0.17   | -         | -                   | -         |  |
| procyanidin dimer              | -                      | 1.06±0.38  | -          | 0.34±0.34   | 1.96 ± 1.71 | 5.62±4.31 | 2.70±0.04  | 1.42±0.99   | 1.30±0.00   | -         | -                   | -         |  |
| procyanidin B1                 | -                      | -          | -          | -           | -           | -         | -          | -           | -           | -         | -                   | -         |  |
| procyanidin B2                 | 5.25±3.78              | -          | 2.96±2.42  | 3.37±2.01   | -           | -         | -          | 16.18±2.66  | 1.23±0.00   | -         | -                   | -         |  |
| procyanidin B3                 | -                      | -          | -          | -           | -           | -         | 11.00±9.22 | 3.68±2.45   | 1.87±0.22   | -         | -                   | -         |  |
| procyanidin B4                 | 5.38±2.14              | -          | -          | -           | -           | -         | 5.21±5.21  | -           | -           | -         | -                   | -         |  |
| procyanidin                    | -                      | -          | -          | -           | -           | -         | 0.30±0.30  | 2.57±1.04   | 0.93±0.87   | -         | -                   | -         |  |
| procyanidin gallate            | -                      | -          | -          | 0.43±0.37   | -           | -         | 2.18±0.88  | 4.39±1.59   | 1.70±0.22   | 2.13±0.45 | -                   | -         |  |
| catechin                       | 1.6±1.09               | -          | 1.01±0.56  | -           | -           | -         | 0.52±0.52  | 2.87±2.56   | 0.97±0.74   | -         | -                   | -         |  |
| epigallocatechin               | -                      | -          | -          | -           | -           | -         | 3.00±3.00  | -           | -           | -         | -                   | -         |  |
| <b>Flavonols</b>               |                        |            |            |             |             |           |            |             |             |           |                     |           |  |
| quercetin-3-glucoside          | -                      | -          | -          | -           | 8.76±7.03   | 9.55±2.58 | -          | 24.38±15.27 | 25.62±23.08 | 9.60±9.29 | -                   | 1.92±0.86 |  |
| quercetin-3-galactoside        | -                      | 4.99±4.37  | -          | -           | -           | -         | 0.37±0.37  | 10.7±4.91   | 1.57±0.00   | -         | 1.99±0.66           | -         |  |
| quercetin-3-rutinoside         | -                      | 4.63±4.01  | -          | -           | 9.19±6.91   | 7.94±3.76 | 5.57±4.81  | 22.68±3.46  | 3.05±1.40   | -         | -                   | -         |  |
| kaempferol-3-glucoside         | 0.50±0.48              | 0.38±0.29  | 0.07±0.06  | 0.20±0.12   | -           | -         | 0.13±0.13  | 1.38±0.71   | 0.55±0.00   | -         | -                   | -         |  |
| kaempferol-3-galactoside       | -                      | 0.80±0.71  | -          | -           | 0.74±0.46   | 0.77±0.16 | 0.81±0.81  | 3.69±1.47   | 1.84±1.79   | 4.01±3.42 | -                   | 0.26±0.17 |  |
| Kaempferol-3-rutinosido        | 0.35±0.33              | -          | -          | -           | -           | -         | 0.09±0.00  | -           | 0.90±1.52   | -         | -                   | -         |  |
| <b>Hydroxycinnamic Acid</b>    |                        |            |            |             |             |           |            |             |             |           |                     |           |  |
| cis-neochlorogenic acid        | -                      | -          | 4.41±1.66  | -           | -           | -         | -          | -           | -           | -         | -                   | -         |  |
| trans-neochlorogenic acid      | 1.39±0.47              | -          | -          | -           | -           | -         | 3.70±3.70  | 6.31±3.36   | -           | -         | -                   | -         |  |
| cis-chlorogenic acid           | 24.59±3.30             | -          | 20.2±6.00  | 18.78±8.36  | 20.72±12.45 | 7.17±6.86 | -          | 6.93±4.20   | 2.66±0.28   | -         | -                   | -         |  |
| trans-chlorogenic acid         | 15.89±13.80            | 19.54±8.34 | 16.61±7.86 | 21.27±9.49  | 19.95±14.51 | 8.56±5.76 | -          | 12.69±3.93  | 2.93±1.25   | 0.70±0.34 | -                   | -         |  |
| cis-ferulic acid ester         | -                      | -          | 0.11±0.10  | 0.22±0.02   | -           | 0.04±0.03 | 0.28±0.28  | 0.62±0.47   | 0.07±0.00   | -         | -                   | -         |  |
| trans-ferulic acid ester       | 0.21±0.19              | 0.16±0.14  | -          | -           | -           | -         | -          | 0.71±0.23   | 0.20±0.17   | -         | -                   | -         |  |
| cis-ferulic acid               | -                      | -          | -          | -           | -           | -         | 0.19±0.06  | 0.45±0.42   | -           | -         | -                   | -         |  |
| cis-p-coumaric acid ester      | -                      | -          | -          | -           | -           | -         | -          | 0.47±0.45   | -           | -         | -                   | -         |  |
| trans-p-coumaric acid ester    | 0.23±0.03              | -          | 0.25±0.00  | 0.21±0.04   | -           | 0.21±0.02 | 0.25±0.25  | 0.46±0.43   | -           | -         | -                   | -         |  |
| <b>Stilbenes</b>               |                        |            |            |             |             |           |            |             |             |           |                     |           |  |
| trans-resveratrol              | -                      | -          | -          | -           | -           | -         | 0.50±0.50  | 0.32±0.03   | 0.03±0.00   | -         | -                   | -         |  |
| <b>Tanins</b>                  |                        |            |            |             |             |           |            |             |             |           |                     |           |  |
| elagitanine                    | -                      | 0.61±0.00  | 0.54±0.08  | 0.6±0.13    | 0.77±0.41   | 0.51±0.17 | 1.07±0.00  | 0.96±0.38   | 1.31±0.33   | 0.59±0.22 | 0.34±0.11           | 0.43±0.14 |  |

Peach control fruits correspond to commercial varieties (Alice Col: nectarine with yellowish flesh; White Flesh: nectarine variety with white mesocarp; Romea: cling peach variety). SD: represents the average of three samples. -: not determined for the equipment

### *Contents and profiles of micro- and macroelements in fruits*

The micro- and macroelement analyses in the mesocarp and epicarp of the fruits of the blood-flesh peaches and the control peach varieties (Alice Col, White Flesh, and Romea) are presented in Supplementary Tables 1-4. The macroelements found in more significant quantities included potassium and phosphorus, and the most abundant microelements were boron and zinc. However, no statistically significant differences were detected between blood-flesh peaches and the other commercial varieties analyzed, and the concentrations tended to remain within the same ranges in all varieties.

### *Contents of carotenoids in blood-flesh peach fruits*

The contents of carotenoids present in the blood-flesh peaches, which have an intense purple mesocarp (Figure 1b), were determined (Supplementary Table 5); one measurement is shown because the values were the same for the nine samples. In the epicarp and mesocarp, two types of carotenoids could be found: lutein and  $\beta$ -carotene. According to their chemical structures, they are carotenes or hydrocarbon carotenoids, i.e.,  $\beta$ -carotene, and xanthophylls or oxycarotenes, i.e., lutein (Beltrán, 2012). According to the results,  $\beta$ -carotene was present at higher levels than lutein in both tissues. Compared to commercial varieties, the contents of  $\beta$ -carotene and lutein were always lower in the blood-flesh peaches (Supplementary Table 5).

## **Discussion**

The Peach v.2 (16K) array (RosBREED Peach v2) revealed a moderate level of heterozygosity ( $H_o = 0.32$ ) for the 14 commercial varieties, similar to those reported for occidental varieties by Li et al. (2013), Micheletti et al. (2015), and Akagi et al. (2016). In this regard, Micheletti et al. (2015)

analyzed 1,240 peach accessions from European and Asiatic germplasm collections using this chip (9K). They ultimately analyzed 4,271 SNPs that showed heterozygosity levels that ranged between 0.003 and 0.680, with an average of  $H_o = 0.286$ . Akagi et al. (2016) found heterozygosity values between 0.220 and 0.264 in 67 cultivars using 5,180 SNPs. In contrast, the blood-flesh peaches showed low heterozygosity (9.0%), which was inferior to that reported for occidental varieties by Li et al. (2013).

The partitioning of genetic variability carried out by AMOVA indicated high genetic differentiation between blood-flesh heritages and commercial varieties of peaches (56%). The commercial varieties showed close genetic similarity, which was expected because the development of new peach varieties has been based on a reduced number of founding parental lines (Aranzana et al., 2010; Aranzana et al., 2019). The commercial peach varieties had a high heterozygosity level, in agreement with previously reported investigations on this species ( $H_o = 0.3$  and  $0.5$ ; Li et al., 2013; Micheletti et al., 2015). In this regard, commercial peach varieties are produced by crossing two elite parental lines to obtain a hybrid F1 generation from which the best phenotypes are selected. Subsequently, the best F1 phenotypes are grafted and evaluated for several years, with the ultimate goal of releasing a new variety (Carrasco et al., 2013). The low genetic variability observed in the local varieties of blood-flesh peaches can be partially explained by the self-pollination and crosses between related trees over many years. Simultaneously, propagation via seeds and genetic segregation could support the more significant genetic differences between local varieties of blood-flesh peaches. Similar results have been reported for peach heritages in Spain (Pérez et al., 2020).

Many studies on bioactive and antioxidant compounds have been published on yellow- and white-flesh peaches and nectarines, the most commercial cultivars worldwide (Tomas-Barberán

**Table 5.** Comparison of phenolic compound concentrations in blood-flesh peach cultivars

| Reference                  | Red-fleshed Peach Cultivar  | Phenolics Contents                                 | Concentration   | Methodology                |
|----------------------------|---|--|---|----------------------------|
| Jiao et al. 2014           | Beijingyixianhong (Germplasm Resource Center of Peach, Nanjing, China)        | Cyanidin-3-glucoside                               | Epicarp: 120 mg/100g FW<br>Mesocarp: 50 mg/100g FW                    | HPLC                       |
|                            |   | Cyanidin-3-rutinoside                              | Epicarp: nd<br>Mesocarp: nd   |                            |
|                            | Heiyoutao (Germplasm Resource Center of Peach, Nanjing, China)                | Cyanidin-3-glucoside                               | Epicarp: 115 mg/100g FW<br>Mesocarp: 15 mg/100g FW                    |                            |
|                            |   | Cyanidin-3-rutinoside                              | Epicarp: 45 mg/100g FW<br>Mesocarp: 5 mg/100g FW                      |                            |
| Zhao et al. 2015           | Wujingzaobai Feng (Zhuanghang Integrated Experiment Station, Shanghai, China) | Cyanidin-3-glucoside                               | Epicarp: 220.30±11.40 mg/100g DW<br>Mesocarp: 184.81±10.14 mg/100g DW | HPLC-DAD and LC-ESI-MS/MS  |
|                            |   | Chlorogenic acid                                   | Epicarp: 674.38±25.83 mg/100g DW<br>Mesocarp: 175.89±5.84 mg/100g DW  |                            |
|                            |   | Quercetin-3-O-galactoside                          | Epicarp: 69.38±2.77 mg/100g DW<br>Mesocarp: 4.90±0.02 mg/100g DW      |                            |
|                            |   |  | Quercetin-3-O-glucoside   |                            |
|                            |   | Quercetin-3-O-rutinoside                           | Epicarp: 172.73±6.07 mg/100g DW<br>Mesocarp: nd                       |                            |
|                            |   | Yan et al. 2017                                    | Dahongpao (National Peach Germplasm Repository, Nanjing, China)       |                            |
| Aubert et Chalot, 2020     | Monsan 1 (Star Fruits, France)  | Cyanidin-3-glucoside                               | Whole peach: 14.38 mg/100g FW   | HPLC (Rocket, Genevac Ltd) |
|                            |   | Cyanidin-3-rutinoside                              | Whole peach: 2.38 mg/100g FW  |                            |
|                            |   | Quercetin-3-O-galactoside                          | Whole peach: 18.81 mg/100g FW   |                            |
|                            |   | Quercetin-3-O-glucoside + Quercetin-3-O-rutinoside | Whole peach: 17.66 mg/100g FW   |                            |
|                            | Monsantros, Star Fruits, France   | Chlorogenic acid                                   | Whole peach 40.87 mg/100g FW  |                            |
|                            |   | Cyanidin-3-glucoside                               | 17.53 mg/100g FW  |                            |
|                            |   | Cyanidin-3-rutinoside                              | 3.12 mg/100g FW   |                            |
|                            |   | Quercetin-3-O-galactoside                          | 16.88 mg/100g FW  |                            |
| Corral et al. (this study) | Durazno Betarraga, Constitución, Maule, Chile.                                | Quercetin-3-O-glucoside + Quercetin-3-O-rutinoside | 20.29 mg/100g FW  |                            |
|                            |   | Chlorogenic acid                                   | 18.37 mg/100g FW  |                            |
|                            |   | Cyanidin-3-glucoside                               | Epicarp: 175.9 mg/100g FW<br>Mesocarp: 943.5 mg/100g FW               |                            |
|                            |   | Cyanidin-3-rutinoside                              | Epicarp: 62.9 mg/100g FW<br>Mesocarp: 186.3 mg/100g FW                |                            |

et al., 2001; Cantin et al., 2009; Zhao et al., 2015). However, little information is available regarding the quantity of individual phenolic compounds in blood-flesh cultivars, even when the high contents of these secondary metabolites should be considered a valuable trait for producing nutritious food.

A Chilean heritage of blood-flesh peach (“durazno betarraga”) has been growing for generations in family orchards without commercial agronomical management in the Maule Region (See Figure 1). The evaluations of the morphological and

harvest parameters such as equatorial and edge diameters, weight, firmness and soluble solids (Table 1) revealed that the “durazno betarraga” fruits were smaller and less firm and weighed less at harvest time when compared to commercial varieties. These results may be due to poor agronomical management of the trees or a genetic factor leading to the production of smaller fruits.

One of the compounds that indicates a high antioxidant capacity of a fruit is the presence of anthocyanins. Peach has two distinct red-flesh

phenotypes. One phenotype has been reported in the Harrow Blood cultivar, where anthocyanin accumulates in the mesocarp after pit hardening and leafing (Chaparro et al., 1995). The other phenotype is characterized by anthocyanin accumulation only in the mesocarp during the late stages of fruit development (Zhou et al., 2015). Our study identified one type of anthocyanin linked to two different sugars, cyanidin-3-glucoside (C3G) and cyanidin-3-rutinoside (C3R), with C3G levels that were higher in the mesocarp and epicarp of Chilean blood-flesh peaches than in three commercial varieties. The determination was made by separating the mesocarp and epicarp, and higher concentrations were obtained in the fruit pulp.

The results were contrasted with the commercial peach varieties Alice Col and Romea with yellow mesocarp and White Flesh with white mesocarp. At the ripening stage, the peach fruit changes its background color, which evolves from green to red due to chlorophyll degradation. For the coating color, greenish-yellow to red tones change, generally in response to the accumulation of anthocyanins and carotenoids, both antioxidant compounds (Africano, 2015). In the “Durazno Betarraga” blood-flesh peach, after pit hardening, the mesocarp starts to change from green to an intense purple color, similar to the Harrow Blood phenotype. Altogether, the dark gray with violet hues in the epicarp differentiate the “Durazno Betarraga” from the current commercial peaches available nationally. This purple pigmentation in both tissues is associated with the accumulation of anthocyanins. The results in Table 2 support the above, indicating that the blood-flesh peach reached an average C3G concentration of 943.5 mg 100 g<sup>-1</sup>, with up to 186.3 mg 100 g<sup>-1</sup> of C3R in the mesocarp. In contrast, in the commercial varieties (Alice Col, Romea and White Flesh), no concentrations of any anthocyanin compound were identified in that tissue. The same tendency could be observed with the epicarp contents for C3G and C3R, indicating the high ranges of these two types of anthocyanin in the blood-flesh peaches.

Table 5 shows a comparative analysis among different blood-flesh peach cultivars, including this study. For this analysis, all the results are shown in mg g<sup>-1</sup> rather than µg kg<sup>-1</sup>. The C3G and C3R concentrations in Chilean blood-flesh peach are higher in the mesocarp than in the epicarp. In contrast, the Chinese varieties Beijinggyixianhong, Heiyoutao and Wujingzaobaifeng have higher concentrations in the epicarp than in the mesocarp (Jiao et al., 2014, Zhao et al., 2015). Additionally, our values of C3G and C3R are the highest compared with Beijinggyixianhong, Heiyoutao, Dahongpao and Monsan 1 and Monsantros (French varieties). However, our values cannot be compared with the Wujingzaobaifeng variety because the authors expressed their results as dry weight.

Additionally, the content of phenolic compounds is an indication of potential antioxidant activity. In these analyses, twenty-seven compounds were identified. Phenolic compounds are closely related to food sensory and nutritional quality, contributing to flavor and, to a lesser extent, aroma (Rodrigo-García, 2006). The concentrations of polyphenols can vary significantly according to the extraction method used, which explains, in part, the diversity of results found in the literature. Additionally, differences for the same variety in different geographical regions can explain the differential expression of the phenylpropanoid pathway enzymes responsible for synthesizing phenolic compounds (Rodrigo-García, 2006). Our results show that chlorogenic acid and quercetin were the predominant components detected in both tissues (the epicarp contained higher amounts of low-molecular-weight phenols than the mesocarp). As summarized in Table 5, chlorogenic acid has been found in the Wujingzaobaifeng, Monsan 1 and Monsantros varieties with higher concentrations than in our results.

Our results show high variability in concentrations, ranging from not detected to 24.59 mg 100 g<sup>-1</sup> in mesocarp and 62.95 mg 100 g<sup>-1</sup> in epicarp, which are in the same range as values reported for French varieties (40.87 mg 100 g<sup>-1</sup> for M1 and 18.37 mg 100 g<sup>-1</sup> for M3)(Aubert & Chalot, 2020).

Quercetin-3-O-galactoside, quercetin-3-O-glucoside and quercetin-3-O-rutinoside were all found in Chinese and French varieties and Chilean blood-flesh peaches. The values between French and Chilean varieties were similar, despite more variability detected in the Chilean varieties.

These results showed that different varieties of blood-flesh peaches are rich sources of hydroxycinnamates (chlorogenic acid) and flavonols (quercetin-3-glucoside and quercetin-3-rutinoside) (Zhao, 2015).

All the phenolic compounds found have gained attention due to their potential biological and pharmacological effects on human health. Anthocyanins are known to have beneficial properties for health, such as cardioprotective, antidiabetic and anti-inflammatory properties, among others (Garzón, 2008). Both catechin and procyanidins significantly protect against coronary heart disease (Auger et al., 2004). Chlorogenic acid has antimicrobial, antioxidant and anti-inflammatory properties (Tajik, 2017).

Fruits also have micro- and macroelements that are necessary for a balanced diet. In this study, the macroelements found in higher quantities corresponded to potassium and phosphorus, and the more abundant microelements were boron and zinc. In this study, peach fruits proved to be a good source of macroelements, especially potassium. High potassium intake is positively associated with bone metabolism, decreased blood pressure and reduced morbidity and mortality from cardiovascular disease. Zinc and boron, which are among the essential microelements, were the most abundant in both tissues, with important biological activity linked to the metabolism of human enzymes. Zinc has been reported as a cofactor for more than 200 enzymes involved in immunity, the growth of new cells, acid-base regulation, and other processes and is more concentrated in the epicarp than in the mesocarp (Tylavsky, 2008). However, no statistically significant differences between blood-flesh peaches and the commercial varieties used as controls were found.

In peaches, the color of the mesocarp is among the most popular and commercial criteria for classifying cultivars. Ranging from yellow to orange, these are colors generated by the high contents of carotenoids. The accumulation of carotenoids in the mesocarp causes the difference between yellow and white genotypes. A peculiar and more intense aroma generally characterizes the latter due to the higher formation of volatile compounds derived from the decomposition of the carotenoid skeleton (Giberti, 2019). According to the results presented in Supplementary Table 5,  $\beta$ -carotene predominated over lutein in both tissues (mesocarp and epicarp). However, the blood-flesh peach had a lower content than the commercial varieties. When we compared the color of the mesocarp, the blood-flesh peach and the White Flesh commercial variety did not have significant amounts of this type of pigment, as opposed to what was noted in the varieties of yellow mesocarp (Romea and Alice Col), which had 18 times higher concentrations of  $\beta$ -carotene than the white/red mesocarp.

This work shows that the concentration of anthocyanins in blood-flesh peaches is above the parameters previously reported for white and yellow mesocarp commercial peach varieties, directly associating the purple–reddish pigmentation with a more considerable accumulation of these compounds. Similarly, the low-molecular-weight phenols are present in higher concentrations and are more diverse in the blood-flesh peaches compared to commercial varieties. As mentioned above, it should be noted that blood-flesh peaches have a higher antioxidant potential than the commercial varieties used as a control in this study, as a high content of low-molecular-weight phenolic compounds was found in the epicarp compared to that of the mesocarp, unlike anthocyanins, where this comparison was reversed. Therefore, consumption of this variety may help prevent cardiovascular diseases, cancer and some types of diabetes, among other diseases by eating either the whole fruit (preferably) or removing the epicarp, a practice that is useful for people allergic to the trichomes of this tissue.

In conclusion, the analyses presented in this manuscript detail a phenotypic and genetic characterization of the Chilean heritage blood-flesh peach, revealing several interesting characteristics that make the Chilean blood-flesh peach relevant for future breeding programs and a potential commercial crop with desirable consumer traits. These characteristics include the following:

- The 75 local varieties of red-fleshed heritage peaches showed high genetic similarity with each other and significant genetic differentiation compared with commercial peach varieties.
- The Chilean heritage blood-flesh peach contained higher levels of anthocyanins (cyanidin-3-glucoside (C3G) and cyanidin-3-rutinoside, C3R) compared to the commercial peach varieties used as controls.
- Chilean heritage blood-flesh peach number 56 showed higher cyanidin-3-glucoside (C3G)

levels in the mesocarp and epicarp, followed by peaches from trees 53, 60, and 71.

- The Chilean heritage blood-flesh peach (*Prunus persica* L.) contained a higher diversity of low-molecular-weight phenols than the commercial peach varieties analyzed in this study.

### Compliance with Ethical Standards

The authors declare that they do not have any conflicts of interest.

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### Resumen

**R. Corral, B. Carrasco, C. Ramirez, L. Marchant, A. Peña, J. I. Covarrubias, L. A. Meisel, I. Pacheco, E. R. Bascuñan-Ortiz, y H. Silva. 2022. Análisis fenotípicos de diversidad genética, composición polifenólica y rasgos de calidad de la fruta del durazno betarraga chileno (*Prunus persica* L.). *Int. J. Agric. Nat. Resour.* 169-198.** Este estudio reporta por primera vez la diversidad genética entre duraznos betarraga de Chile, así como la caracterización de los compuestos fitoquímicos y bioactivos presentes en estos frutos. Se realizó un análisis de diversidad genética utilizando 7.934 marcadores SNP. La heterocigosidad promedio observada ( $H_o=0,09$ ) fue muy baja en los 75 durazneros betarraga chilenos, mientras que 14 variedades comerciales de durazno tuvieron niveles significativamente más altos de heterocigosidad ( $H_o=0,32$ ). Además, las líneas de durazno betarraga fueron genéticamente similares entre sí, y todas estas líneas fueron genéticamente diferentes de las variedades comerciales. Se realizó un análisis comparativo, entre epicarpio y mesocarpio de los frutos de durazno. Se evaluaron parámetros de calidad de frutos a la cosecha (peso, tamaño, firmeza y sólidos solubles) y concentraciones de polifenoles totales, antocianinas, carotenoides, así como macro (P, K, Ca, Mg) y microelementos (Fe, Zn, Mn, B, Cu). Estos análisis mostraron que el durazno betarraga tiene altas concentraciones de antocianinas (cianidina-3-glucósido (C3G) y cianidina-3-rutinósido, C3R) en comparación con las variedades comerciales con mesocarpios blancos o amarillos. Se realizó una comparación entre las variedades Chinas, Francesas y

Chilenas que muestran valores similares para los compuestos antioxidantes. No se detectaron diferencias significativas en el contenido de micro y macroelementos en estos frutos de pulpa roja en comparación con las variedades comerciales.

**Palabras claves:** Compuestos bioactivos, desarrollo del fruto, durazno betarraga, Rosacea.

## References

- Africano, K., Almanza, P., & Balaguera, H. (2015). Physiology and biochemistry of peach [*Prunus persica* (L.) Batsch] fruit maturation. *Revista Colombiana de Ciencias Hortícolas*, *9*, 161–172.
- Akagi, T., Hanada, T., Yaegaki, H., Gradziel, T. M., & Tao, R. (2016). Genome-wide view of genetic diversity reveals paths of selection and cultivar differentiation in peach domestication. *DNA Research*, *23*, 271–282. <https://doi.org/10.1093/dnares/dsw014>
- AOAC, Association of analytical communities. (2007). *Official methods of Analysis of Analytical Chemist.* Ed. Patricia Cunniff, Maryland, USA. 1067.
- Aranzana, M. J., Abbassi, el-K., Howad, W., & Arús, P. (2010). Genetic variation, population structure and linkage disequilibrium in peach commercial varieties. *BMC Genet*, *11*, 69–80. <https://doi.org/10.1186/1471-2156-11-69>
- Aranzana, M. J., Decroocq, V., Dirlwanger, E., Eduardo, I., Gao, Z. S., Gasic, K., Iezzoni, A., Jung, S., Peace, C., Prieto, H., Tao, R., Verde, I., Abbott, A. G., & Arús, P. (2019). Prunus genetics and applications after de novo genome sequencing: achievements and prospects. *Hort Res*, *6*, 58–82. <https://doi.org/10.1038/s41438-019-0140-8>
- Aubert, C., & Chalot, G. (2020). Physicochemical characteristics, vitamin C, and polyphenolic composition of four European commercial blood-flesh peach cultivars (*Prunus persica* L. Batsch). *Journal of food composition and analysis*, *86*, 103337. <https://doi.org/10.1016/j.jfca.2019.103337>
- Auger, C., Al-Awwadi, N., Borne, A., Rouanet, J., Gasc, F., Cros, G., & Teissedre, P. (2004). Catechins and procyanidins in Mediterranean diets. *Food Research International*, *37*, 233–245. <https://doi.org/10.1016/j.foodres.2003.11.008>
- Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*, 2633–2635. <https://doi.org/10.1093/bioinformatics/btm308>
- Beltrán, B., Estévez, R., Cuadrado, C., Jiménez, S., & Olmedilla, B. (2012). Base de datos de carotenoides para valoración de la ingesta dietética de carotenos, xantofilas y de vitamina A; utilización en un estudio comparativo del estado nutricional en vitamina A de adultos jóvenes. *Nutrición Hospitalaria*, *27*, 1334–1343. <https://doi.org/10.3305/nh.2012.27.4.5886>
- Berni, R., Cantini, C., Romi, M., Hausman, J-F., Guerriero, G., & Cai, G. (2018). Agrobiotechnology Goes Wild: Ancient Local Varieties as Sources of Bioactives. *International Journal of Molecular Sciences*, *19*, 2248. <https://doi.org/10.3390/ijms19082248>
- Butelli, E., Titta, L., Giorgio, M., Mock, H-P., Matros, A., Peterek, S., Schijlen, E. G. W. M., Hall, R. D., Bovy, A. G., Luo, J., & Martin, C. (2008). Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nature Biotechnology*, *26*, 1301–1308. <https://doi.org/10.1038/nbt.1506>
- Cantin, C. M., Moreno, M. A., & Gogorcena, Y. (2009). Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarines (*Prunus persica* (L.) Batsch) breeding progenies. *J Agric Food Chem*, *57*, 4586–4592. <https://doi.org/10.1021/jf900385a>
- Chaparro, J. X., Werner, D. J., Whetten, R. W., & O'Malley, D. M. (1995). Inheritance, genetic interaction and biochemical characterization of anthocyanin phenotypes in peach. *Journal of*



- Heredity*, 86, 32–38. <https://doi.org/10.1093/oxfordjournals.jhered.a111522>
- Carrasco, B., Meisel, L., Gebauer, M., Garcia-Gonzales, R., & Silva, H. (2013). Breeding in peach, cherry and plum: from a tissue culture, genetic, transcriptomic and genomic perspective. *Biological Research*, 46, 219–230. <https://doi.org/10.4067/S0716-97602013000300001>
- Carrasco, B., Diaz, C., Garcia-Gonzales, R., Avila, P., Lobos, G. A., Silva, H., & Caligari, P. D. S. (2014). Genetic and morphological characterization of the endangered Austral papaya: *Vasconcellea chilensis* (Planch. ex A. DC.) Solms. *Genetic Resources and Crop Evolution*, 61, 1423–1432. <https://doi.org/10.1007/s10722-014-0143-0>
- Dragsted, L. O., Krath, B., Ravn-Haren, G., Vogel, U. B., Vinggaard, A. M., Bo Jensen, P., Loft, S., Rasmussen, S. E., Sandstrom, B., & Pedersen, A. (2006). Biological effects of fruit and vegetables. *Proceedings of the Nutrition Society*, 65, 61–67. <https://doi.org/10.1079/pns2005480>
- Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131, 479–491. <https://doi.org/10.1093/genetics/131.2.479>
- FAO. (2019). The State of the World's Biodiversity for Food and Agriculture, J. Bélanger & D. Pilling (eds.). FAO Commission on Genetic Resources for Food and Agriculture Assessments. Rome. 572 pp.
- Faust, M., & Timon, B. (1995). Origin and dissemination of peach. *Horticultural Reviews* 17, 331–379.
- Garzón, G. (2008). Las antocianinas como colorantes naturales y compuestos bioactivos. *Acta Biológica Colombiana*, 13, 27–36.
- Gay, C. (1865). *Historia Física y Política de Chile, Agricultura Tomo Segundo*. París, Francia.
- Giberti, S., Giovannini, D., & Forlani, G. (2019). Carotenoid cleavage in chromoplasts of white and yellow-fleshed peach varieties. *Journal of the Science and Food Agriculture*, 99, 1795–1803. <https://doi.org/10.1002/jsfa.9372>
- Gil, G. (2001). *Fruticultura: madurez de la fruta y manejo poscosecha*. Santiago. Universidad Católica de Chile. 39–40.
- Glaubitx, J. C., Casstevens, T. M., Lu, F., Harri-man, J., Elshire, R. J., Sun, Q., & Buckler, E. S. (2014). TASSEL-GBS: A High Capacity Genotyping by Sequencing Analysis Pipeline. *PLoS ONE*, 9(2), e90346. <https://doi.org/10.1371/journal.pone.0090346>
- Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontologia Electronica*, 4, 1–9.
- Kong, J. M., Chia, L. S., Goh, N. K., Chia, T. F., & Brouillard, R. (2003). Analysis and biological activities of anthocyanins. *Phytochemistry*, 64, 923–933. [https://doi.org/10.1016/s0031-9422\(03\)00438-2](https://doi.org/10.1016/s0031-9422(03)00438-2)
- Lacoste, P., Yuri, J. A., Aranda, M., Castro, A., Quinteros, K., Solar, M., Soto, N., & Chávez, C. (2011). Variedades de carozos (Chile y Cuyo 1700-1850). *IDESIA*, 29, 131–137.
- Li, X-W., Meng, X-Q., Jia, H-J., Yu, M-J., Ma, R-J., Wang, L-R., Cao, K., Shen, Z-J., Niu, L., Tian, J-B., Chen, M-J., Xie, M., Arus, P., Gao, Z-S., & Aranzana, M. J. (2013). Peach genetic resources: diversity, population structure and linkage disequilibrium. *BMC Genet.*, 14, 84–99. <https://doi.org/10.1186/1471-2156-14-84>
- Marchant, L., Campos, J., Luco, J., Ramirez, C., Barrientos, F., Carrasco, B., & Silva, H. (2022). Potential of traditional Chilean blood-fleshed peach to support livelihood opportunities in local agriculture. *Frontiers in Sustainable Food Systems*, 6, 820811. <https://doi.org/10.3389/fsufs.2022.820811>
- Markandya, A., Salcone, J., Hussain, S., Mueller, A., & Thambi, S. (2021). Covid, the environment and food systems: contain, cope and rebuild better. *Frontiers in Environmental Science*, 9, 674432. <https://doi.org/10.3389/fenvs.2021.674432>
- Meisel, L., Fonseca, B., Gonzalez, S., Baeza-Yates, R., Cambiazo, V., Campos, R., Gonzalez, M., Orellana, A., Retamales, J., & Silva, H. (2005). A rapid and efficient method for purifying high

- quality total RNA from peaches (*Prunus persica*) for functional genomics analyses. *Biological Research*, 38, 83–88. <https://doi.org/10.4067/s0716-97602005000100010>
- Micheletti, D., Dettori, M. T., Micali, S., Aramini, V., Pacheco, I., Da Silva Linge, C., Foschi, S., Banchi, E., Barreneche, T., Quilot-Turion, B., Lambert, P., Pascal, T., Iglesias, I., Carbó, J., Wang, L-r., Ma, R-j., Li, X-w., Gao, Z-s., Naz-zicari, N., Troggio, M., Bassi, D., ... Aranzana, M. J.. (2015) Whole-Genome Analysis of Diversity and SNP-Major Gene Association in Peach Germplasm. *PLoS ONE*, 10(9): e0136803. <https://doi.org/10.1371/journal.pone.0136803>
- Molina, J., & Covarrubias, J. (2019). Influence of Nitrogen on Physiological Responses to Bicarbonate in a Grapevine Rootstock. *Journal of Soil Science and Plant Nutrition*, 19, 305–312. <https://doi.org/10.1007/s42729-019-00030-1>
- Obreque, E., Peña, A., López, R., Zamora, F., Ricardo, J., & Laureano, O. (2010). Comparative Study of the Phenolic Composition of Seeds and Skins from Carménere and Cabernet Sauvignon Grape Varieties (*Vitis vinifera* L.) during Ripening. *Journal of Agricultural and Food Chemistry*, 58, 3591–3599. <https://doi.org/10.1021/jf904314u>
- ODEPA. (2014). Oficina de estudios y políticas agrarias.
- Pandey, A., Misra, P., Khan, M. P., Swarnkar, G., Tewari, M. C., Bhambhani, S., Trivedi, R., Chattopadhyay, N., & Trivedi, P. K. (2014). Co-expression of *Arabidopsis* transcription factor, *AtMYB12*, and soybean isoflavone synthase, *GmIFS1*, genes in tobacco leads to enhanced biosynthesis of isoflavones and flavonols resulting in osteoprotective activity. *Plant Biotechnology Journal*, 12, 69–80. <https://doi.org/10.1111/pbi.12118>
- Peakall, R., & Smouse, P. E. (2006) genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Resources*, 6(1), 288–295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Peña, A., Cáceres, A., & Pastenes, C. (2007) Low molecular weight phenolic and anthocyanin composition of grape skins from cv. Syrah (*Vitis vinifera* L.) in the Maipo Valley (Chile): Effect of clusters thinning and vineyard yield. *Food Science and Technology International*, 13, 153–158. <https://doi.org/10.1177/1082013207077920>
- Pérez, V., Larrañaga, N., Abdallah, D., Wunsch, A., & Hormaza, J. I. (2020). Genetic Diversity of Local Peach (*Prunus persica*) Accessions from La Palma Island (Canary Islands, Spain). *Agronomy*, 457, 1–13. <https://doi.org/10.3390/agronomy10040457>
- Rodrigo-García, J., Álvarez-Parrilla, E., de la Rosa, L., Mercado, G., & Herrera, B. (2006). Valoración de la capacidad antioxidante y actividad polifenol oxidasa en duraznos de diferentes áreas de producción. I Simpósio Ibero-Americano de Vegetais Frescos Cortados, San Pedro, SP Brazil.
- Shen, Z. J., Confolent, C., Lambert, P., Poëssel, J. L., Quilot-Turion, B., Yu, M., Ma, R., & Pascal, T. (2013) Characterization and genetic mapping of a new blood-flesh trait controlled by the single dominant locus DBF in peach. *Tree Genetics and Genomes*, 9, 1435–1446. <https://doi.org/10.1007/s11295-013-0649-1>
- Shin, W. H., Park, S. J., & Kim, E. J. (2006). Protective effect of anthocyanins in middle cerebral artery occlusion and reperfusion model of cerebral ischemia in rats. *Life Sciences*, 79, 130–137. <https://doi.org/10.1016/j.lfs.2005.12.033>
- Stange, C., Pizarro, L., Fuentes, P., Handford, M., Cifuentes, V., & Stange, C. (2013). Levels of lycopene  $\beta$ -cyclase 1 modulate carotenoid gene expression and accumulation in *Daucus carota*. *PLoS ONE*, 8, 1–13. <https://doi.org/10.1371/journal.pone.0058144>
- Tajik, N., Tajik, M., Mack, I., & Enck, P. (2017). The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. *Eur J Nutr*, 56, 2215–2244. <https://doi.org/10.1007/s00394-017-1379-1>
- Thornton, C. (2015). Series: Rare and heritage fruit cultivar; set 1-29. Red-fleshed peaches. Leaves of gold Press, Victoria, Australia.
- Tylavsky, F., Spence, A., Harkness, L. (2008). The importance of calcium, potassium, and acid-base

- homeostasis in bone health and osteoporosis prevention. *Journal of Nutrition*, *138*: 164S–165S. <https://doi.org/10.1093/jn/138.1.164S>
- Verde, I., Abbott, A. G., Scalabrin, S., Jung, S., Shu, S., Marroni, F., Zhebentyayeva, T., Dettori, M. T., Grimwood, J., Cattonaro, F., Zuccolo, A., Rossini, L., Jenkins, J., Vendramin, E., Meisel, L. A., Decroocq, V., Sosinski, B., Prochnik, S., Mitros, T., Policriti, A., Cipriani, G., ... Rokhsar, D. S. (2013). The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. *Nature Genetics*, *45*, 487–494. <https://doi.org/10.1038/ng.2586>
- Wang, Z. H., & Zhuang, E. J. (2001). The China fruit plant monograph-peach flora. Chinese Forest Press, Beijing 10:1–51.
- Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., Jonell, M., Clark, M., Gordon, L. J., Fanzo, J., Hawkes, C., Zurayk, R., Rivera, J. A., De Vries, W., Sibanda, L. M., Afshin, A., Chaudhary, A., ... Murray, C. J. L. (2019). Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet*, *393*, 447–492. [https://doi.org/10.1016/S0140-6736\(18\)31788-4](https://doi.org/10.1016/S0140-6736(18)31788-4)
- Williams, C. M., Mohsen, M., Vauzour, D., Rendeiro, C., Butler, L. T., Ellis, J. A., Whiteman, M., & Spencer, J. P. E. (2008). Blueberry-induced changes in spatial working memory correlate with changes in hippocampal CREB phosphorylation and brain-derived neurotrophic factor (BDNF) levels. *Free Radical Biology and Medicine*, *45*(3), 295–305. <https://doi.org/10.1016/j.freeradbiomed.2008.04.008>
- Yan, J., Cai, Z., Shen, Z., Ma, R., & Yu, M. (2017). Proanthocyanidin monomers and cyanidin 3-o-glucoside accumulation in blood-flesh peach (*Prunus persica* (L.) Batsch) fruit. *Arch Biol Sci*, *69*, 611–617. <https://doi.org/10.2298/ABS161212006Y>
- Zhang, Z., Shen, Z. J., Ma, R. J., Yu, M. L., & Cai, Z. X. (2015). Diversity, population structure, and evolution of local peach cultivars in China identified by simple sequence repeats. *Genetics and Molecular Research*, *14*, 101–117. <https://doi.org/10.4238/2015.January.15.13>
- Zhao, X., Zhang, W., Yin, X., Su, M., Sun, C., Li, X., & Chen, K. (2015). Phenolic Composition and Antioxidant Properties of Different Peach [*Prunus persica* (L.) Batsch] Cultivars in China. *International Journal of Molecular Science*, *16*, 5762–5778. <https://doi.org/10.3390/ijms16035762>
- Zhou, H., Lin-Wang, K., Wang, H., Gu, C., Dare, A. P., Espley, R. V., He, H., Allan, A. C., & Han, Y. (2015). Molecular genetics of blood-fleshed peach reveals activation of anthocyanin biosynthesis by NAC transcription factors. *Plant Journal*, *82*, 105–121. <https://doi.org/10.1111/tpj.12792>
- Zimmerer, K. S., & de Haan, S. (2020). Informal food chains and agrobiodiversity needs strengthening – not weakening – to address food security amidst the COVID-19 crisis in South America. *Food Sec.*, *12*, 891–894. <https://doi.org/10.1007/s12571-020-01088-x>

