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Abstract

The objective of this study was to identify transcripts or hormone-based biomarkers to define the physiological age of “Hass” avocado fruit and to elucidate the changes at the level of metabolic pathways and their regulation. “Hass” avocado fruit from orchards in different agroclimatic zones were collected during two harvest periods. Fruit were stored for 30 d under controlled atmosphere and regular air conditions and then transferred to shelf-life conditions at 20 °C. The physiological age as represented by the initial state of a hypothetical enzyme system (E_0) of each fruit was obtained through a mechanistic softening model for Chilean “Hass” avocado previously developed. Fruit from three different E_0 ranges (low, intermediate and high) were selected for transcriptome and hormone analyses. Sequencing data were processed by partial least squares regression analysis, which revealed 46 genes correlated to E_0. Different metabolic pathways were over expressed between low and high E_0 fruit. Low E_0 fruit showed overexpression of genes related to DNA replication, auxin transport, cell wall remodeling, gibberellin synthesis, brassinosteroids and flavonols. On the other hand, fruit with high E_0 revealed genes related to ethylene and abscisic acid biosynthesis and related responses and phenylpropanoid biosynthesis. Likewise, targeted hormone analysis revealed higher concentrations of active gibberellin and jasmonic acid for low E_0 fruit and for high E_0 fruit higher concentrations of abscisic acid, salicylic acid, indole acetic acid and cytokinin trans-zeatin, only the latter two being significant for this phenotype. This study reveals the relationship between transcripts and hormones during fruit maturation that is key to evaluate the physiological age of “Hass” avocado fruit.

Keywords: *Persea americana*, heterogeneity, firmness, hormones, maturation, transcripts.

1. Introduction

The cultivation of avocado cv. Hass in Chile is of significant economic importance, thanks to the large planted area, reaching 30,000 hectares in 2020 (ODEPA, 2021). During the 2019-2020 season, 168,000 ton fruit was produced, of which 72 % was exported; mainly to Europe, the United States, China and Argentina. Over 70 % of the "Hass" avocado production in Chile is concentrated in the Valparaíso Region (ODEPA, 2021). The low water resources have reduced the production area planted with avocado cv. Hass by 20 % over recent years (ODEPA, 2021). Thus, competition with neighbouring exporting countries that present more favourable hydrological conditions solely based on production volume is not possible but requires differentiation towards a high-quality product.

The main challenge to provide the market with a high-quality product (in terms of colour, firmness, days to reach eating quality) is the high heterogeneity evidenced postharvest (Hernández et al., 2017; Pedreschi et al., 2019). Although the Chilean "Hass" avocado industry relies on a minimum 23 % dry matter as commercial harvest index, previous studies have demonstrated that neither dry matter content nor at-harvest firmness are good indicators of the physiological age of the fruit and thus of its ripening behaviour (Rivera et al., 2017; Pedreschi et al., 2014; Hernández et al., 2021). Previous research has developed kinetic models capable of using simplified physiological concepts to predict the loss of firmness and ripening behaviour of different batches of avocado cv. Hass (Ochoa-Ascencio et al., 2009; Gwanpua et al., 2018; Hernández et al., 2021) as to early segregate batches of fruit into fast and slow ripening. Recently, Hernández et al. (2021) developed a mechanistic model based on the premises of Ochoa-Ascencio et al. (2009) but incorporated real measurements of non-destructive firmness at harvest, in addition to correlating polar metabolites content at harvest with the model parameter E_0 reflecting the physiological or

biological age of the fruit. In addition, Monte Carlo simulations, revealed that segregation of fruit based on the E_0 value reflecting their physiological age would have a tremendous positive effect on improved firmness retention for distant markets (low E_0 – premium fruit vs high E_0 mainstream fruit) and on reducing ripening heterogeneity. The GC-MS polar metabolite profile revealed potential as a biochemical phenotyping technique to assess the physiological age of the "Hass" avocado fruit at an early stage, as did previous research by García et al. (2018 and 2019) where they used metabolomics to find early biomarkers of the browning of freshly cut lettuce. However, other omics platforms such as transcriptomics through RNA sequencing allow the profiling of the entire transcriptome, providing a much broader coverage compared to the metabolome. For instance, the study reported by Nielson et al. (2017) successfully used gene expression profiles with selection of a set of genes as predictive biomarkers of cold-induced sweetening in potatoes. Therefore, due to the complexity of the maturation process of the “Hass” avocado due to the multiple interactions that exist among different levels of cellular control, transcriptomics in conjunction with targeted hormone analysis could bring more solid early correlations with E_0 (reflecting the physiological age of the fruit).

Due to the climacteric nature of “Hass” avocado most research has focused on the role of ethylene during fruit ripening (Adato and Gazit, 1977; Jeong and Huber, 2004; Kumar et al., 2014) and only recently the role of abscisic acid (ABA) has been reported (Meyer et al., 2017). In addition, Vincent et al. (2020) have recently reported a complex hormonal interplay during ripening of avocado revealing the participation of other hormones such as abscisic acid (ABA), jasmonates, gibberellins and auxins. In addition, Uarrota et al. (2019) reported large proteome differences at harvest associated with differences in physiological age affecting ripening behavior. Ripening is a high complex

process that involves the activation and interaction of several metabolic pathways, gene expression and regulatory mechanisms. Therefore, a transcriptomics approach could provide an additional platform to search for early biomarkers of physiological age of "Hass" avocado. The nuclear genome of *P. americana* var. drymifolia and *P. americana* var. Hass has been sequenced relatively recently (Rendón-Anaya et al., 2019). De novo transcriptomics has been used to study lipid biosynthesis during fruit development of avocado cv. Hass (Vergara-Pulgar et al., 2019) revealing potential biomarkers of fruit development. To our knowledge, previous biomarker studies on fruit maturity of "Hass" avocado relied on proteomics or metabolomics platforms (Fuentealba et al., 2017; Pedreschi et al., 2019; Uarrota et al., 2019; Hernández et al., 2021) but did not explore the potential of transcriptomics to identify marker genes correlating with the physiological age of the fruit at commercial harvest.

RNA-seq allows the creation of entire transcriptome profiles, providing a much more extensive coverage of the metabolic pathways and networks involved in "Hass" avocado at its different physiological stages (as indicated by E_0), especially when complemented with a targeted hormone analysis. The present study aims: (*i*) to propose early biomarkers of physiological age at the level of transcripts and hormones and (*ii*) to elucidate the differences at the level of metabolic pathways and their regulation between avocado cv. Hass fruit with different physiological age (as indicated by E_0).

2. Material and methods

2.1 Conditions of sampling, fruit storage and biopsy sampling

The plant material, sampling and storage conditions used correspond to those detailed in Hernández et al. (2021). Briefly, two hundred "Hass" avocado fruit from 12 orchards from three different agroclimatic zones were used. The sampling considered early

harvest fruit (≥ 23 % - 26 % dry matter content) and middle harvest fruit (> 26 % to 30 % dry matter content). One hundred fruit from each batch were stored in controlled atmosphere (CA) conditions of 4 kPa O_2 and 6 kPa CO_2 at 5 °C for 30 d. The other 100 remaining fruit from each orchard were stored in regular air (RA) at 5 °C for 30 d. After storage in RA or CA, fruit were brought to shelf-life conditions at 20 °C until each fruit reached the read-to-eat stage (4-8 N). Biopsy sampling conditions and non-destructive firmness measurements of each evaluated fruit were performed as described by Hernández et al. (2021).

2.2 Mechanistic model used - estimation of physiological age at fruit level

The estimated E_0 values for each fruit analyzed were obtained from the mechanistic model developed by Hernández et al. (2021). The experimental data were analyzed using the mechanistic model based on ordinary differential equations (ODE), to describe the softening of the "Hass" avocado from different agroclimatic zones and harvests (early and middle). The model presents a generic approach based on batches and another specific based on individual fruit. The physiological basis of the model used is based on a simplified representation of the participation of an enzyme complex (E_0) in autocatalytic processes, including the exponential increase in the activity of E_0 during ripening and on the action of E_0 on firmness retention.

The estimation of the E_0 parameter at the batch and individual fruit level is further detailed in the study by Hernández et al. (2021). Briefly, the enzyme complex (E in arbitrary units) is responsible for the breakdown of firmness (F in N):

$$F + E \xrightarrow{kf} E \quad (1)$$

In a simplified way, the autocatalytic process of the enzyme complex is explained from a limited inactive precursor resource (E_{pre}):

$$E_{pre} + E \xrightarrow{k_e} 2 \cdot E \qquad \text{with rate constant } k_e \text{ (in } d^{-1}\text{)} \qquad (2)$$

Then, to explain the changes in firmness and in the enzyme complex, three ordinary differential equations are elaborated that can be derived from equations (1) and (2).

$$\frac{dF}{dt} = -k_f \cdot E \cdot (F - F_{fix})$$

$$\frac{dE}{dt} = k_e \cdot E \cdot E_{pre}$$

$$\frac{dEpre}{dt} = -k_e \cdot E \cdot E_{pre} \qquad (3)$$

With the following initial at-harvest values (t = 0 d):

$$E(0) = E_0$$

$$F(0) = F_0$$

$$E_{pre}(0) = E_{tot} - E_0 \qquad (4)$$

In these equations it is considered that avocados lose their firmness until edible ripeness around 4 - 8 N (F_{fix} in N). E_{tot} is in arbitrary units. The rate constants k_f and k_e are assumed to be temperature dependent following Arrhenius's law.

2.3. At harvest transcriptomics analysis: RNA extraction and library construction

A total of 36 biopsies were selected from three different physiological age ranges (in terms of E_0). These three ranges corresponded to: 12 samples for the lowest range ($E_0 <$ 5), 12 samples for the intermediate range ($5 < E_0 < 10$) and 12 samples for the highest range ($E_0 > 10$). Total RNA was extracted from 100 mg of frozen tissue using a Spectrum™ Plant Total RNA kit (Sigma-Aldrich, St. Luis, USA) following the manufacturer's instructions and stored at -80 °C. The quantity and purity of RNA were evaluated with a Qubit®2.0

fluorometer (Invitrogen™, Carlsbad, CA, USA) using a Qubit™ RNA BR assay kit. RNA integrity and concentration were assessed by capillary electrophoresis using an automated CE Fragment Analyzer™ system (Agilent Technologies, Santa Clara, CA, USA) with the RNA kit DNF-471-0500 (15nt). The RNA quality number (RQN value) was used to identify the integrity of the RNA. RNA samples with an RQN value beyond 7.0 were used for the following steps. Total RNA-seq libraries were prepared according to the TruSeq Stranded Total RNA Kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions. The concentration of the libraries was determined with a Qubit®2.0 fluorometer (InvitrogenTM, Carlsbad, CA, USA) using a Qubit™ dsDNA BR assay kit and the size and integrity of the library was evaluated with capillary electrophoresis using the Automated CE Fragment Analyzer™ (Agilent Technologies, Santa Clara, CA, USA) with DNF-474-0500 HS NGS Fragment Kit. The constructed libraries were sequenced using Macrogen sequencing services (Seoul, Korea) in paired end mode on a HiSeq4000 sequencer.

2.4. RNA data analysis

For total RNA differential expression analysis, a quality check was first performed on the raw data files with FASTQC software (Andrews, 2010) to assess the most appropriate read quality filtering and clipping. The following criteria were used with Flexbar (Dodt et al., 2012): (1) remove adapter sequences; (2) eliminate reads with a quality score less than 30; and (3) eliminate reads with a length of more than 60 nucleotides. The STAR aligner software (Dobin et al., 2013) was used to align the filtered reads against *Persea americana* var. drymifolia genome v3.0. For each library, the featureCounts software from the Rsubread package (Liao et al., 2019) was applied to assign

186 expression values to each uniquely aligned fragment. Differential gene expression analysis
187 was performed using the Bioconductor R edgeR package (Robinson et al., 2010).
188 Differentially expressed genes (DEG) were selected with a false discovery rate less than
189 0.05 and a log 2-fold change (FC) larger than 1.0 or smaller than -1.0.

190 Differential expression data were subjected to a statistical analysis with a
191 multivariate approach to find potential physiological age biomarkers for the different
192 harvests (early and middle). Before multivariate analysis, gene count data was pre-
193 processed by filtering unexpressed genes, inconsistent genes, and then normalized using the
194 "Variance Stabilizing Transformation (VST)" method. Samples were checked for quality
195 and adjusted to eliminate putative conformity factors from the expression data. The
196 analyzes were carried out in the R software (R Core Team, 2021), for the preprocessing
197 stage the DaMiRseq package was used (Chiesa et al., 2018). Factoextra
198 (Kassambara&Mundt, 2020) was used to perform the PCA and a mix of packages were
199 used for the partial least squares regression (PLS-R) including mixOmics (Rohart et al.,
200 2017), mdatools (Kucheryavskiy, 2020), pls (Mevik et al., 2020), caret (Kuhn, 2020) and
201 tidyverse (Wickham et al., 2019). Candidate genes for PLS-R were selected in *DaMiRseq*
202 R package (Chiesa & Piacentini, 2020). A series of functions enables data cleaning by
203 filtering genomic features and samples, data adjustment by identifying and removing the
204 unwanted source of variation selecting the best predictors for modelling. Gene filtering was
205 done by setting up the minimum number of read counts permitted across samples and by
206 removing hyper variants (i.e., those genes that present anomalous read counts) by
207 comparing to the mean value across the samples and finally filtered by low correlation.

208 Based on the results obtained in the differential expression analysis, the differences
209 were compared at the level of metabolic pathways using the most contrasting E_0 categories

only (high vs low). To search for genetic functions and pathways overrepresented in the DEG lists, genetic enrichment analyzes were performed using the Genetic Ontology (GO) database with AgriGO v2.0 (Du et al., 2010) and the reconstruction of the metabolic pathways was performed using the Kyoto Encyclopedia of Database of Genes and Genomes (KEGG) (Kanehisa et al., 2021).

2.5. Validation of RNA-seq by qRT-PCR analysis

To perform a technical validation of DEG analysis, primers for F-ACP housekeeping and for 6 candidate genes PIN1, TRN1, NAC072, EXPA8, XTH5 and BR6ox1 were designed using the software Primer 3Plus (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/) based on the RNA sequences aligned. First, from the same input used to construct the libraries, total RNA was treated with DNase I (Fermentas, Thermo Fisher Scientific, Waltham, MA, USA) according to the standard protocol. The first strand cDNA was obtained by reverse transcription using the Superscript II RT system (Invitrogen, Carlsbad, CA, USA). The cDNA concentration was obtained by measuring absorbance at 260 nm. Each cDNA sample was diluted to 20 ng μL^{-1} before being used in qRT-PCR assays. The qRT-PCR assays were performed in a AriaMx real-time PCR system (Agilent Technologies, Santa Clara, CA, USA) with KAPPA SYBR® Fast suitable for qPCR (Sigma-aldrich, Saint Louis, MO, USA) to measure the DNA product derived from RNA.

2.6 Hormone analysis at harvest

A total of 18 samples of avocado cv. Hass were analyzed at harvest from the two most contrasting E_0 groups: 9 samples corresponded to the lowest E_0<5 group and the other 9

samples to the highest E_0> 10 group. The quantification of abscisic acid (ABA), jasmonic acid (JA), gibberellins (GA_1), auxin as indole acetic acid (IAA), cytokinins (trans-zeatinor TZ) and salicylic acid (SA) were identified and quantified by UHPLC–ESI–MS/MS following the protocol of Seo et al. (2011). After extraction, samples were analyzed in a Q-Extractive mass spectrometer applying electrospray ionization (ESI) followed by selected ion monitoring. The hormones were quantified based on internal deuterated standards by construction of calibration curves. Each hormone is expressed in ng g^{-1} DW (dry weight).

3. Results and discussion

3.1 At harvest-relevant transcripts and their correlation with physiological age: in the search of early biomarkers of the physiological age of the fruit

The thirty-six samples subjected to transcriptome analysis corresponded to 12 different orchards in three agroclimatic zones (interior, intermediate and coast) and two harvests (early and middle). These samples represent the high biological variability of the typical Chilean "Hass" avocado production (Hernández et al., 2021). Due to the significant number of genes found in the sequencing of the samples (22,929 genes), the samples were processed using an unsupervised approach through principal component analysis (PCA), to reduce the dimensionality of the experimental data. PCAs were carried out considering both harvests together and each harvest independently (Figure 1). When both harvests were analyzed together, no clear separation of the samples could be observed by PCA (Figure 1a). Samples from the early harvest (Figure 1b) presented a more pronounced separation between the three categories of E_0 (low, medium and high) as compared to the samples of middle harvest (Figure 1c). This is evidenced by the larger amount of explained variation

by the first two PCs (38.92 % and 16.57 % respectively). Previous studies have reported early harvest "Hass" avocado to be the most problematic in terms of ripening heterogeneity (Fuentealba et al., 2017; Hernández et al., 2017 and Uarrota et al., 2019). Part of this is related to the fact that commercial maturity (dry matter content ≥ 23 %) is determined based on few fruit only (10-20 fruit). Thus, early harvest fruit within a single batch can display very different physiological/biological age including fruit that might not even have reached physiological maturity, then a sufficient large sample size is crucial to make the correct harvesting decision.

One of the objectives of this work was to identify early transcriptomic biomarkers of physiological age that correlate with the estimated parameter (E_0) provided by the model developed by Hernández et al. (2021) for each individual fruit. The total of genes sequenced (22,929 genes) was used to perform partial least squares regression analysis (PLS-R) as described in the materials and methods section. This model was able to explain with the first two latent variables 30.49 % and 21.04 % of the X variance and 45.37 % and 18.02 % of the Y variance (Figure 2). Model cross-validation (i.e., the ability to select the correct number of components) was done by dividing the data into segments (CV) and the validation results presented by the Root Mean Squared Error of Prediction (RMSEP) with two cross-validation estimates: the ordinary CV estimate, and a bias-corrected CV estimate, obtaining a total accumulated value of $R^2 = 0.634$ and $Q^2 = 0.395$, respectively with the two selected first components. Feature selection analysis revealed 46 candidate genes and partial least squares regression (PLS-R) showed that 25 displayed a positive correlation with E_0 and 21 displayed a negative correlation. Other previous studies (Neilson et al., 2017; García et al., 2018; García et al., 2019) have used the same multivariate approach to

find early biomarkers of specific quality traits that occurs after the storage period. The gene expression or metabolite profiles developed in these investigations were established as predictive biomarkers, so that later the physiological basis behind these biomarkers can be clarified. In these investigations, as in the current study, the number of biomarkers is quite high, which could make the prediction process difficult. To reduce the high number of biomarkers, Neilson et al. (2017) and García et al. (2018) proposed the use of a multiple regression analysis, obtaining a model with a high R^2 (0.92) and with a fine selection of biomarkers. Our approach based on partial least squares regression (PLS-R) and feature selection could be further optimized in subsequent studies in order to reduce even more the number of transcriptomic biomarkers (46 genes) that predict the biological age of the "Hass" avocado.

The partial least squares regression analysis, in addition to revealing an interesting number of potential biomarkers, the variance explained in this analysis was higher than the PLS-R analysis performed by Hernández et al. (2021), which used the GC-MS polar metabolite profile and explained only 8.81 % and 12.82 % of the X variance and 17.35 % and 16.85 % of the Y variance, so our transcriptomic analysis proved to be more robust to search for early-stage biomarkers of the physiological age of "Hass" avocado. In an earlier study by Vergara-Pulgar et al. (2019) using RNA-seq, the authors reported genes related to lipid biosynthesis and the development of "Hass" avocado fruit, as potential candidate biomarkers to monitor fruit development and harvest index, however, these candidates were not validated. The RNA-seq technique creates profiles of the entire transcriptome clearly providing more relevant information as compared to other omics technologies addressing the proteome or the metabolome.

3.2. Sequencing and mapping of the "Hass" avocado transcriptome of different biological ages

To know the dynamics of the "Hass" avocado transcriptome of different biological ages (as reflected by their E_0), RNA libraries for 3 different E_0 ranges (low, medium, and high) were constructed and sequenced using a HiSeq 4000 sequencer (illumine Inc). The sequencing of the 36 samples produced an average of 61,703,640 read (SRA codes https://www.ncbi.nlm.nih.gov/bioproject/PRJNA754775/) for each sample and after performing the quality filtering of each sample, the number of readings remained on average at 99 % (Supplementary Table 1). As mentioned above, the principal component analysis (PCA) showed a better separation of the E_0 categories for early harvest fruit (Figure 1b), confirming that differences in physiological maturity are more problematic for early harvest fruit characterized by a lower dry matter content (≥ 23-26 % DM). Based on these results, only early season fruit was included in the differential expression analysis of the next section.

Early harvest fruit displayed greater heterogeneity in terms of biological age compared to middle harvest as revealed by the principal component analysis (PCA) of Figure 1 that considered the three E_0 categories (low, medium and high) corresponding to E_0 ranges (low, $E_0 < 5$; medium, $5 > E_0 < 10$; high, $E_0 > 10$), also revealed that only marked separated groups were obtained between low and high E_0 samples. Therefore, to biologically interpret the differentially expressed genes, we will now focus on the two most contrasting classes of E_0, the low and high ranges observed for early harvest being fruit that were also the most clearly differentiated in the PCA analysis (Figure 3a), and in this way to be able to estimate the variability of global gene expression between these more extreme categories. This PCA

analysis (Figure 3a) revealed an evident separation between the low E_0 and high E_0 samples and explained 55.01 % and 11.33 % of the total variance with the first two components.

Although the partial least squares regression analysis revealed 46 candidate genes related to the biological age (in terms of E_0) of the "Hass" avocado fruit, these biomarkers are not capable to provide a physiological/biological explanation related to the differences in biological age of the fruit since they were determined considering the three categories and the actual E_0 estimates from the model. Thus, we decided to perform a differential expression analysis between the most contrasting E_0 categories. Results are displayed in Figure 3b based on a Pearson correlation that shows a high similarity between the samples of the same category (low and high E_0 categories) being consistent with the PCA analysis displayed in Figure 3a. In both cases (PCA analysis and correlation matrix), a homogeneity is shown between the samples of each E_0 category, but there are significant changes in the expression of certain genes that compose the transcriptome. A total of 1806 genes with log fold changes (FC) > $\left|1\right|$ (Supplementary Table 1) were differentially expressed between the contrasting E_0 categories (low and high). These genes were used for genetic ontology (GO) analysis and were associated with different GO terms. After this GO analysis, it was possible to observe two groups of labeled GO terms for each E_0 category (low and high) that have a close biological relationship with each category (Figure 4). Subsequently, from the 1806 genes, a selection of 26 genes were further used for the biological interpretation of the differences. These genes were selected for presenting a marked difference in their expression between the E_0 categories (low and high) with log fold changes ranging from -5.5 to 4.3 and an interesting biological functionality (Table 1).

As previously described, to find and select potential early E_0 biomarkers, partial least squares regression analysis (PLS-R) considering the estimated values from the model of the 36 E_0 samples and all genes was performed and after selection only 46 candidate genes remained with a high amount of explained variance. To gain insight into the biological interpretation of the differences between the most contrasting low E_0 and high E_0 categories, differential expression analysis only considering these two categories were used and revealed 1806 differentially expressed genes. Both approaches shared 13 genes (Supplementary Table 3). These 13 common genes displayed lower log FC (-4.04 to 2.33) than the 26 genes selected by differential expression analysis (Table 1). For the biological interpretation contrasting the high and low E_0 categories, only the 26 genes selected by the differential expression analysis were considered.

3.3 Evaluation of expression differences between high vs low E_0 fruit samples *over-expressed metabolic pathways*

Avocado cv. Hass does not ripen on the tree, with fruit within a tree representing very different maturities that only become evident after harvest (Hernández et al., 2016). Our attempt to search for biomarkers of biological age at harvest is challenging since the transition from growth to maturation is quite discrete involving shifts in phytohormones profiles to stop fruit expansion and promote ripening (Forlani et al., 2019; Fenn et al., 2021). The differences in pathways over expressed between the low vs high E_0 samples nicely represent these changes from growth to maturation as can be seen in Figure 5. The low E_0 category, revealed higher expression of genes related to DNA replication (POL2A, FC = -2.2; N.N, FC = -2.6; POLA2, FC = -3), auxin transport (PIN4, FC = -2.4; PIN1, FC = - 2.7; TRN2, FC = -3.7; TRN1, FC = -4.2), cell wall remodeling (EXPA1, FC = -2.2;

EXPA8, FC = -2.3; XTH33, FC = -3.0; XTH5, FC = -4.3; EXPA8, FC = -3.3; N.N, FC = -4.3; N.N, FC-3,7), synthesis of gibberellins (GA3ox4, FC = -5.5), synthesis of brassinosteroids (BR6OX1, FC = -2.3) and flavonols (F3H, FC = -5.4). During fruit growth, the processes of cell division and expansion occur simultaneously (Inzé and Veylder, 2006). For these two processes to take place, the DNA replication process must occur beforehand. Results of this study revealed higher expression of genes related to this process (POL2A, N.N, POLA2) in the low E_0 samples, thus indicative that the low E_0 category samples are still in the stage of full cell division and expansion. Avocado growth is different from the growth of other fleshy fruits since cell division continues over a relatively long development period, decreasing as the fruit reaches maturity (Cowan et al., 2001). The samples belonging to the low E_0 category (E_0<5) in addition presented a higher expression of genes related to metabolic pathways related to fruit growth including cell division and expansion (Figure 5) both regulated by auxin and gibberellins and with input from other hormones including brassinosteroids (Fenn et al., 2021). The BR6OX1 (brassinosteroid-6-oxidase 1) gene showed higher expression in the low E_0 samples compared to the high E_0 samples (FC = -2.3) and participates in the metabolic pathway of brassinosteroid biosynthesis. Brassinosteroids (BR) are plant hormones that participate in cell division and elongation (Hu et al., 2000), presenting a close relationship with the functionality of expansins (EXPA5) (Park et al., 2010). In addition, many of the hormonal functions of auxins have been shown to act in synergy with the functions of brassinosteroids (Park et al., 2010). Our results, next to revealing a higher expression of BR6OX1 in the low E_0 samples, also showed higher expression of genes involved in auxin transport (PIN1, FC = -2.7; TRN2, FC = -3.7; TRN1 (FC = -4.2) (Table 1). Another gene that was observed higher expressed in fruit with low E_0 was related to gibberellin

biosynthesis (GA3ox4, FC = -5.5). The function of gibberellins (GA) is to promote cell elongation and / or division (Xu et al. 2016). According to Chapman et al. (2012) gibberellin biosynthesis is necessary for the normal auxin response; therefore, it could be that IAA acts interdependently with gibberellin pathways to regulate the expression of growth-associated genes during cell expansion. In tomato, during the onset of fruit cell expansion, increased expression of GA3ox and GA20ox has been reported (McAtee et al., 2013; Kumar et al., 2014). Fenn et al. (2021) indicates that GA promotes cell expansion in a synergistic manner with auxins in a large number of fleshy fruits.

It is important to mention that target genes of auxin and GA include cell wall remodeling enzymes including expansins and pectate lyases (McAtee et al., 2013; Kumar et al., 2014; Fenn et al., 2021). Our results showed many genes related to expansins and pectin lyases to be higher expressed in low E_0 samples. For instance, three genes (EXPA1, EXPA8, and EXPA8) related to expansin functionality displayed fold changes of -2.2, -2.3 and -3.3 (higher expressed in low E_0 samples), respectively (Table 1). Expansins are capable of loosening cell walls in a non-enzymatic but pH-dependent manner (Marowa et al., 2016). This relationship is attributed to the ability of auxins to stimulate the synthesis of proton pumps that causes the acidification of the apoplast and therefore promote the activity of expansins (Majda and Robert. 2018). The plasma membrane hyperpolarization process caused by stimulation of H + -ATPase proton pumps has also been reported to be regulated by auxin-inducible SMALL AUXIN UP-RNA (SAUR) proteins (Spartz et al., 2014), this gene was found in our study among the 1088 genes displaying differential expression (Supplementary Table 2). Although the genes found with the highest expression in the low E_0 fruit were related to auxin transport rather than their functionality, these

actions are related to each other. In addition to a higher expression of expansin genes in low E_0 samples, an overexpression of xyloglucan endotransglycosylase (XTH33, FC = -3.3) and XTH5 (FC = -4.3) were also found (Table 1). These are also cell wall remodeling enzymes involved in cell expansion. Pectin lyase genes (FC=-3.7) were also higher expressed in the E_0 samples. Remodeling enzymes such as expansins, endotransglycosylase and pectinases have been reported to participate in phytohormone mediated cell expansion, since fruit growth require loosening of the cell wall matrix for the deposition of new cell wall components (Sánchez-Rodríguez et al., 2010; Cosgrove, 2016).

Genes related to flavonoid biosynthesis (F3H, FC = -2.1 and F3H, FC = -5.4) were also higher expressed in the low E_0 fruit category. Although it has been reported by Xoca-Orozco et al. (2019) that in general the greatest biosynthesis of phenolic compounds such as flavonoids or phenylpropanoids occurs when the avocado fruit reaches physiological maturity. The content of phenolics in the avocado mesocarp varies considerably depending on the levels of abiotic and biotic stress to which this fruit was subjected, regardless its size and maturity (Trujillo-Mayol et al., 2020). According to the study carried out by Figueroa et al. (2018) the main flavanols in avocado are rutin, isorhamnetin, narirutin and quercetin, acting as powerful free radical stabilizers. Campos et al. (2020) has reported that the profile and content of phenolic compounds increases and changes as the fruit transits from maturity to edible ripeness, finding sixteen new phenolic compounds, of which p-coumaric acid, caffeic acid and their derivatives are the most important. Research work carried out by Di Stefano et al. (2017) and Hurtado-Fernández et al. (2011) reported the same trend on the evolution of phenolic compounds in avocado but with some differences in the reported compounds. This may be due to factors, such as genetics (avocado cultivars), climatic

conditions or management conditions of the crop. In the recent research by Hernández et al. (2021) one of the 17 metabolites with the highest correlation to the E_0 parameter was quinic acid, showing a positive correlation. Quinic acid is an intermediate in the shikimic acid pathway (Dewick, 2002), the main pathway in the biosynthesis of flavonoids. The low E_0 category presented higher expression of a gene (F3H), this enzyme is part of the flavonoid biosynthesis pathway (Table 1).

The over-expressed metabolic pathways for the high E_0 category are displayed in Figure 5 and are mostly stress-related genes such as biosynthesis and response to abscisic acid (ZEP, FC = 2.7), ethylene biosynthesis and response (ACS, FC = 4.3 and ERF110, FC = 3.3) and phenylpropanoid biosynthesis (CAD9, FC = 3.3). These results coincide with the higher potential of high E_0 fruit to trigger the different metabolic pathways activated during ripening. The high E_0 category represents mature fruit which has the competence to ripen but has not yet started the ripening process (McAtee et al., 2013; Kumar et al., 2014). Auxin and cytokinins seem to be the key regulators of fruit maturation (McAtee et al., 2013) based on tomato mutant studies displaying the non-ripening phenotype that maintained higher levels of auxins and cytokinins at the breaker stage compared to the wild type fruit (Rolle and Chism, 1989) and on apple where suppression of a rin-like MADs-box gene resulted in high auxin concentrations during maturation and fruit did not ripen (Ireland et al., 2013). Our results only revealed genes related to auxin transport but as indicated in the next section on the targeted hormone analysis, auxin and cytokinin concentrations were higher in high E_0 samples. ABA together with ethylene play a crucial role in inducing ripening (Zhang et al., 2009). Our results showed higher expression of genes related to the biosynthesis (ACS1, FC=4.3) and perception (ERF110, FC=3.3) of ethylene and ABA

462 (ZEP, FC=2.7) in the high E_0 samples. Even though, the role of ethylene and ABA during
463 ripening of climacteric fruit has been extensively reported, studies on the role of both
464 hormones during fruit maturation are still scarce (Kumar et al., 2014).

465 Avocado, being a climacteric fruit, is characterized by presenting an increase in
466 respiration rate and ethylene production at the beginning of ripening (Seymour et al., 1993).
467 Previous studies on differences at harvest displaying different ripening behavior could not
468 associate these differences to endogenous ethylene levels but to other metabolites such as
469 amino acids (Pedreschi et al., 2014; Fuentealba et al., 2017; Uarrota et al., 2019). Up to
470 now, the information on the interaction of hormonal signals and the changes in the primary
471 metabolism during the ripening of “Hass” avocado is quite limited (Pedreschi et al., 2019).
472 Despite this, ethylene has been widely studied as the hormone responsible for the ripening
473 process, but other hormones can act sequentially and / or synergistically with ethylene in
474 controlling fruit ripening. ABA has been shown to interact with ethylene production,
475 improving its production in various climacteric fruits, although the way the interaction
476 occurs currently is not entirely clear (Meyer et al. 2017). In a study carried out on "Granny
477 Smith" apples, it was observed that 1 to 2 months before the commercial harvest begins, a
478 noticeable increase in 1- aminocyclopropane-1-carboxylic acid (ACC) and in parallel the
479 increase in ABA and endogenous ethylene occurs (Lara and Vendrell, 2000). This could
480 indicate that the increase in ACC synthesis may be the point that signals the transition from
481 immature preclimacteric to mature preclimacteric fruit and if endogenous ABA is involved
482 in ACC synthesis it could be used as a maturity marker in "Granny Smith" apples (Lara and
483 Vendrell, 2000). Due to the high heterogeneity reported in “Hass” avocado (Pedreschi et
484 al., 2014; Hernández et al., 2017; Fuentealba et al., 2017) it is very likely that fruit with a

485 high E_0 are in a more advanced stage of preclimaterium maturity and for that reason have a
486 higher content of ABA and ethylene.

487 In addition to the genes related to ABA and ethylene (ZEP, ACS, and ERF110), also
488 genes pertaining to the phenylpropanoids machinery (CAD9, FC = 3.3) were over-
489 expressed in the high E_0 category. These metabolites are being synthesized when the
490 avocado reaches physiological maturity with metabolites such as epicatechin and persin
491 becoming more relevant. These metabolites decrease as ripening progresses in the peel
492 (Xoca-Orozco et al., 2019); however, Campos et al. (2020) have recently reported that as
493 ripening progresses some other phenolics are synthesized in the mesocarp.

494 *3.4 Internal validation of candidate genes as potential early biomarkers of biological age*

495 In order to validate the genes proposed as candidates biomarkers of biological age
496 (as reflected by their E_0) that presented a marked differential expression between the high
497 E_0 and low E_0 categories, an internal validation was also carried out by quantitative real-
498 time PCR (qRT-PCR).

499 Five genes (PIN1, TRN1, EXPA8, XTH5 and BR6OX1) that presented higher
500 expression in the low E_0 category and one gene (NAC072) that presented a higher
501 expression in the high E_0 category were selected. Results were completely consistent with
502 the transcriptomic data. Pearson's correlations ($R > 0.77$) showed that there is a positive
503 correlation between the two methods, validating the transcriptome analysis (Figure 6).

504

505 *3.5At harvest-relevant hormones and their correlation with E_0*

506 Hormone analysis was performed on 18 randomly selected samples that belong to the
507 high and low E_0 categories of biological age. The hormone quantification (expressed on a
508 dry weight basis as ng g^{-1}) of six relevant hormones included an auxin – indole acetic acid
509 (IAA), an active gibberellin (GA_1), abscisic acid (ABA), jasmonic acid (JA), salicylic acid
510 (SA) and the cytokinin *trans*-zeatin (TZ). Selection of these six hormones was based on the
511 results of Vincent et al. (2020) that reported these hormones to be relevant from
512 commercial maturity, during room and/or cold storage and ripening of avocado mesocarp
513 (cv. Bacon).

514 The results were analyzed using a *t*-test with a significance level of $p < 0.05$. Only IAA
515 and TZ were significant between the high E_0 and low E_0 categories, with higher
516 concentrations of both hormones in the high E_0 category (Figure 7). Previous studies have
517 reported that auxin and cytokinins are key regulators of fruit maturation (McAtee et al.,
518 2013). Mature fruit maintains high concentrations of both auxin and cytokinins during fruit
519 maturation, but fruit does not ripen (Ireland et al., 2013). Thus, our results point to high E_0
520 samples having reached maturity and having the potential to ripen.

521 For the other four hormones, no significant differences were observed between the low
522 and high E_0 categories although showing trends of increasing (ABA and SA) and
523 decreasing (JA and GA_1) levels with increasing levels of E_0. Our results at the hormone
524 level agree with the results found at the gene level, where genes related to ethylene
525 biosynthesis and perception and ABA were higher expressed in the high E_0 category. The
526 current work did, to our knowledge for the first time, study the involvement of hormones
527 other than auxin and cytokinins (that remain high during maturation) during maturation of
528 “Hass” avocado fruit. While jasmonic acid has been reported to act antagonistically to ABA

in non-climacteric fruit (Garrido-Bigotes et al., 2018), our results at maturation seem to reveal similar observations of a negative correlation between ABA and JA, with high E_0 samples displaying a trend towards lower concentrations of JA.

Several works on different climacteric fruits have reported the action of ABA and ethylene in relation to fruit ripening (Forlani et al., 2019; Meyer et al., 2017). For avocado fruit ripening, most of the work has focused on ethylene and to a minor extent on ABA (Meyer et al., 2017). Despite the many studies on the ripening of climacteric fruits, the signs of the onset of ripening remain unknown, but the idea that the ripening of the fruit is regulated by the interaction of a set of hormonal factors becomes more documented (Giovannoni et al., 2017).

Auxins and gibberellins promote fruit softening by activating enzymes that promote cell expansion, observing a clear increase in their concentration as the avocado fruit ripens (Majda and Robert, 2018; Guzmán et al., 2021). Vincent et al. (2020) reported increases of auxin and GA_1 content during avocado softening at 25°C, as well as cytokinin accumulation (iP and *trans*-zeatin riboside). Jasmonic and salicylic acid are known to actively participate in the plant immune system (Xoca-Orozco et al., 2019). Additionally, jasmonic acid could be involved in ripening, as its precursor 12-oxo-phytodienoic acid (OPDA) increases during ripening or over-ripening. However, the study of Vincent et al. (2020) did not report a direct correlation with free or conjugated jasmonates in avocado fruit during ripening. Therefore, the role OPDA could play during the ripening process is not very clear. Finally, there are no recent studies clearly indicating the role of cytokinins during avocados ripening. Also, the most recent study of Vincent et al. (2020) only observed a sporadic increase in cytokinins (including isopentenyl adenine (2-iP) and its

552 precursor isopentenyl adenosine (IPA), as well as trans-zeatin riboside (ZR) during
553 ripening). Although more studies are needed to elucidate the behavior of cytokinins during
554 avocado fruit ripening, the current study revealed a significantly higher content of cytokinin
555 (trans-zeatin) in high E_0 fruit compared to the low E_0 fruit which could be an important lead
556 for future research on the activity of this hormone in avocado fruit. It might be relevant to
557 indicate that fruit maturity has been reached and the fruit has developed all the required
558 machinery needed to trigger ripening.

559 **4. Conclusions**

560 Using the RNA-seq technique it was possible to provide robust information in the
561 search for possible biological age markers of "Hass" avocado. Through partial least squares
562 regression analysis (PLS-R), a total of 46 candidate genes were obtained, which showed a
563 significant correlation with biological age (in terms of E_0). This indicates that the
564 transcriptomics platform can provide robust information to find biomarkers of E_0,
565 compared to other omics platforms such as metabolomics or targeted hormone analysis. In
566 addition, it was observed as in previous works, that early harvest batches continue to
567 present greater problems in terms of heterogeneity, as greater differences in biological ages
568 were observed. With respect to the biological age (in terms of E_0) of the samples, a clear
569 difference in the metabolic pathways expressed in samples with high E_0 and low E_0 could
570 be observed. Through differential expression analysis, it was observed that low E_0 fruit
571 showed a higher expression of genes associated with DNA replication together with a
572 higher auxin transport and increased synthesis of gibberellins, cell wall remodeling and
573 flavonols. On the other hand, high E_0 fruit showed a higher expression of ABA, ethylene
574 and phenylpropanoid synthesis. Likewise, targeted hormone analysis revealed higher

concentrations of GA_1 and JA in the low E_0 phenotype and higher but not significant concentrations of ABA and SA in high E_0 and significant for trans-zeatin and IAA in this phenotype. This is the first study in "Hass" avocado to reveal the complex hormonal interplay during maturation, where the transition from fruit growth to fruit maturation is key to assess biological age of the fruit potentially enabling improved postharvest management of the commercial batches.

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References

Adato, I., Gazit, S., 1977. Changes in the initiation of climacteric ethylene in harvested avocado fruits during their development. J. Sci. Food Agric. 28, 240–242.

Andrews, A., 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Campos, D., Teran-Hilares, F., Chirinos, R., Aguilar-Galvez, A., García-Ríos, D., Pacheco-Avalos, A. and Pedreschi, R., 2020. Bioactive compounds and antioxidant activity from harvest to edible ripeness of avocado cv. Hass (*Persea americana*) throughout the harvest seasons. Int J Food Sci Technol. 55: 2208-2218.

595 Cosgrove, D. 2016. Plant cell wall extensibility: connecting plant cell growth with cell wall
596 structure, mechanics, and the action of wall-modifying enzymes. J. Exp. Bot. 67, 463-
597 476.

598 Cowan, A.K., Cripps, R.F., Richings, E.W., Taylor, N.J., 2001. Fruit size: Towards
599 understanding of the metabolic control of fruit growth using avocado as a model
600 system. Physiol. Plant. 111, 127–136

601 Chapman, E.J., Greenham, K., Castillejo, C., Sartor, R., Bialy, A., Sun, T.P., Estelle, M.,
602 2012. Hypocotyl transcriptome reveals auxin regulation of growth-promoting genes
603 through GA-dependent and -independent pathways. PLoSOne 7, e36210.

604 Chiesa, M., Colombo. G.I., Piacentini, L., 2018. "DaMiRseq - an R/Bioconductor package
605 for data mining of RNA-Seq data: normalization, feature selection and classification."
606 Bioinformatics, 34(8), 1416-1418.

607 Dewick, P. M., 2002. Medicinal natural products: a biosynthetic approach: John Wiley &
608 Sons.

609 Di Stefano, V., Avellone, G., Bongiorno, D., Indelicato, S., Massenti, R., Lo Bianco, R.,
610 2017. Quantitative evaluation of the phenolic profile in fruits of six avocado (*Persea*
611 *americana*) cultivars by ultra-high-performance liquid chromatography-heated
612 electrospray-mass spectrometry. Int J Food Prop. 20, 1302-1312.

613 Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P.,
614 Chaisson, M., Gingeras, T.R., 2013. STAR: Ultrafast universal RNA-seq aligner.
615 Bioinformatics. 29: 15-21.

Dodt, M., Roehr, J.T., Ahmed, R., Dieterich, C., 2012. Flexbar – Flexible barcode and adapter processing for next-generation sequencing platforms. Biology. 1: 895-905.

Du, Z., Zhou, X., Ling, Y., Zhang, Z., Su, Z., 2010. agriGO: a GO analysis toolkit for the agricultural community. Nucleic Acids Res. 38: W64-W70.

Fenn, M., Giovannoni, J., 2021. Phytohormones in fruit development and maturation. Plant J. 105, 446-458.

Figueroa, J.G., Borrás-Linares, I., Lozano-Sánchez, J., Segura-Carretero, A., 2018. Comprehensive characterization of phenolic and other polar compounds in the seed and seed coat of avocado by HPLC-DAD-ESI-QTOF-MS. Food Res. Int.105, 752–763.

Forlani, S., Masiero, S., Mizzotti, C., 2019.Fruit ripening: the role of hormones, cell wall modifications, and their relationship with pathogens. J. Exp. Bot. 70:11606, 2993–3006.

Fuentealba, C., Hernández, I., Olaeta, J.A., Defilippi, B., Meneses, C., Campos-Vargas, R., Lurie, S., Carpentier, S., Pedreschi, R., 2017.New insights into the heterogeneous ripening in Hass avocado via LC MSMS proteomics. Postharvest Biol. Technol. 132, 51-61.

García, C.J., Gil, M.I., Tomas-Barberan, F.A., 2018. LC–MS untargeted metabolomics reveals early biomarkers to predict browning of fresh-cut lettuce. Postharvest Biol. Technol. 146, 9–17.

636 García, C.J., Gil, M.I., Tomas-Barberan, F.A., 2019. Targeted Metabolomics Analysis and
637 Identification of Biomarkers for Predicting Browning of Fresh-Cut Lettuce. J.
638 Agric. Food Chem. 67, 5908−5917.
639 Garrido-Bigotes, A., Figueroa, C.R. and Figueroa, P.M., 2018. Jasmonate metabolism and
640 its relationship with abscisic acid during strawberry fruit development and
641 ripening. J. Plant Growth Regul. 37, 101–113.
642 Giovannoni, J., Nguyen, C., Ampofo, B., Zhong, S., Fei, Z., 2017. The Epigenome and
643 Transcriptional Dynamics of Fruit Ripening. Annu Rev Plant Biol. 28, 68: 61-84.
644 Guzmán, Y., Pugliese, B., González, C.V., Travaglia, C., Bottini, R., Berli, F., 2021. Spray
645 with plant growth regulators at full bloom may improve quality for storage of
646 'Superior Seedless' table grapes by modifying the vascular system of the bunch.
647 Postharvest Biol. Technol. 176, 111522.
648 Gwanpua, S. G., Qian, Z., East, A., 2018. Modelling ethylene regulated changes in ‘Hass’
649 avocado quality. Postharvest Biol. Technol. 136, 12–22.
650 Hernández, I., Fuentealba, C., Olaeta, J.A., Poblete, C., Defilippi, B., González, M.,
651 Campos-Vargas, R., Lurie, S., Pedreschi, R., 2017. Postharvest abiotic stresses and
652 ripening synchronization of Hass avocados. Sci. Hort. 225, 408–415.
653 Hernández, I., Uarrota, V., Paredes, D., Fuentealba, C., Defilippi. B.G., Campos-Vargas,
654 R., Meneses, C., Hertog, M.L.A.T.M., Pedreschi, P., 2021. Can metabolites at harvest
655 be used as physiological markers for modelling the softening behaviour of Chilean
656 “Hass” avocados destined to local and distant markets?. Postharvest Biol. Technol.
657 174, 111457.

Hu, Y., Bao, F., Li, J., 2000. Promotive effect of brassinosteroids on cell division involves a distinct CycD3-induction pathway in Arabidopsis. Plant J. 24, 693–701.

Hurtado-Fernández, E., Carrasco-Pancorbo, A., Fernández-Gutiérrez, A., 2011.Profiling LC-DAD-ESI-TOF MS Method for the Determination of Phenolic Metabolites from Avocado (*Persea americana*). J. Agric. Food Chem. 59, 6, 2255–2267.

Inzé, D., Veylder, L.D., 2006. Cell Cycle Regulation in Plant Development. Annu. Rev. Genet. 40, 77 – 105.

Ireland, H. S., Yao, J. L., Tomes, S., Sutherland, P. W., Nieuwen-huizen, N., Gunaseelan, K., 2013. Apple SEPALLATA1/2-like genes control fruit flesh development and ripening. Plant J. 73, 1044–1056.

Jeong, J., Huber, D.J., 2004. Suppression of avocado (*Persea americana* Mill.) fruit softening and changes in cell wall matrix polysaccharides and enzyme activities: differential responses to 1-MCP and delayed ethylene application. J. Am. Soc. Hortic. Sci. 129, 752–759.

Kassambara, A., Mundt, F., 2020. factoextra: Extract and Visualize the Results of Multivariate Data Analyses. R package version 1.0.7.

Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., and Tanabe, M., 2021. KEGG: integrating viruses and cellular organisms. Nucleic Acids Res. 49, D545-D551.

Kucheryavskiy, S., 2020. “mdatools - R package for chemometrics”._Chemometrics and Intelligent Laboratory Systems_, *198*.

679 Kuhn, M., 2020. Caret: Classification and Regression Training. R package version 6.0-86.

680 Kumar, R., Khurana, A., Sharma, A.K., 2014. Role of plant hormones and their interplay in
681 development and ripening of fleshy fruit. J. Exp. Bot. 65, 4561–4575

682 Lara, I., and Vendrell, M., 2000. Development of Ethylene-synthesizing Capacity in
683 Preclimacteric Apples: Interaction between Abscisic Acid and Ethylene. Soc. Hort.
684 Sci. 125 (4): 505–512.

685 Liao, Y., Smyth, G.K., Shi, W., 2019. The R package Rsubread is easier, faster, cheaper
686 and better for alignment and quantification of RNA sequencing reads. Nucleic Acids
687 Res. 47: e47.

688 McAtee, P., Karim, S., Schaffer, R., David, K., 2013. A dynamic interplay between
689 phytohormones is required for fruit development, maturation and ripening. Front.
690 Plant Sci.4, article 79.

691 Majda, M., Robert, S., 2018.The role of auxin in cell wall expansion. Int. J. Mol. Sci. 19,
692 951.

693 Marowa, P., Ding, A., & Kong, Y., 2016. Expansins: roles in plant growth and potential
694 applications in crop improvement. Plant Cell Rep. 35(5), 949–965.

695 Mevik, B.H., Wehrens, R., HovdeLiland, K., 2020. pls: Partial Least Squares and Principal
696 Component Regression. R package version 2.7-3.

697 Meyer, M.J., Chope, G.A., Terry, L.A., 2017. Investigation into the role of endogenous
698 abscisic acid during ripening of imported avocado cv. Hass. J. Sci. Food Agric. 97,
699 3656–3664.

Neilson, J., Lagüe, M., Thomson, S., Aurousseau, F., Murphy, A. M., Bizimungu, B., Deveaux, V., Bègue, Y., Jacobs, J., & Tai, H. H., 2017. Gene expression profiles predictive of cold-induced sweetening in potato. Funct. Integr. Genomics, 17(4), 459–476.

Oficina de Estudios y Política Agrarias (ODEPA)., 2021. Catastro Frutícola Cirén – Odepa.https://www.odepa.gob.cl/estadisticas-del-sector/catastros-fruticolas/catastro-fruticola-ciren-odepa. Visited march 17, 2021.

Ochoa-Ascencioa, S., Hertog, M.L.A.T.M., Nicolaï, B.M., 2009. Modelling the transient effect of 1-MCP on 'Hass' avocado softening: A Mexican comparative study. Postharvest Biol. Technol. 51, 62–72.

Park, C. H., Kim, T. W., Son, S. H., Hwang, J. Y., Lee, S. C., Chang, S. C., Kim, S. H., Kim, S. W., & Kim, S. K., 2010. Brassinosteroids control AtEXPA5 gene expression in Arabidopsis thaliana. Phytochemistry. 71(4), 380–387.

Pedreschi, R., Muñoz, P., Robledo, P., Becerra, C., Defilippi, B., van Eekelen, H., Mumm, R., Westra, E., de Vos, R., 2014. Metabolomisc analysis of postharvest ripening heterogeneity of Hass avocados. Postharvest Biol. Technol. 92, 172–179.

Pedreschi, R., Uarrota, V., Fuentealba, C., Alvaro, J., Olmedo, P., Defilippi, B., Meneses, C., Campos-Vargas, R., 2019. Primary metabolism in avocado fruit. Front. Plant Sci. 10, article 795.

R Core Team, 2021. R: a Language and Environment for Statistical Computing. RFoundation for Statistical Computing, Vienna, Austria. URL.

721 Rendón-Anaya, M., Ibarra-Laclette, E., Méndez Bravo, A., Lan, T., Zheng, C., Carretero-
722 Paulet, L., Perez-Torres, C.A., Chacón-López, A., Hernandez-Guzmán, G., Chang,
723 T.H., Farr, K.M., Barbazuk, W.B., Chamala, S., Mutwil, M., Shivhare, D., Alvarez-
724 Ponce, D., Mitter, N., Hayward, A., Fletcher, S., Rozas, J., Sánchez-Gracia, A.,
725 Kuhn, D., Barrientos-Priego A.F., Salojärvi, J., Librado, P., Sankoff, D., Herrera-
726 Estrella, A., Albert, V.A., Herrera-Estrella, L., 2019. The avocado genome informs
727 deep angiosperm phylogeny, highlights introgressive hybridization, and reveals
728 pathogen-influenced gene space adaptation. Proc. Natl. Acad. Sci. 34, 17081–
729 17089.

730 Rivera, S. A., Ferreyra, R., Robledo, P., Selles, G., Arpaia, M.L., Saavedra, J., Defilippi,
731 B.G., 2017. Identification of preharvest factors determining postharvest ripening
732 behaviors in 'Hass' avocado under long term storage. Sci. Hort. 216, 29–37.

733 Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a bioconductor package for
734 differential expression analysis of digital gene expression data. Bioinformatics. 26:
735 139-140.

736 Rohart, F., Gautier, B, Singh, A., Lê Cao, K. A., 2017. mixOmics: an R package for 'omics
737 feature selection and multiple data integration.

738 Rolle, R. S., and Chism, G. W., 1989. Kinetic comparison of cytokinin nucleosidase
739 activity isolated from normally ripening and mutant tomato varieties. Plant Physiol.
740 91, 148–150.

741 Sánchez-Rodríguez, C; Rubio-Somoza, I; Sobout, R; Persson, S., 2010. Phytohormones and
742 the cell wall in Arabidopsis during seedling growth. Trends Plant Sci. 15, 291-301.

743 Seo, M., Jikumaru, Y., Kamiya, Y., 2011. Profiling of hormones and related metabolites in
744 seed dormancy and germination studies. In: Allison R. Kermode (ed.), Seed
745 Dormancy: Methods and Protocols, Methods Mol. Biol. 773, 99-111.

746 Seymour, G. B., Taylor, J. E., and Tucker, G. A. (eds.)., 1993. Biochemistry of Fruit
747 Ripening. (Netherlands: Springer), 454.

748 Spartz, A.K., Ren, H., Park, M.Y., Grandt, K.N., Lee, S.H., Murphy, A.S., Sussman, M.R.,
749 Overvoorde, P.J., Gray, W.M., 2014. SAUR Inhibition of PP2C-D Phosphatases
750 Activates Plasma Membrane H+-ATPases to Promote Cell Expansion in
751 Arabidopsis. Plant Cell. 2014: 26, 2129–2142.

752 Trujillo-Mayol. I., Badillo-Muñoz, G., Céspedes-Acuña, C., Alarcón-Enos, J., 2020. The
753 Relationship between Fruit Size and Phenolic and Enzymatic Composition of
754 Avocado Byproducts (*Persea americana* Mill.): The Importance for Biorefinery
755 Applications. Horticulturae. 6, 91.

756 Uarrota, V.G., Fuentealba, C., Hernández, I., Defilippi, B.G., Meneses, C., Campos-Vargas,
757 R., Lurie, S., Carpentier, S., Poblete-Echeverría, C., Pedreschi, R., 2019. Integration
758 of proteomics and metabolomics data of early and middle season Hass avocados
759 under heat treatment. Food Chem. 289, 512–521.

760 Vergara-Pulgar, C., Rothkegel1, K., González-Agüero, M., Pedreschi, R., Campos-Vargas,
761 R., Defilippi, B., Meneses, C., 2019. De novo assembly of *Persea americana* cv.
762 'Hass' transcriptome during fruit development. BMC Genom. 20:108.

763 Vincent, C., Mesa, T., Munné-Bosch, S., 2020. Hormonal interplay in the regulation of fruit
764 ripening and cold acclimation in avocados. J. Plant. Physiol.251, 153225.

765 Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R.,
766 Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller,
767 E., Bache, S.M., Müller, K., Ooms, J., Robinson, D., Seidel, D.P., Spinu, V.,
768 Takahashi, K., Vaughan, D., Wilke, C., Woo, K., Yutani, H., 2019. Welcome to the
769 tidyverse. J. Open Res. Softw. 4 (43), 1686.

770 Xoca-Orozco, L.A., Aguilera-Aguirre, S., Vega-Arreguí, J., Acevedo-Hernández, G.,
771 Tovar- Pérez, E., Stoll, A., Herrera-Estrella, L., Chacón-López, A., 2019. Activation
772 of the phenylpropanoid biosynthesis pathway reveals a novel action mechanism of
773 the elicitor effect of chitosan on avocado fruit epicarp. Food. Res. Int. 121, 586 –
774 592.

775 Xu, Q., Krishnan, S., Merewitz, E., Xu, J., Huang, B., 2016. Gibberellin regulation and
776 genetic variations in leaf elongation for tall fescue in association with differential
777 gene expression controlling cell expansion. Sci Rep 6:30258.

778 Zhang, M., Yuan, B., and Leng, P., 2009. The role of ABA in triggering ethylene
779 biosynthesis and ripening of tomato fruit. J. Exp. Bot. 60, 1579–1588.

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