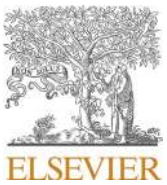
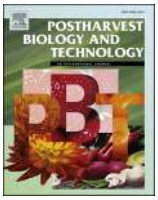
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Ripening-related gene expression analysis revealed the molecular impact of 1-MCP application on apricot fruit softening, color, aroma, and antioxidant capacity

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ABSTRACT

Apricot (*Prunus armeniaca* L.) is globally the third most cultivated stone fruit, prized for its flavourful and nutritious fruit. However, its climacteric nature impacts postharvest behaviour, shortening shelf-life. While the role and interaction of key genes and proteins involved in the ripening process of this species have been extensively studied over the years, research on ethylene suppressors like 1-methylcyclopropene (1-MCP) and cold storage is still limited. Fruit samples from 10 genotypes were collected at commercial maturity from the CEBAS-CSIC research centre and divided into three groups: control (T0), one application of 1-MCP at harvest (T1), and two applications of 1-MCP at harvest and after seven days of cold storage (T2). The study aimed to assess the effects of these treatments on fruit quality traits, such as skin color, soluble solids, acidity, firmness, and ethylene production, after various cold storage periods (10, 20, and 30 days) at 1°C, followed by 48 hours at 20°C. Cold storage combined with 1-MCP effectively reduced fruit softening and ethylene production, with varying impacts on fruit quality traits depending on the genotype. '906–12' and 'Goldrich' were identified as the most contrasting genotypes in terms of ethylene production and fruit softening. Additionally, the application of 1-MCP reduced the expression of genes responsible for fruit softening, coloration and some organoleptic characteristics while enhancing those promoting antioxidant activity during ripening. This study sheds light on the molecular processes involved in apricot fruit ripening and highlights the potential of this approach for enhancing postharvest preservation.

1. Introduction

Stone fruit trees have served as a vital source of food, providing fiber, vitamins, antioxidants, and nutritional compounds, thus contributing to a balanced diet, and offering various health benefits over the centuries (Bento et al., 2022). Through classical genetic breeding and modern molecular biology technologies, or a combination of both, new cultivars have emerged, tailored to meet the demands of the current market in terms of fruit quality and consumer preference (Bassi and Audergon, 2006; Ruiz et al., 2012). Simultaneously, with the globalized market and the increasing demand for fruits worldwide, there has been a growing necessity to extend the shelf-life of fruits for an extended commercial window. However, we must bear in mind that the genetic background of the different fruit species means that many of these species have little scope for improvement in terms of postharvest behavior, characterized by a shelf-life of just for a few weeks.

This is particularly evident in the climacteric species, which are known for their ethylene production, allowing fruits to continue ripening even after harvest. Notably, fruits such as apricots have one of the shortest shelf-life extensions, lasting only 1–4 weeks when stored at temperatures between –0.5°C to 1°C and at 90 ± 5 % relative humidity (Fan et al., 2000). Hence, the current challenge lies in discovering new apricot cultivars that facilitate an extension of the fruit's shelf-life. Achieving this goal necessitates a comprehensive exploration of the physiological processes occurring during postharvest process (Chen et al., 2018). This challenge has been addressed from different perspectives at the genomic, transcriptomic, proteomic and metabolomic levels. At genomic level, (Salazar et al., 2020) identified several QTLs linked to fruit softening in the LGs 4 and 5 in related species such as Japanese plum. Likewise, other authors (Carrasco-Valenzuela et al.,

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2019) conducted similar studies in peach. Furthermore, they successfully confirmed expression QTLs (eQTLs) linked to candidate genes associated with fruit flesh softening rate in peach. At transcriptomic level several works have delved to discern the main expression genes linked to the fruit maturation in apricot after ethylene-related treatments (Fan et al., 2018; Salazar et al., 2022a,2022b). On the other side in an apricot monitoring from green to orange vearison *CHS* was considered as the main predictor for anthocyanins content, *CCD4* and *ZDS* for carotenoids content, and *LOX2* and *MADS-box* linked to the beginning and end of the ripening process (García-Gómez et al., 2022). As for proteomics, over one hundred proteins were identified as the main enzymes involved in biochemical processes during fruit maturation, such as ethylene biosynthesis and cell wall restructuring (D'Ambrosio et al., 2013; Jiang et al., 2014).

Therefore, considering the ongoing investigations, it is crucial to delve into the influence of ethylene-related phytohormones (Payasi and Sanwal, 2010), placing particular emphasis on understanding the mechanisms that regulate fruit degradation. Such investigations are instrumental in gaining a deeper comprehension of the intricate processes underlying fruit storage.

It is important to note that among fruit conservation techniques, the most common method to extend the shelf-life of any fruit species is through cold storage preservation (Infante et al., 2008). On the other hand, in recent years, various chemical treatments have been employed, either in combination with cold storage or independently (Ghasemnezhad et al., 2010; Martínez-Romero et al., 2002; Hayama et al., 2008; Moradinezhad and Jahani, 2016; Arnao and Hernández-Ruiz, 2020; Verde et al., 2022). 1-MCP is a cycloalkene, with the molecular formula C_4H_6, capable of inhibiting ethylene action. Being a structural analogue, it has the property of binding to the receptor, thus preventing ethylene from binding to it. In this way, 1-MCP inhibits fruit ripening (Candan et al., 2011). These treatments serve not only to determine the most effective combination for extending shelf-life but also to elucidate the intricate molecular-level mechanisms involved in this complex physiological process (Stanley et al., 2013).

With this background, further studies are necessary to explore the application of products such as 1-MCP in new collections of cultivars within apricot genetic breeding programs, using combined treatments of cold and 1-MCP. These studies serve two main objectives: (1) to obtain valuable information on cultivars that respond best to the 1-MCP treatments, and (2) to evaluate the expression of genes highly expressed during the ripening process and analyze their response to the combination of 1-MCP and cold storage in apricot fruits.

2. Material and methods

2.1. Plant material

Apricot fruits were harvested from the experimental orchard of the CEBAS-CSIC research centre located in Cieza-Calasparra (Murcia, southeastern Spain, 37° N latitude, 1° W longitude, and 450 m altitude). The assay was conducted on a total of ten genotypes, including the cultivars 'Cebas Red', 'Primorosa', 'Mirlo Naranja', 'Valorange', 'Micaelo', 'Capricho', 'Deseo', as well as the accession '906–12' from the apricot breeding program at the Plant Breeding Department of the CEBAS-CSIC centre (Table 1). Furthermore, 'Goldrich' and 'Moniquí' were assessed as reference cultivars based on their postharvest behavior. 'Goldrich' was considered to have good postharvest performance, in contrast to the Spanish traditional cultivar 'Moniquí', known for its rapid fruit softening (Marty et al., 2005). All genotypes were grafted onto 'Real Fino' rootstock.

2.2. Experimental design and postharvest evaluation

Apricot cultivars and accessions were harvested at two stages of maturation: (1) optimum eating ripeness (consumption maturity) and (2) before reaching full maturity for consumption (commercial maturity) for subsequent treatment and evaluation (Table 1, Fig. 1). Harvest timing at optimum eating ripeness was established based on skin color and firmness (Salazar et al., 2013; Salazar et al., 2022), where the fruits displayed complete skin color coverage and achieved a fruit firmness below 30 N. Commercial maturity was determined when the fruits had also developed their skin color and exhibited a fruit firmness close to or exceeding 40 N (Contador et al., 2016), aiming to prolong the shelf-life period. Sampling was conducted randomly from various sides of the tree, selecting the most representative tree. A total of 200 fruits were collected per genotype. Subsequently, the fruits were transported to the CEBAS-CSIC laboratories for further analysis. Out of the collected fruits, 150 uniform ones were selected and divided into three sets (control, T0; one application of 1-MCP, T1; two applications of 1-MCP, T2). Initially, all fruit sets were stored at 1°C for the first 24 hours. During this period, the second and third fruit sets underwent a 1-MCP treatment. The third fruit set received an additional 1-MCP application after 7 days of cold storage. The application of 1-methylcyclopropene (1-MCP, SmartFresh™) was conducted in a 400 L sealed chamber, utilizing a 1-MCP tablet submerged in a liquid activator provided by the AgroFresh Inc. (Barcelona, Spain), emitting 1-MCP gas at 625 ppb.

Throughout the storage period, the fruit were evaluated after ten-day intervals at cold storage of 1°C and two-day of shelf-life period at 20°C (on days 10, 20, and 30 at 1°C + 2 days at 20°C). Various fruit quality attributes, including fruit weight, red blush coverage, red blush color, skin color, flesh color, soluble solid content, and titratable acidity, were measured. Concurrently, physiological traits associated with shelf-life, such as fruit firmness and ethylene production, were determined. Fruit weight was measured in grams using a Blauscal digital balance (model AH-600). Red blush coverage was visually assessed on a scale ranging from 0 to 4 (representing 0–100 % of skin coverage). Red blush, skin, and flesh color were analyzed with a Minolta Chroma Meter colorimeter (CR-300; Minolta, Ramsey, NJ, USA) using the CIELAB scale, including L*, a*, and b* values, as well as the Hue angle [hº=arctangent (b*/a*)] (Brown and Walker, 1990). Soluble solids content was determined using a handheld digital refractometer (ATAGO®). Titratable acidity (g.

Table 1
Apricot cultivars studied including the pedigree, origin and main agronomic characteristics (self-compatibility, sharka resistance, flowering and ripening time and skin colour).

Cultivar	Pedigree	Self-compatibility	Sharka resistance (PPV-D)	Flowering	Ripening	Skin colour
'Capricho'	Moniquí × Pepito del Rubio	Self-compatible	No	Medium	Medium	Light Yellow/Pink
'Cebas Red'	1100–5–26 × Colorao	Self-compatible	Yes	Early	Very Early	Orange/Red
'Deseo'	Orange Red × Tardif de Bordaneil	Self-compatible	No	Late	Medium-Late	Orange/Red
'Goldrich'	Sunglo × Perfection	Self-incompatible	Yes	Late	Late	Orange
'Micaelo'	Orange Red × Tardif de Bordaneil	Self-compatible	Yes	Medium	Medium	Orange/Red
'Mirlo Naranja'	Rojo Pasión × Búlida Precoz	Self-compatible	Yes	Early	Early	Light Orange/Red
'Moniquí'	Unknown	Self-incompatible	No	Medium	Medium	Light Yellow/Pink
'Primorosa'	Rojo Pasión × Búlida Precoz	Self-compatible	Yes	Early	Very Early	Light Orange/Red
'Valorange'	Orange Red × Currot	Self-compatible	No	Medium	Medium	Orange/Red
'906–12'	Orange Red × Z211–18	Self-compatible	Yes	Medium	Medium	Orange/Red

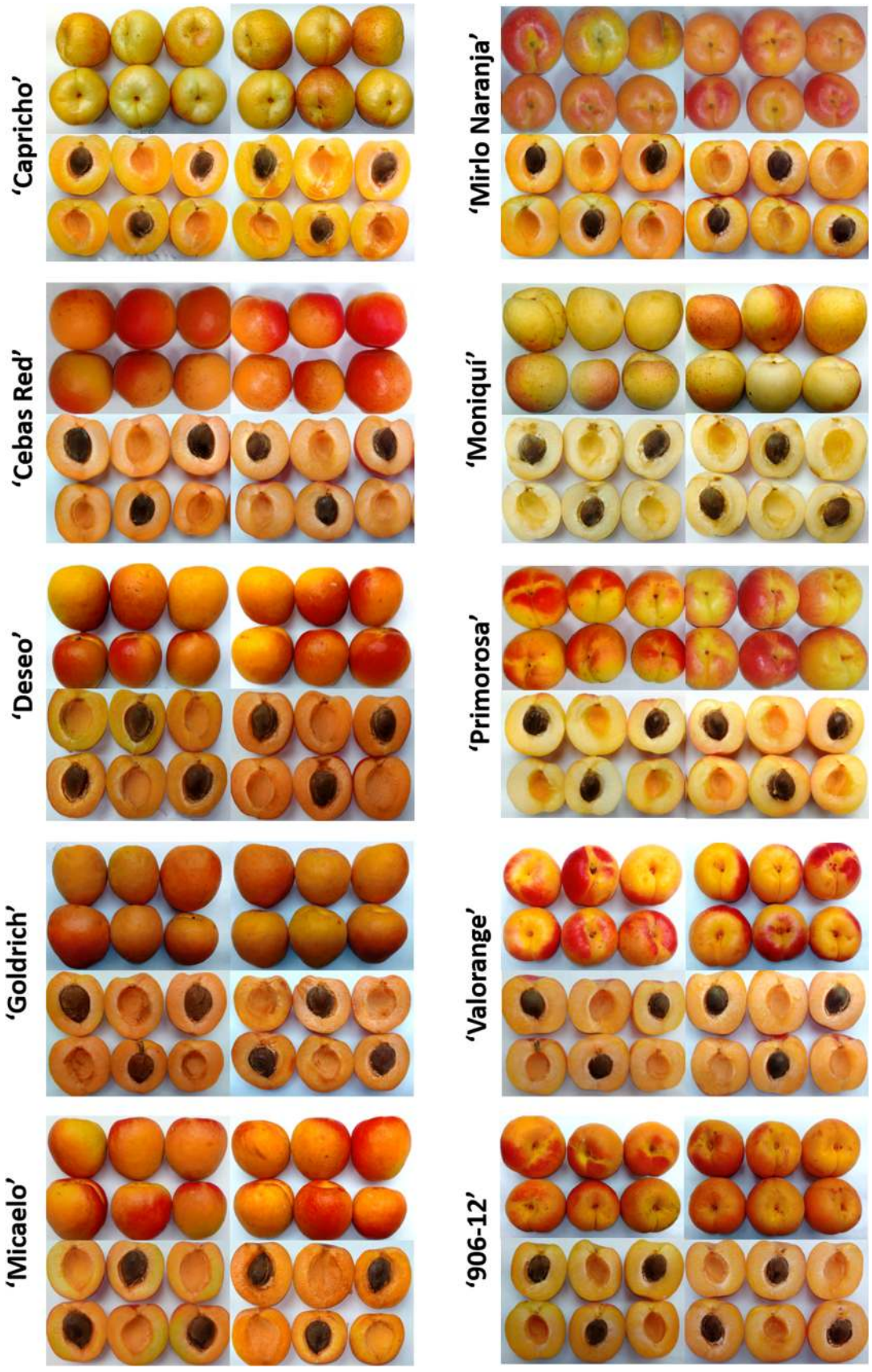


Fig. 1. Details of the sample fruits collected at commercial (left) and consumption (right) maturity.

malic/100 mL) was measured by acid-base titration using a Metrohm® titrator coupled to a carousel, involving 2 g of the sample diluted in 30 mL of distilled water. Fruit firmness (N) was assessed by applying a 5 mm compression using a 75 mm cylinder (TA.XT plus, Texture Technologies, Hamilton, MA, USA). Ethylene production (µl kg-1 h-1) was quantified using gas chromatography (ethylene chromatograph Konik®), with each fruit individually enclosed in 500 mL jars for 2 hours, followed by injecting 1 mL of the gaseous sample into the chromatograph. The experimental unit comprised ten fruits per cultivar, storage day, and treatment, except for ethylene production, which utilized 5 fruits as biological replicates. ANOVA analysis and Tukey's multiple comparison test (p-value ≤ 0.05) were conducted to identify significant differences between treatments and storage periods. These statistical analyses were performed using INFOSTAT v18 software (Universidad Nacional de Córdoba, Argentina)

2.3. RNA isolation and purification and RT-qPCR analysis

Samples of fruit from each genotype, storage day, and treatment were frozen at −80°C for subsequent RNA extractions. Subsequently, the two most contrasting cultivars were selected for gene monitoring, and a total of 9 fruit samples were used for each genotype, considering three biological replicates per treatment (T0, T1, and T2) and day (day1 at 1°C+2 days at 20°C, day 10 at 1°C+2 days at 20°C, and day 20 at 1°C+2 days at 20°C).

Initially, approximately 200 mg of each sample was ground using liquid nitrogen and a ceramic pestle. Total RNA was then extracted following the protocol outlined by (Le Provost et al., 2007), utilizing CTAB buffer at 2 %, Chloroform-isoamyl-alcohol 24:1, and LiCl 10 M. Following the RNA extractions, the samples were purified using the RNeasy® Power Plant Kit, according to the manufacturer's instructions. The quantity and quality of RNA were determined using a spectrophotometer and agarose gel electrophoresis to select the highest quality samples for RT-qPCR.

RT-qPCR for gene monitoring was carried out considering three biological and two technical replicates. The oligonucleotide primers of 10 postharvest-related genes were previously designed in *Prunus species* (Salazar et al., 2022b). The raw sequence reads archive (SRA) is accessible in the NCBI database under the bioproject number PRJNA752255. The assessed genes include key pathways closely associated with fruit ripening: *Pectin methylesterase* [EC:3.1.1.11] and *Polygalacturonase* [EC:3.2.1.15] (Pentose and glucuronate interconversions); *Auxin-responsive protein*, *IAA* (Plant hormone signal transduction); *Beta-carotene 3-hydroxylase*, *CRTZ* (Carotenoid biosynthesis); *GDP-L-galactose phosphorylase*, *VTC2* (Ascorbate and aldarate metabolism); *3-hydroxyisobutyryl-coa hydrolase, HIBCH* (Carbon metabolism); *Sucrose synthase, SUS* (Starch and sucrose metabolism); *1-aminocyclopropane-1-carboxylate synthase*, *ACS* (Cysteine and methionine metabolism); *Myo-inositol-1-phosphate synthase*, *INO1* (Inositol phosphate metabolism); *Ferredoxin*, *PETF* (Photosynthesis) (Table S3). Several housekeeping reference genes were examined, with RNA polymerase II (RPII) and Ubiquitin 10 (UBQ10) serving as internal controls in both species (Tong et al., 2009). The cDNA was synthesized using SuperScript III Reverse Transcriptase (Thermo Fisher Scientific), and RT-qPCR experiments were performed using the One Step Plus real-time PCR system (Applied Biosystems). Each RT-qPCR reaction consisted of a 10 µL mixture, comprising 5 µL of Power SYBR® Green PCR Master Mix (Applied Biosystems), 0.5 µL of each primer (5 µM), and 2 µL of cDNA (2.5 ng/µL). Amplification conditions included an initial step of 10 minutes at 95 °C, followed by 40 cycles of 15 seconds (sec) at 95 °C and 1 minute at 60 °C. The melt curve analysis involved 15 sec at 95 °C, 1 min at 60 °C, and a gradual increase of 0.3 °C until 95 °C for 0.15 sec. Normalized Relative Quantification (NRQ) for gene expression levels was determined using the modified Pfaffl method (Pfaffl, 2001; Vandesompele et al., 2002), which relied on the comparison of the expression levels of the target gene with those of a housekeeping gene.

3. Results

3.1. Evaluation of the pomological traits in apricot fruits after 1-MCP treatments

The obtained results demonstrated substantial variability in fruit quality traits, indicating significant differences among genotypes for all traits. However, attributes such as red blush coverage, red blush color, and soluble solids content did not exhibit differences per storage day, while malic acid did not display significant differences per treatment (Table S1 and S2). Considering the results presented in Fig. 2, it becomes apparent that all the cultivars were consistently harvested at a uniform size for each day and treatment. Notably, 'Goldrich' stood out as the largest, weighing over 100 g, while 'Deseo' averaged around 40 g, making it the smallest. In terms of red blush coverage, although this trait depends on the tree's location, it is worth mentioning that 'Micaelo,' 'Cebas Red,' and 'Primorosa' achieved an average coverage of approximately 40 %, particularly in the consumption timing. In contrast, 'Goldrich' reached a maximum coverage of approximately 25 % on the last day of cold storage. Regarding the red blush color, 'Capricho' and 'Moniquí' recorded the highest h° value of approximately 75, corresponding to a lighter red blush, in contrast to 'Deseo' and 'Micaelo' during the last days, and 'Cebas Red' at the time of consumption maturity, which measured around 40 %, indicating a more intense red blush (Fig. 2).

Concerning skin color, 'Capricho' and 'Moniquí' displayed the highest h° value, exceeding 90, which corresponds to a light-yellow skin color at commercial maturity. Conversely, 'Cebas Red' and 'Micaelo', consistently exhibited values below 70, suggesting an intense orange skin color at any period and treatment excluding commercial maturity (Fig. 2). With regards to flesh color, consistent results were observed across all cultivars, with the majority falling within the 70–80 range, indicating an orange color, except for 'Capricho' and 'Primorosa,' which registered h° values between 80 and 90 (orange to yellow), and 'Moniquí,' which recorded values above 90 (light yellow). Furthermore, a noticeable trend was observed in the case of 'Cebas Red', 'Deseo', 'Micaelo', 'Mirlo Naranja', and 'Primorosa' (Fig. S1ab) with a visible decrease in skin color over time, gaining more orange tones. In terms of soluble solid content, the highest values were generally observed at consumption maturity, with 'Moniquí' and 'Deseo' recording the highest levels at approximately 16°Brix (Fig. 2). Conversely, the earlier cultivars, 'Cebas Red,' 'Primorosa,' and 'Mirlo Naranja,' ranged between 10 and 12°Brix. In terms of acidity levels, malic acid exhibited a decreasing trend over time (Fig. S2ab), with the highest value recorded at commercial maturity (Fig. 2). 'Goldrich' stood out as the most acidic cultivar, reaching nearly 3 g/100 mL, while 'Valorange' and 'Mirlo Naranja' recorded around 1 g/100 mL. With regard to the SSC-acidity balance, 'Valorange' and 'Capricho' achieved the highest values at approximately 20, while 'Goldrich' obtained the lowest value at around 5 (Fig. 2). Regarding fruit firmness, to ensure a longer shelf-life, all cultivars were harvested above a firmness of 40 N or close to this value which would correspond to commercial ripening. It should be noted that the main criterion in the sampling was color change, and therefore in this sense, 'Cebas Red' stands out as one of the cultivars obtaining the highest values, indicating its high firmness at harvest even though it is among the cultivars with the most pronounced orange tones (Fig. 3). Therefore, considering the most relevant traits linked to the fruit's shelf-life, significant differences among genotypes, days, and treatments (p-value <0.05) were observed for the firmness (N) and ethylene production. Subsequently, we analyzed the results obtained for each storage day and between treatments using the Tukey's homogeneous groups test, where we are able specifically to see where significant differences occurred (Fig. 3).

Regarding fruit firmness, all cultivars and accessions experienced a significant softening between the harvest day and the initial cold storage, particularly within the first 10 days. Overall, in spite of differences

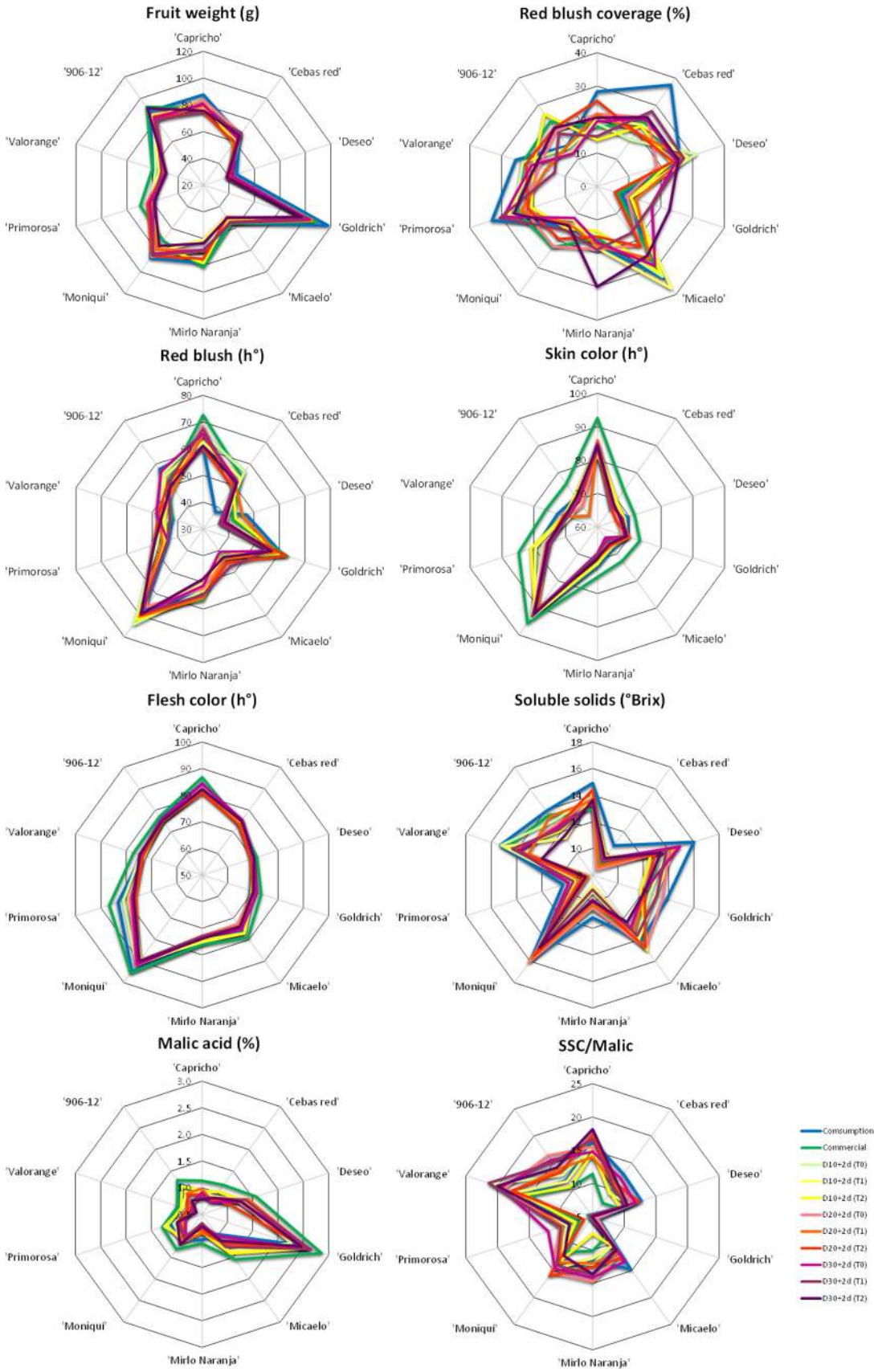


Fig. 2. Pomological analysis of ten apricot cultivars under different conditions: consumption, commercial maturity (storage condition), D10+2d (ten days at 1°C and 2 days at 20°C), D20+2d (twenty days at 1°C and 2 days at 20°C), and D30+2d (thirty days at 1°C and 2 days at 20°C). T0, control; T1, one application of 1-MCP; T2, two applications of 1-MCP.

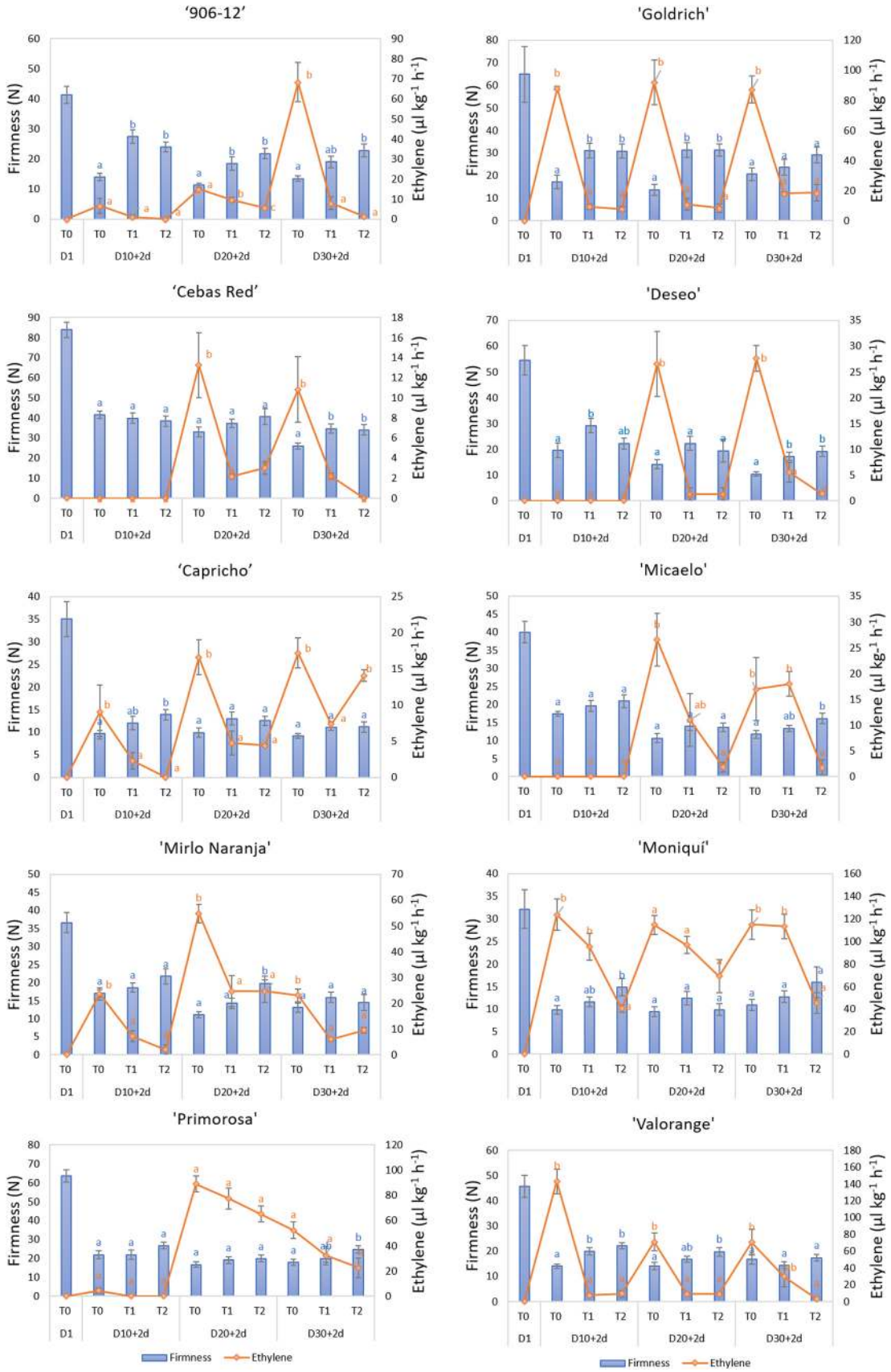


Fig. 3. Monitoring of fruit firmness (blue bars) and ethylene production (orange lines) per day (D1, D10+2d, D20+2d, and D30+2d) and treatment (T0, control; T1, one application of 1-MCP; T2, two applications of 1-MCP) for ten apricot genotypes. Different homogeneous groups are indicated based on Tukey's test (p-value<0.05) between treatments for each day.

between treatments and days were shown, the differences between control and treatments were clearly significant for '906–12', 'Goldrich', 'Deseo', 'Capricho', and 'Valorange' (Fig. 3). However, '906–12' and 'Goldrich' exhibited the most pronounced differences between the 1-MCP treatments and the control, particularly after the 10th and 20th day. Specifically, '906–12' displayed a decrease in firmness from approximately 40 to 15 N after 10 days of storage under the control condition, whereas both 1-MCP treatments-maintained values around 30 N. Similarly, 'Goldrich' started at roughly 60 N, but its firmness decreased to 15 N after 10 days in the control, whereas the 1-MCP treatments-maintained values around 30 N (Fig. 3). Furthermore, after 30 days of cold storage, the differences between the treatments and the control were less pronounced, with no discernible differences between dosages of 1-MCP applications.

On the other hand, concerning ethylene production, the highest emission typically aligned with the control treatment, except for those cultivars where no ethylene production was observed after 10 storage days in either the control or treatment, such as 'Cebas Red', 'Deseo', and 'Micaelo'. These three cultivars, along with 'Capricho', exhibited the lowest ethylene production after 20 days of storage, ranging from 0 to 25 μl kg^{-1} h^{-1}, while 'Goldrich' consistently displayed high values of approximately 60 μl kg^{-1} h^{-1} throughout the storage period under the control condition. Furthermore, 'Moniquí' displayed even higher values, exceeding 120 μl kg^{-1} h^{-1} each storage day in the control (Fig. 3). Notably, 'Valorange' stood out as the cultivar with the highest ethylene production rate, surpassing 150 μl kg^{-1} h^{-1}, but only after 10 days of storage. Conversely, 'Primorosa' and '906–12' exhibited values around 70–80 μl kg^{-1} h^{-1} after 20 and 30 days of storage, respectively.

In the principal component analysis (PCA) depicted in Fig. 4, the comprehensive impact of genotype, time, and treatments on the evaluated traits becomes apparent. Regarding genotype, the PCA indicated a contrasting association between 'Cebas Red' and 'Moniquí' concerning firmness and ethylene production, as well as skin and flesh color, explaining 52.6 % of the variance along the x-axis. With regard to the PCA concerning the progression over time (days), on day 1, malic acid, firmness, skin color, and flesh color were grouped together, whereas ethylene exhibited an opposing trend on days 10, 20, and 30, explaining 87.5 % of the variance along the x-axis. In terms of treatments, a single application of 1-MCP and two applications of 1-MCP demonstrated a distinctly opposite effect compared to the control regarding soluble solids content, ethylene, malic acid, skin color, flesh color, and firmness, elucidating 87.8 % of the variance along the x-axis.

3.2. Ethylene-related gene expression analysis in fruits after the 1-MCP treatments

In lights of the previous results concerning fruit firmness, we selected '906–12' and 'Goldrich' as the best candidates for a more comprehensive exploration of gene expression by qPCR. This focused analysis will specifically target key ripening-related genes, considering the influence of ethylene-related treatments. For gene monitoring we selected 10 genes previously identified and designed by Salazar et al. (2022b).

In the present study, we analyzed the gene encoding the enzyme *3-Hydroxybutyryl-CoA-hydrolase (HIBCH)*, which is involved in the oxidative degradation of the amino acid valine, leucine and isoleucine. This gene showed minimal expression for 1-MCP applications of both genotypes, while it was overexpressed in the control (Fig. 5). Other important genes according to their significant expression were encoding genes for *Polygalacturonase (PG, EC:3.2.1.15)* and *Pectin methylesterase (PME, EC:3. 1.1.1.11)* typically involved in fruit softening, as well as the enzyme *Aminocyclopropane-1-carboxylate synthase (ACS)*, responsible for ethylene biosynthesis, exhibited noteworthy expression levels.

Concerning the previous results, genotypes '906–12' and 'Goldrich' exhibited a decrease in fruit firmness over time, in contrast to fruits treated with 1-MCP as illustrated in Fig. 3. Therefore, we can assure that the application of 1-MCP significantly delayed fruit softening.

The decrease on fruit softening attributed to 1-MCP treatments could be linked to a reduction in *PME* and *PG* enzyme activities. This is supported by the observation that the greater loss of firmness in both genotypes coincided with an increase in the expression of both genes, specifically in the control treatment (Fig. 5).

In terms of ethylene production, the combined effect of cold and 1-MCP treatment resulted in reduced rates of ethylene production during the postharvest period for both genotypes (Fig. 3). For the 'Goldrich' cultivar, ethylene production in the control exhibited a climacteric peak, which coincided with an increase in the expression of the gene coding for *ACS* (Fig. 5), whereas the suppression of *ACS* in the 1-MCP-treated fruit can be attributed to the inhibitory effect of 1-MCP on its synthesis. On the other side, another encoding gene of *Auxin-responsive protein (IAA)* which plays an important role in both fruit set and fruit growth, was monitored. In our assay, this enzyme seems to be overexpressed by 1-MCP treatments more linked to earlier maturation condition.

Regarding the evolution of fruit skin color, another gene encoding *Beta-carotene-3-hydrolase (CRTZ)* was monitored (Fig. 6). As observed in the previous results, '906–12' and 'Goldrich' show a slight trend towards more orange shades over time. However, following the applications of 1-MCP, '906–12' on day 10 or 'Goldrich' on days 10 and 20 exhibited slightly lighter orange shades compared to the control treatment (Fig. S1ab). Therefore, the reduction of skin color hue linked to more orange shades may be attributed to increased *CRTZ* activity over time. Conversely, higher hue values associated with lighter orange skin color were associated to decreased *CRTZ* activity due to 1-MCP treatments (Fig. S1a and Fig. 6).

Additionally, we assessed the expression of the gene encoding *Sucrose synthase (SUS)*, which is involved in starch and sucrose metabolism. As depicted in Fig. 6, '906–12' on day 10 and 'Goldrich' on days 10 and 20 displayed a slight decrease in *SUS* transcript levels in the fruits treated with 1-MCP compared to the control fruits. Nevertheless, this observation did not reveal a distinct trend in the evolution of the soluble solids content either over time or between different treatments (Fig. S2ab).

In this study, we have also analyzed the expression level of the gene coding for the enzyme *Myo-inositol-1 phosphate synthase (INO1)*, involved in Myo-inositol-1-phosphate synthase pathway and linked to stress tolerance. Likewise, the gene encoding this enzyme showed no significant differences in its expression among treatments (T0, T1 and T2). However, for the 'Goldrich' cultivar, a significant increase was observed in fruits treated with 1-MCP (T2) by day 10 (Fig. 6).

Other evaluated genes have been associated to the oxidative processes such as genes encoding for *Ferredoxin (PETF)* and *GDP-L-galactose (VTC2)*. We observed that in '906–12' and 'Goldrich' there was generally minimal expression over time. The application of 1-MCP resulted in significant differences between treatments only in a few days for both genotypes. For the accession '906–12' an increase in *PETF* expression was observed at day 10 and *VTC2* at day 10 (T1 and T2) and 20 (T2). While, in the 'Goldrich' cultivar, there were differences in the expression of f *PETF* at day 10 (T2) and GDP-L-galactose phosphorylase at day 10 (T2) and day 20 (T1) (Fig. 6).

4. Discussion

4.1. Monitoring of pomological traits in relation to fruit quality and postharvest behavior

Fruit quality plays a pivotal role in agricultural production, as it directly impacts consumer satisfaction. Attaining superior fruit quality demands a blend of suitable genetic background, effective cultural practices, meticulous postharvest handling, and adherence to established quality benchmarks (Martínez-Gómez et al., 2021).

Therefore, the condition of the fruit upon reaching the consumer is influenced by post-cold storage factors. Consequently, it is imperative to closely monitor traits pertaining to fruit quality, including skin color,

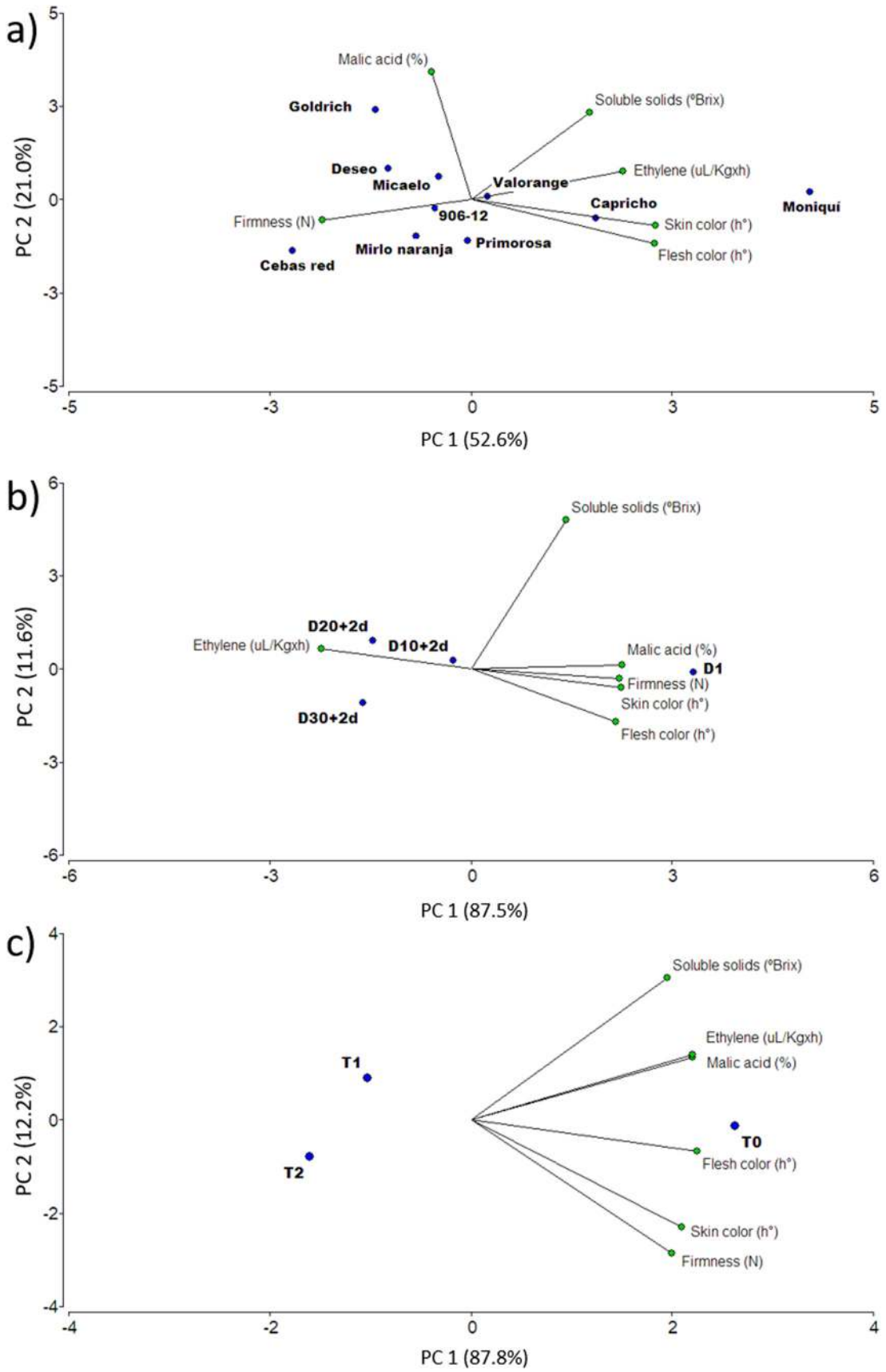


Fig. 4. Principal component analysis by genotype (a), day (b), and treatment (c) of the key pomological and fruit quality traits.

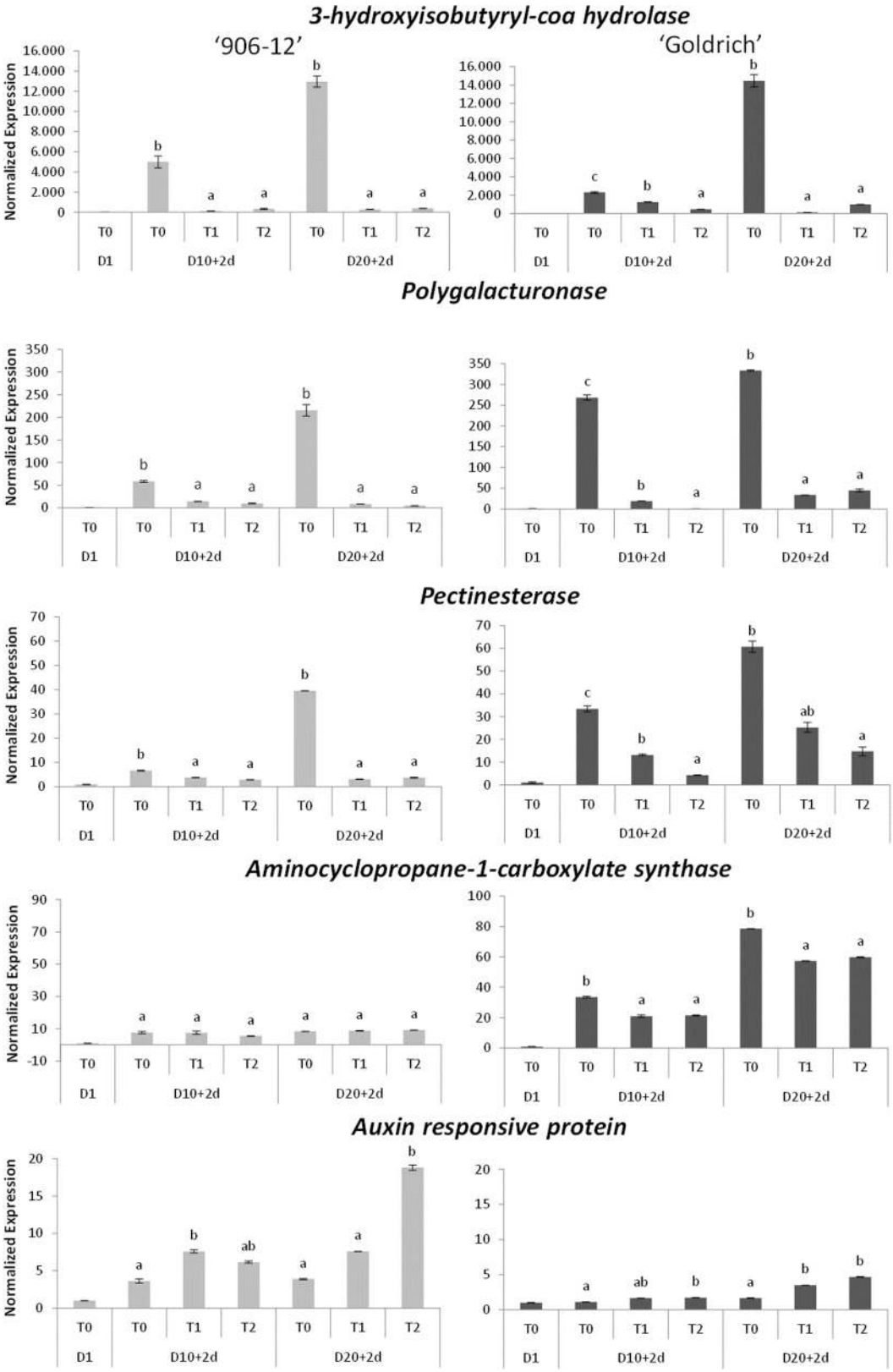


Fig. 5. Normalized Relative Quantification (NRQ) by RT-qPCR of 5 genes using three biological and two technical replicates for each day (D1, D10+2d, D20+2d) and treatment (T0, control; T1, one application of 1-MCP; T2, two applications of 1-MCP). The light grey color shows genes analyzed in apricot '906–12, while dark grey color shows genes analyzed in apricot 'Goldrich': *3-hydroxyisobutyryl-coa hydrolase* (*HIBCH*), *Polygalacturonase* (*PG*), *Pectinesterase* (*PME*), *1-aminocyclopropane-1-carboxylate synthase* (*ACS*), and *Auxin-responsive protein* (*IAA*). The different homogeneous groups are shown according to Tukey's test (p-value<0.05). Letters above each bar indicate differences between treatments on each day.

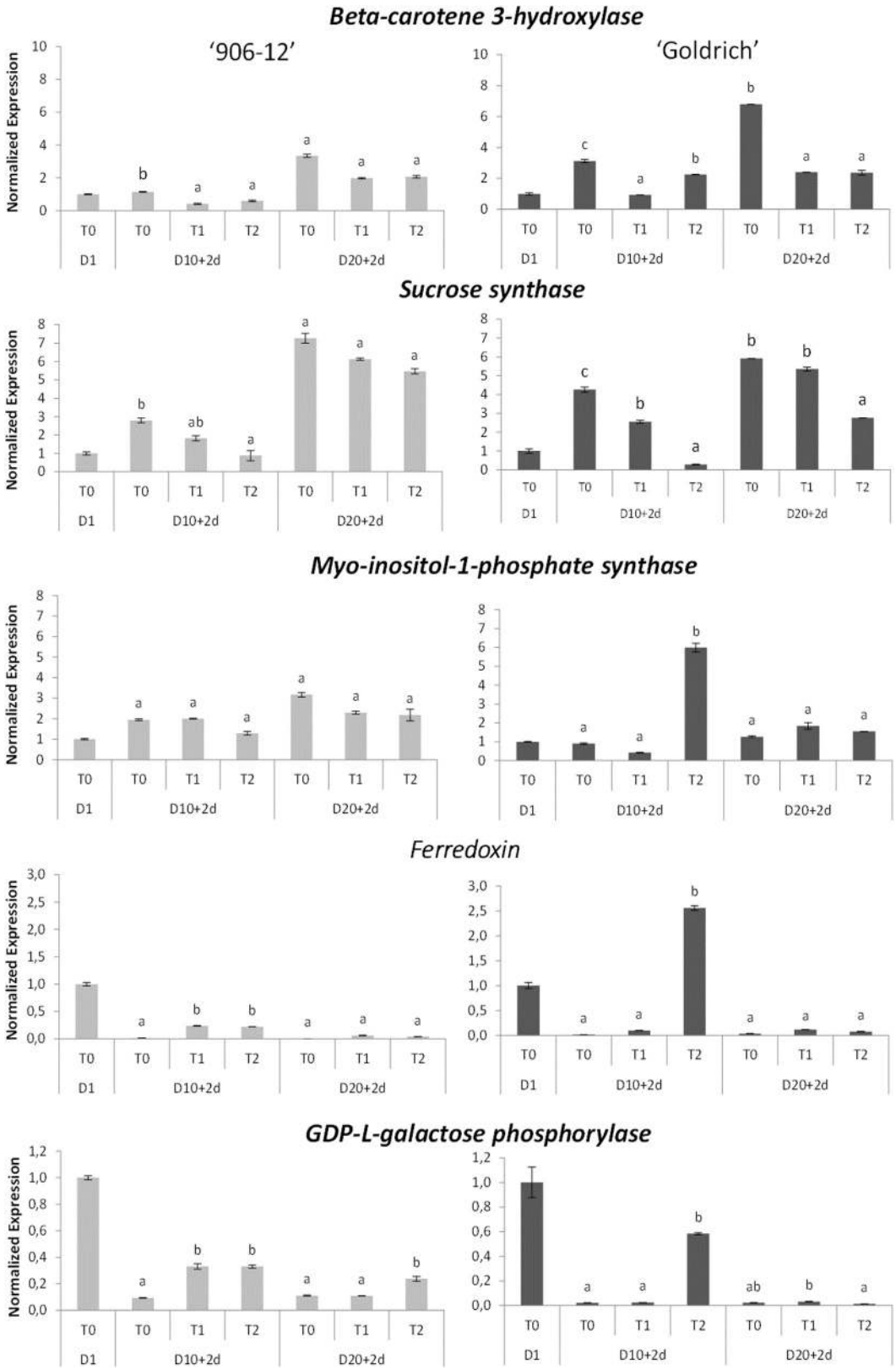


Fig. 6. Normalized Relative Quantification (NRQ) by RT-qPCR of 5 genes using three biological and two technical replicates for each day (D1, D10+2d, D20+2d) and treatment (T0, control; T1, one application of 1-MCP; T2, two applications of 1-MCP). The light grey color shows genes analyzed in apricot '906–12, while dark grey color shows genes analyzed in apricot 'Goldrich': *Beta-carotene 3-hydroxylase* (*CRTZ*), *Sucrose synthase* (*SUSY*), Myo-inositol phosphate metabolism (INO1), Ferredoxin (PET), and GDP-L-galactose phosphorylase (VTC2_5). The different homogeneous groups are shown according to Tukey's test (p-value<0.05). Letters above each bar indicate differences between treatments on each day.

flesh color, soluble solids, acidity, as well as postharvest attributes such as fruit firmness and ethylene production. This monitoring facilitates the identification of the optimal cold storage conditions for specific cultivars and, in our study, underscores the advantages of 1-MCP applications. The combination of cold storage and 1-MCP applications aims to extend the fruit's shelf-life, striving to meet the minimum fruit quality standards (Egea et al., 2010; Minas et al., 2013). Thus, the analysis of fruit quality traits represents a good approximation in terms of quality since they define very well the visual appearance (color), ripening stage (firmness) and flavor (soluble solids and acidity) of the fruit (Ruiz and Egea, 2008). It is important to study them since they are largely conditioned by fruit softening and ethylene production, both crucial factors in determining the shelf-life of climacteric fruits like apricots.

In the PCA (Fig. 4), we can easily discern the effect of treatments and time on the evaluated traits. The results reflect a clear influence of the inhibitor 1-MCP on ethylene, and consequently slowing down fruit softening. Furthermore, these results align with other studies, such as the one conducted by (Valdes et al. (2009), in which 1-MCP was applied to the apricot cultivar 'Modesto', being softening and fruit color evolution the most influenced parameters, showing a significant reduction following the application of 1-MCP. Likewise, the treatment with 1-MCP at low temperature on the apricot cultivar 'Xiaobai' had an effective impact on delaying ethylene production, respiration rates, soluble solids content, and maintaining firmness, titratable acidity, and ascorbic acid content during storage (Lv et al., 2021).

Summarizing, the effect of cold storage and 1-MCP combination was genotype dependent, being '906–12' and 'Goldrich' those who responded best to treatments in terms of fruit softening. Additionally, all genotypes end the 30 days of storage period close to or over 20 N, excepting 'Capricho', 'Moniquí', and 'Valorange'. In general, all genotypes responded to the 1-MCP treatments, especially in traits closely linked to the fruit's shelf-life, such as firmness and ethylene production. However, overall, the same did not occur for the rest of the fruit quality traits, and no differences were observed among the different applications of 1-MCP (Figs. 2 and 3).

4.2. Expression of genes related to fruit ripening

In the current assay, a total of 10 ripening-related genes have been monitored, which have been previously related to fruit development (*IAA*), fruit quality traits such as fruit color (*CRTZ*), sugar content (*SUS*), or even odor (*HIBCH*). Additionally, ethylene-related genes linked to ethylene production (*ACS*) and fruit softening (*PG* and *PME)* were also monitored (García-Gómez et al., 2020), as well as other important genes linked to oxidative stress (*INO1, VTC2,* and *PETF*).

Fruit growth and development is a preceding and complex process that directly influences fruit ripening, indicating that genes related to fruit growth could potentially influence the ripening process. In various studies, such as Manganaris et al. (2011), an up-regulation of genes involved in the biosynthesis of *Auxin-sensitive protein* (*IAA*) has been observed during early ripening of apricots, as demonstrated in our study using 1-MCP (Fig. 5), which reinforces the notion that this protein plays a crucial role in fruit ripening. Therefore, applications of 1-MCP appear to promote increased expression of the gene encoding *IAA*, in contrast to the consistently lower gene expression observed in the control. These results are supported by the study of alterations in the expression of the *PmIAA* genes in response to treatment with 1-MCP during fruit ripening (Iqbal et al., 2022).

On one hand, the external appearance of the fruit, including skin color, stands out as a crucial visual quality factor for fresh consumption, influencing varying levels of consumer acceptance. Apricot skin color varies from yellow to orange or even orange-reddish as ripening occurs. *Beta-carotene-3-hydrolase* is an enzyme that participates in the biosynthesis of xanthophylls, carotenoid pigments that together with anthocyanins provide fruit color changes during ripening (Tanaka et al., 2008). In our study, we can affirm that after treatment with 1-MCP the synthesis of pigments that provide color changes of the fruit during ripening was reduced. In fact, 1-MCP treatments could probably have slowed down the orange skin color gain affecting its external appearance.

On the other hand, fruit flavor is determined by the concentration of sugars, organic acids, aromatic compounds, and other water-soluble substances, including amino acids. *Sucrose synthase (SUS)* is an enzyme that catalyzes the reversible reaction of sucrose and UDP to UDP-glucose and fructose (Stein and Granot, 2019), with these sugars playing a crucial role in defining sweetness (Itai and Tanahashi, 2008). In another study conducted by Yu et al. (2017), in peach fruits treated with 1-MCP, exhibited a decrease in sucrose degradation throughout fruit ripening, associated in turn with an increase in glucose and fructose levels. This increase in sucrose was positively correlated with increased expression and synthesis activity of both *SUS* (*Sucrose synthase*) and *SPS* (*Sucrose phosphate synthase*) and negatively related to enzymes involved in sucrose catabolism, *SUS*-mediated sucrose cleavage activity (*Sucrose synthase*), *Acid invertase (AI)* and *Neutral invertase (NI)*. Although we did not measure the activity of the enzyme towards sucrose synthesis or degradation, we can attribute that its expression is influenced by the action of ethylene, playing an important role in sugar content during fruit ripening. In our assay, the gene encoding *Sucrose synthase* clearly increase its expression over time, however, no differences were found between treatments as happen for soluble solid content. This finding shows that 1-MCP treatment may be is not being detrimental in terms of flavor according to taste linked to sweetness.

According to fruit aroma, this is determined by a mixture of various volatile compounds, playing metabolic pathways of lipid degradation such as amino acid degradation an important role in the biosynthesis of aromatic compounds (Zhang et al., 2019). Highlighting among others; branched-chain volatile alcohols, aldehydes, and esters (Li et al., 2023). More concretely, the enzyme *3-hydroxyisobutyryl-CoA* contributes to the synthesis of *Succinyl-CoA*, a compound that participates in the Krebs Cycle, a metabolic pathway involved in cellular respiration and from which energy is synthesized for fruit development and ripening. In different studies such as Zhou et al. (2018), an increase in the expression of this enzyme is observed during peach ripening. In our study, we obtained results in agreement with this research, since the most mature fruits of the 2 genotypes evaluated exhibited higher gene expression, consistently aligning with the control treatment. In contrast, fruits at a less advanced ripening stage due to 1-MCP treatments, as well as on day 1 demonstrated minimal expression. Therefore, as shown in Fig. 5, the 1-MCP treatments with one application (T1) and two (T2) resulted in a significant decrease in the expression of this gene for both genotypes. In addition, an increase in gene expression over time was also observed, especially in the controls. In summary, it appears that 1-MCP applications might influence the aroma of the fruit. However, it is crucial to assess whether these differences would be perceptible to consumers from a sensory perspective.

Regarding fruit firmness, the activities of the enzymes *PME* and *PG* stand out since the greater loss of firmness of both genotypes coincided with an increase in the expression of both enzymes. The softening of stone fruits is typically associated with the disassembly of the cell wall, in which the depolymerization of pectins catalyzed by *endo-polygalacturonase* (*endo-PG*) plays a main role (Ghiani et al., 2011; Paniagua et al., 2014). Other important cell wall-degrading enzymes are *pectinesterase*, *cellulase* and *β-galactosidase* (Lin et al., 2018). These enzymes participate in the modification of the pectins that make up the cell wall, specifically the homogalacturonans. Initially, the *PME* enzyme acts by dissociating the methyl group from galacturonic acid. Subsequently, the resulting demethyl esterified pectins are hydrolyzed at the *α-1,4 bond of galacturonic acid* by *PG*. Once the pectins are demethyl esterified, hydration of the wall occurs and consequently relaxation is increased leading to a loss of fruit wall firmness. In the present study, 1-MCP treatments led to a decrease in the expression of both enzymes. However, there were no significant differences between T1 (one dose of

1-MCP) and T2 (two doses of 1-MCP), so it seems that the application of a second dose of the product did not mean a greater reduction in the activity of these enzymes, which is in accordance with fruit firmness value. Other studies have demonstrated that 1-methylcyclopropene (1-MCP) has a significant impact on delaying the ripening of harvested kiwifruit, apricot, plums or peach (Fan et al., 2018; Luo et al., 2009; Mworia et al., 2012; Zheng et al., 2023).

With regard to ethylene production, the combination of cold and 1-MCP favored lower ethylene production rates during the postharvest period for both genotypes. This decrease in ethylene production may be attributed to the fact that 1-MCP interferes with the autocatalytic production of ethylene, since it acts by irreversibly inhibiting ethylene receptors (Sisler et al., 1996). In other studies, it has been observed that despite 1-MCP blocking ethylene receptors, new ones are generated after a period (Jiang et al., 1999). Therefore, the effect of 1-MCP seems not to be permanent.

Thus, the decrease in ethylene production, can be attributed to the 1-MCP application which results in the reduction of the activity of the enzyme *aminocyclopropane-1-carboxylate synthase (ACS)*, involved in ethylene synthesis. In another study carried out by (Khan and Singh (2007) where 1-MCP is applied to the plum cultivar 'Tegan Blue' at different increasing concentrations during fruit ripening, a reduction in the activity of this enzyme is shown as the concentration of 1-MCP increases. Conversely, Muñoz-Robredo et al. (2012) characterized three *1-aminocyclopropane-1-carboxylic-acid synthase (ACS)* genes, but only the expression of *ACS2* was highly reduced by ethylene inhibition. In our study, we observe a similar pattern, as the fruit treated with 1-MCP, while not displaying differences among the 1-MCP treatments, exhibited lower gene expression compared to the control treatments, especially in the 'Goldrich' cultivar. Overall, *PME*, *PG*, and *ACS* enzymes showed higher expression in the control treatments compared to the 1-MCP-treated fruit.

In addition, other genes encoding metabolites linked to stress responses such as myo-inositol were monitored. This compound is a versatile metabolite that plays an important role in plant biochemistry and physiology, which is involved in fruit development (Taji et al., 2006, Wang et al., 2008, Loewus, 2006). In a study conducted by Hu et al. (2020) in apple fruit, to learn more about the physiological role of myo-inositol, the expression level of the enzyme *Myo-inositol-1-phosphate synthase (MIPS)* was altered using transgenic technology. In summary, this study suggests that a reduction in myo-inositol levels in apple tree is associated with an increase in reactive oxygen species-induced programmed cell death, involving pathways dependent on salicylic acid and ethylene. In a previous study on a closely related species, such as plum (Farcuh et al., 2018), it was demonstrated that 1-MCP applications resulted in an increase in myo-inositol levels, revealing a negative correlation between this compound and ethylene. In our apricot fruit assay, the application of 1-MCP did not yield definitive evidence supporting the previously observed trends for *Myo-inositol-1-phosphate synthase*, except in the case of 'Goldrich,' where the second application of 1-MCP led to an increase in the levels of this compound.

In relation to oxidative stress, it has been observed that the synthesis of reactive oxygen species (ROS) is irremediable during the metabolism of living beings (Mubarakshina et al., 2006). In fact, it has been contemplated that the major source of H_2O_2 is the photosynthetic electron transport chain in plants (Ivanov and Khorobrykh, 2003; Mubarakshina et al., 2006). *Ferredoxin* is a ferrosulfur protein associated with the photosystem I (PSI), yielding an electron to oxygen and producing the superoxide anion radical (O_2^-), which subsequently dismutates to hydrogen peroxide (H_2O_2) (Asada, 1999). In these studies, electrons from H_2O in photosystem 2(PSII) are yielded to O_2 in PSI for reduction to H_20, thereby reducing the level of ROS. On the other side, L-ascorbate also called vitamin C is an essential antioxidant in plants (Fenech et al., 2019), synthesized through the Smirnoff-Wheeler (SW) pathway (Wheeler et al., 1998), where the enzyme GDP-L-galactose phosphorylase acts as the main control point (Laing et al., 2015). In our assay, in spite of *Ferredoxin (PETF)* and *GDP-L-galactose phosphorylase* (*VTC2)* decrease over the time, 1-MCP treatments maintains high expression levels compared to the control, therefore based on previous studies we can attribute a beneficial effect to treatment with 1-MCP, reducing oxidative stress during ripening.

In short, employing ethylene suppression treatments like 1-MCP along with cold storage, extends the apricot fruit shelf-life since ethylene production and fruit softening were delayed. This, coupled with optimal management practices, extends the fruit's shelf life, and ensures favorable consumer acceptance in terms of quality. These management practices depend on the commercial cultivar, as the ripening process varies among different cultivars. However, in order to achieve a good balance between shelf-life and sensory attributes, harvest time is crucial. Fruits should be harvested when they reach a color variation that ensures the effectiveness of the 1-MCP treatment in slowing down fruit ripening. However, it should also be considered that the fruit is sufficiently advanced in its ripening process to continue ripening and acquire suitable sensory attributes, albeit at a slower pace. For this reason, sometimes it is unavoidable to lose some organoleptic characteristics after 1-MCP applications. Additionally, there are evidences that the shelf-life related genes such as *PG*, *PME*, *ACC* or *IAA* are affected by 1-MCP applications, as well as other genes including *HIBCH*, *CRTZ*, and *SUS* or those linked to oxidative stress such as *INO1*, *PETF* and *VTC2*.

5. Conclusions

This research enables a deeper exploration of the interrelationships among fruit quality, postharvest traits, and the impact of key ripening genes. The application of 1-MCP significantly decreased ethylene production and favored greater fruit firmness with respect to the control in all genotypes, indicating that the 1-MCP treatments generally fulfilled their goal of suppressing ethylene production. This delay in ripening and senescence processes varied depending on the genotype, as the response to treatment is genotype-dependent.

Concerning the expression of genes involved in fruit ripening, 1-MCP treatment suppressed the expression of genes encoding *PME*, *PG*, and *ACS*, enzymes mainly involved in fruit softening and ethylene production. In addition, it favored the expression of *IAA*, involved in fruit growth and fruit set. Applications of 1-MCP slightly affected fruit color, which was due to the suppression by 1-MCP of the expression of *Beta-carotene-3-hydrolase* (*CRTZ*), an enzyme involved in fruit color change during ripening. In turn, 1-MCP applications could affect fruit aroma, since they inhibited the expression of genes encoding *3-hydroxyisubitil-CoA-hydrolase* (*HIBCH*). However, concerning flavor-related traits such as soluble solids content, no clear trend was observed, and slight differences were found for the gene encoding sucrose synthase (*SUS*). Therefore, it is likely that 1-MCP treatments do not have a negative impact on the fruit flavor. However, it would be necessary to verify whether these differences would be appreciable for consumers from a sensory point of view. On the other side, the antioxidant activity could be favored by 1-MCP treatments since some related genes encoding for *Myo-inositol-1-phosphate synthase* (*INO1*), *Ferredoxin* (*PETF*) and *GDP-L galactose phosphorylase* (*VTC2*), were overexpressed. This study represents a great advance in the knowledge of apricot post-harvest behavior, particularly in the expression of genes associated with shelf-life in a species known for its perishable nature, *Prunus armeniaca* L. Emphasizing the importance of continued research using diverse treatment approaches is crucial for gaining a more comprehensive understanding of the intricate mechanisms involved in the ripening process. This deeper insight is essential for developing apricot cultivars that not only meet optimal nutritional standards but also fulfill commercial requirements effectively.

Author Statement

I, Juan Alfonso Salazar, affirm that this manuscript is an original work and has not been submitted elsewhere for publication. I have contributed significantly to the research, design, and writing of this work and approve the final version for publication. All sources of support and collaborators have been properly acknowledged. Additionally, I declare no conflicts of interest related to this publication.

CRediT authorship contribution statement

Pedro Martínez-Gómez: Writing – review & editing, Supervision. **Maravillas Fernández:** Methodology, Data curation. **Germán Ortuño-Hernández:** Writing – original draft, Formal analysis. **Juan A Salazar:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **David Ruiz:** Resources, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2024.113037.

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