Identification of biomarkers associated to ‘Gala’ apples ripening and postharvest quality

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Abstract

Apple is, sociocultural and economically, on of the most important species in the world, and stands out for its high storage potential. However, the monitoring of factors that could result in fruit quality modifications during postharvest is essential to ensure the acceptability and for the development of new storage technologies in order to increase fruit shelf life. Approaches focused on molecular biology, such as RT-qPCR have been used to better understand the mechanisms involved in fruit development and maturation. In this study the use of RT-qPCR to monitoring apple quality during ripening and development in different postharvest conditions such as room temperature, cold storage with or without control of atmosphere and the 1-methylcyclopropene usage were proposed. The potential of genes involved in ethylene biosynthesis and response, cell wall modification and degradation, sugar and aroma metabolisms for employment as biomarkers of fruit development and quality were evaluated. Thus, MdEXP4 is highlighted as biomarker for development and MdACO1, MdPG1, MdAF1, MdAF3 and MdAAT2 as potential biomarkers for ripening. MdACO1 and MdPG1 appear as suitable markers for quality, conservation technologies and storage time in apples. This work suggests that the study of gene expression by RT-qPCR may be an alternative for a better fruit characterization during the development and postharvest period.

Keywords: Cold storage, gene expression, Malus x domestica, molecular analysis

Identificação de biomarcadores associados ao amadurecimento e qualidade pós-colheita em maçãs ‘Gala’

Resumo

A maçã é, sociocultural e economicamente, uma das espécies mais importantes do mundo, destacando-se por seu alto potencial de armazenamento. Contudo, o monitoramento dos fatores que alteram a qualidade dos frutos durante a pós-colheita é essencial para garantir a aceitabilidade dos mesmos, e para o desenvolvimento de novas tecnologias de armazenamento com o intuito de aumentar a durabilidade dos frutos. Técnicas de Biologia Molecular, como a RT-qPCR têm sido empregadas para melhor entendimento dos mecanismos envolvidos com o desenvolvimento e maturação dos frutos. Este estudo propôe a utilização de RT-qPCR para o monitoramento da qualidade de maçãs durante o desenvolvimento e amadurecimento em diferentes condições de pós-colheita, como temperatura ambiente, armazenamento refrigerado com ou sem controle da atmosfera, e uso do 1-metilciclopropeno. Para isso foi avaliado o potencial de genes envolvidos com a biossíntese e resposta ao etileno, modificação e degradação da parede celular e metabolismo de açúcares e aromas para uso como marcadores de desenvolvimento e qualidade dos frutos. Assim, destacaram-se os genes MdEXP4 como bom marcador de desenvolvimento e MdACO1, MdPG1, MdAF1, MdAF3 e MdAAT2 como potenciais marcadores de amadurecimento. MdACO1 e MdPG1 aparecem ainda como bons marcadores de qualidade, tecnologias de conservação e tempo de armazenamento. Sugere-se o estudo da expressão gênica como alternativa para melhor caracterização dos frutos durante os períodos de desenvolvimento e pós-colheita.

Palavras-chave: Armazenamento refrigerado, expressão gênica, Malus x domestica, análises moleculares
Introduction

The technological advances have allowed whole genome sequencing of several species. A large number of genome sequences from fruit species, such as apple, is currently available (Gapper et al., 2014). The increasing availability of genome sequences of fruit species has paved the way for functional genomics approaches including analyses of fruit transcriptome, proteome and metabolome.

The transcription analyses are an important tool to investigate the molecular changes during the postharvest period. Different technologies are available for transcriptome exploration, such as reverse transcription quantitative polymerase chain reaction (RT-qPCR) which is characterized by its rapidity, high sensitivity, specificity, precision, reproducibility (Gachon et al., 2004) and lower cost in comparison with other transcription analyses techniques (VanGuilder et al., 2008).

In others fields of study, transcriptional analyses are been used with classical techniques or replacing them in phenotyping analysis (Valdés et al., 2013). Likewise, RT-qPCR can be employed in postharvest with classic techniques for fruit quality determination or replacing more expensive biochemical approaches that would require specific equipment, not always available in molecular biology laboratories.

In this context, the use of biomarkers is promising in monitoring fruit quality. RT-qPCR has been shown an important tool for biomarker monitoring, where, previously identified targets can be assayed in a very large numbers of samples (VanGuilder et al., 2008).

Apple (Malus x domestica Borkh.) is one of the most important fruit species, socially and economically (Both et al., 2014; Zhu et al., 2013). Molecular biology tools have allowed scientific advances in the field and have been applied to numerous studies in apples (Ireland et al., 2014; Muñoz-Bertomeu et al., 2013; Nobile et al., 2011; Visser et al., 2014; Zheng et al., 2013). Some of these studies correlated gene expression patterns with biochemical and physiological changes taking place in fruit development (Atkinson et al., 2012, Dandekar et al., 2004). Thus, genes associated to fruit ripening can be used as biomarkers for physiological disorders, storage time and marketing decisions. The transcriptional profile of these genes can be used to monitor fruit development and to propose novel alternatives for storage, increasing fruit shelf life.

In this context, the current study aimed to identify biomarkers associated to the ripening process, condition and period of storage in ‘Gala’ apples. Apple genes with known behavior in fruit ripening and during postharvest storage, along with novel genes, for which the transcriptional profile is less studied, were analyzed under standard conditions, such as ripening at room temperature (RT) and under cold storage (CS). Moreover, fruit development and ripening under controlled atmosphere (CA) conditions, where the mechanisms responsible for fruit modifications are not well established, were analyzed. The current study highlights the use of RT-qPCR to monitor fruit quality.

Material and Methods

Plant Material

‘Gala’ apples from the clone Baigent, on M9 rootstocks, from a commercial five-year old orchard in the region of Caxias do Sul, RS, Brazil, during the 2013/2014 growing season were used. In Experiment I – ‘Apple developmental stages’, the samples were harvested with intervals of 15 days, starting at full bloom up to 105 days after anthesis (DAA).

For Experiment II – ‘Ripening evolution in apples’, fruit were harvested at physiological maturity (an average of 87 N of firmness) and split into two lots. One group was submitted to treatment with 1 µL L⁻¹ of 1-methylcyclopropene (1-MCP) and the other remained without treatment. Subsequently, treated and control fruit were kept at room temperature (25°C) for 12 days to investigate ripening in the presence and absence of the ethylene inhibitor. Fruit were sampled at two days intervals.

In Experiment III – ‘Influence of storage conditions on apples quality’, the fruit were harvested when physiologically ripe and, subsequently, split into two groups, one receiving treatment with 1 ppm of 1-MCP and the other, remaining untreated. Fruit treated with 1-MCP...
and untreated controls were submitted to cold storage (0ºC ±0.5ºC, 90% UR ± 5%) and distinct controlled atmosphere conditions (0.5% and 1.5% O₂ with 2% CO₂) for 9 months. Fruit were evaluated at removal from cold storage and after seven days.

For the treatment with the ethylene inhibitor the commercial product SmartFresh™ (Agro Fresh, Rohm and Haas, PA, USA) was used, which contains 0.14% of the active principle. The product was diluted in water and the released gas was applied to the fruit for 24 hours in hermetically sealed boxes.

Physicochemical analyses

Flesh firmness (FF) was investigated using an automatic penetrometer (Fruit Texture Analyzer), with cylindrical tip of 11 mm. The measurements were taken from the equatorial region of the fruit, at opposite sides, after the skin removal. Soluble solids content (SSC) was determined using a digital refractometer (PR 101 Atago) with automatic temperature compensation.

Statistical analyses

Physicochemical data were submitted to statistical analyses with the aid of the software WinStat v. 2.0 (Machado & Conceição, 2003). For the experiment III, the effect of each storage condition was evaluated by Tukey’s test (p≤0.05), the effect of each treatment was investigated by t test (p≤0.05) and the effect of days at room temperature was evaluated by the t test (p≤0.05).

Gene expression

A group of 10 genes associated to fruit ripening was investigated (Table 1). The sequences were obtained from Malus x domestica (Borkh.) coding sequences available at Genome Database for Rosaceae (GDR). Primers were designed using the software Primer3Plus (Untergasser et al., 2007). Primers were selected according to Applied Biosystems™ recommendations. Total RNA was extracted from a pool of ten fruit, using the protocol described by Zeng and Yang (2002), with minor modification. After nucleic acid isolation, RNA quality was verified spectrophotometrically (BioTekEpoch™, TAKE3™) and with the aid of 1% agarose gel electrophoresis. After treatment with DNase I (Invitrogen™) and confirmation of DNA elimination by PCR using primers for constitutively expressed genes, cDNA was synthesized from 1 µg of RNA, using the SuperScript III/RNase Out Mix (Invitrogen™).

Amplification by qPCR was carried out using StepOne™ Real-Time PCR Systems (Applied Biosystems™), employing the reagent SYBR Green PCR Master Mix (Applied Biosystems™). A pool of cDNA from all samples was used to validate the primer pairs, with a 1:10 dilution and five points. Expression analyses were carried out exclusively using primers with efficiency close to 100% and dissociation curves with a single peak. Reference genes were chosen based on the comparison of transcription stability throughout the investigated experimental conditions, as described by Storch et al. (2015a). Thus, the reference genes employed for Experiment I were MdACT, MdPDI and MdUBC, for Experiment II MdH1, MdUBC and MdACT, and the Experiment III was normalized with the reference genes MdUBC, MdPDI and MdH1. The amplification conditions for the genes of interest were: 95ºC for 10 min, followed by 40 cycles at 95ºC for 15 s and 60ºC for 1 min, followed by dissociation curve generation. The relative expression level was obtained for each gene. Expression levels at harvest were employed as calibrators for gene expression experiments investigating ripening evolution and fruit quality after different storage conditions. For the experiment involving developmental stages, the expression levels at 105 DAA were used as calibrators.
<table>
<thead>
<tr>
<th>Metabolism</th>
<th>Gene description/acronym</th>
<th>M. domestica locus</th>
<th>Primers sequence</th>
<th>Forward Melting Tm (°C)</th>
<th>Reverse Melting Tm (°C)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethylene</strong></td>
<td>1-aminocyclopropane-1-carboxylic acid oxidase 1 (ACO1)</td>
<td>MDP0000195885</td>
<td>CAATGCACCACTCCATTGTC</td>
<td>58</td>
<td>TCCCCATCCGAGTGAGCATTTC</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Ethylene Insensitive 2 (EIN2)</td>
<td>MDP0000152033</td>
<td>GCACACCAGCTGAATACAGAAG</td>
<td>57</td>
<td>CCCITTCGACAAGGAGATTGC</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>Ethylene Insensitive 3 (EIN3)</td>
<td>MDP0000136668</td>
<td>AAAGACAAACATGGCCACACA</td>
<td>58</td>
<td>TGGTTTCTTGGCTCTCCAT</td>
<td>58</td>
</tr>
<tr>
<td><strong>Cell wall</strong></td>
<td>Endo Polygalacturonase (PG1)</td>
<td>MDP0000326734</td>
<td>TCACGGTAACTGCACACCAAG</td>
<td>62</td>
<td>CTTTGAGCCACACTCA</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>α-L-arabinofuranosidase 1 (AF1)</td>
<td>MDP0000065078</td>
<td>TGAGATGGAACGCATGCACCAC</td>
<td>70</td>
<td>ACCCGCCTTCATGGGTAATGC</td>
<td>72</td>
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<tr>
<td></td>
<td>α-L-arabinofuranosidase 3 (AF3)</td>
<td>MDP0000140483</td>
<td>ATTTCACAAGGTCCATATCG</td>
<td>56</td>
<td>CATGTCACCAATITCCAG</td>
<td>54</td>
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<tr>
<td></td>
<td>Expansin 4 (EXPA4)</td>
<td>MDP0000681724</td>
<td>ACCCGGTCCTCAGCACAATGG</td>
<td>59</td>
<td>ACCCCCTTGGAGAAACAGAGT</td>
<td>59</td>
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<tr>
<td><strong>Flavor</strong></td>
<td>Alcohol dehydrogenase (ADH)</td>
<td>MDP00000594290</td>
<td>CGATTCGTCTCTTTCGTTT</td>
<td>59</td>
<td>CTGGTCAAACAGGCAAACA</td>
<td>59</td>
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<tr>
<td></td>
<td>Alcohol acyl-transferase 2 (AAT2)</td>
<td>MDP0000166457</td>
<td>CGTGAATGCACTITTCCTGCAATG</td>
<td>60</td>
<td>CATTCCAACCTTTGGATAACAGC</td>
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<tr>
<td><strong>Carbohydrate</strong></td>
<td>Sucrose synthase (SUSY3)</td>
<td>MDP0000126946</td>
<td>AGTAGGCAACCGTGAGCTTT</td>
<td>60</td>
<td>CATAAATTCGAGCGTGAGCA</td>
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<td>Actin (ACT)</td>
<td>MDP0000170174</td>
<td>GGCTCTATCACCACCATCCA</td>
<td>60</td>
<td>TAGAAGCAATGCACCACAC</td>
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<td>Histone 1 (H1)</td>
<td>MDP0000223691</td>
<td>CATATTTGGCAGGACCAAGA</td>
<td>58</td>
<td>CTGGTACCAACTCAGATCCA</td>
<td>60</td>
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<td></td>
<td>Nucleosome assembly 1 protein (NAP1)</td>
<td>MDP0000272485</td>
<td>CAAACTTGGCCCCCTCCTCATT</td>
<td>58</td>
<td>CCAGCCTTCGTGAAATTTT</td>
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<tr>
<td></td>
<td>Protein Disulfide isomerase (PDI)</td>
<td>MDP0000233444</td>
<td>TGCGTGACACGCCAACGAT</td>
<td>60</td>
<td>CATCITTAGCGCCGTATCC</td>
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<td></td>
<td>Ubiquitin conjugating enzyme E2 (UBC)</td>
<td>MDP0000205182</td>
<td>TGCTGTTGACCTCTGCACT</td>
<td>60</td>
<td>AGACCAACCCTAATCCCCGTCT</td>
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</tbody>
</table>
Results and Discussion

Experiment I

The expression profiles of ten genes, whose function is associated to ripening, storage quality, condition and period were investigated during apple development (Table 1).

The expression of MdACO1 was low throughout the investigated developmental stages (Figure 1), suggesting that its transcriptional regulation is exclusively associated to ethylene biosynthesis during fruit ripening (Yang et al., 2013). Thus, the absence of MdACO1 transcription during apple development indicates that it can be used as a biomarker for ripening studies in apple. The genes MdEIN2 and MdEIN3 coding for proteins associated to ethylene signaling, exhibit variable expression throughout fruit development (Figure 1) that appears to be associated to basal ethylene production during the period.

The expression of MdPG1 was increased during anthesis and during the subsequent 15 days. The absence or low level of MdPG1 transcription in the remaining investigated developmental stages demonstrates that, although the gene codes for an essential enzyme in cell wall degradation during ripening (Wang et al., 2009), it is not associated to posterior developmental fruit changes. However, the observation of the increase in MdPG1 transcription during anthesis is recent and appears to be associated to the formation of the flower abscission zone and pollen maturation (Iglesias-Fernández et al., 2007). The transcriptional behavior of MdPG1 reinforces the use of the gene as biomarker in apple ripening studies.

The gene MdAF3 exhibits a transcriptional behavior similar to that of MdPG1 (Figure 1), suggesting that they may act concomitantly during anthesis. Due to its expression profile, the first is an important biomarker candidate, associated to apple ripening.

In contrast to the observations for MdPG1 and MdAF3, the expression levels of MdAF1 were low during fruit development. Therefore, it can also be used as a ripening marker. The transcription of MdEXP4 increased after full bloom and lasts during the entire period of fruit development (Figure 1), suggesting its involvement in cell wall changes during development, as previously shown by Wakasa et al. (2003). Due to its transcriptional behavior, MdEXP4 is not considered a suitable biomarker for ripening. However, the gene can be used as biomarker for fruit development.

The expression of MdSUSY3 was low throughout fruit development (Figure 1). The activity of the enzyme coded by the gene is likely to be more important at the postharvest period, where sugar catabolism occurs in the absence of photosynthesis, making it necessary to cleave sucrose in the cytosol where it is converted to fructose and UDP-glucose by SUSY. The expression profile of MdSUSY3 suggests that it is a suitable marker for ripening in apple.

The levels of transcripts for MdAAT2 and MdADH increased in the final stages of fruit development (Figure 1). This profile may be associated to the biosynthesis of volatiles occurring in the later stages of development and initial stages of fruit ripening. Thus, these genes can be used as biomarkers associated to fruit ripening.

Experiment II

In control fruit, the expression of MdACO1 was as described previously, with increased expression along maturation (Figure 2) (Binnie & McManus, 2009; Ireland et al., 2012; Wiersma et al., 2007; Yang et al., 2013; Zhu et al., 2008), where the authors have demonstrated the association of the transcription of the gene with ethylene biosynthesis during apples ripening. In contrast, the treatment with 1-MCP repressed the transcription of MdACO1 (Figure 2), in agreement with the effective role of the inhibitor in preventing ethylene biosynthesis in apples (Watkins, 2006, 2008). The transcriptional profile of
MdACO1 demonstrates that it is a potent marker associated to ripening in apple, as suggested by its transcriptional behavior during fruit development. Therefore, the gene expression pattern can be used to replace ethylene chromatographic analyses in laboratories of molecular analyses with lack of chromatography platforms. Additionally, in a study evaluating the effect of 1-MCP in apples, it was observed that chromatographic methods were not able to detect very low ethylene contents, whereas the transcription of MdACO1 by RT-qPCR was detected in fruit with low amounts of ethylene (Storch et al., 2015b). Although the use of 1-MCP is known to reduce ethylene synthesis, basal levels of the hormone may occur (Hiwasa et al., 2003).

In contrast, the expression of MdEXPA4 occurred throughout fruit ripening, regardless the 1-MCP application (Figure 2), suggesting that the transcription of the gene is not regulated by ethylene. These findings are interesting, since the majority of the expansins associated to fruit ripening are characterized by their ethylene dependent transcriptional regulation (Gaete-Eastman et al., 2009; Sane et al., 2005; Trivedi and Nath, 2004; Wakasa et al., 2003). Previous studies carried out by Wakasa et al. (2003) demonstrated the importance of MDEXP1 (hereby referred as MdEXPA4) in fruit development, also showing its ethylene independent transcription. In the current study, we have demonstrated that, regardless its ethylene independent regulation, MdEXP4 may play a relevant role during fruit ripening. Due to the involvement of expansins in fruit cell wall modifications leading to tissue softening (Hiwasa et al., 2003; Sane et al., 2005), we suggest that the biological function of the product of MdEXPA4 is associated to ethylene independent softening (Guis et al., 1997). The transcriptional behavior of MdEXPA4 rules out its use as ripening biomarker, as shown previously.

The transcription of MdSUSY3 remained low throughout ripening, regardless the 1-MCP application. It may be associated to the lack of changes in soluble sugars under these conditions. Although the transcription of MdSUSY3 is correlated to sugar metabolism, the gene is not considered a suitable marker for ripening, since its expression remained constant throughout ripening, in disagreement with the proposed behavior.

The gene MdATT2 was expressed in all investigated conditions, however, in untreated fruit the transcript accumulation was higher, suggesting a possible inductive role of ethylene, as shown by previous works (Zhu et al., 2008). The observed profile suggests that the gene MdATT2 functions in the production of esters, the main volatiles during apple ripening (López, 1997). The observed behavior of MdATT2 suggests that the
gene can be used as a suitable biomarker for ripening. In contrast, the expression of MdADH was low throughout the ripening process in treated and control fruit, thus, ruling out its use as a biomarker for ripening, as previously hypothesized.

**Experiment III**

Low levels of MdACO1 transcription were observed at removal from cold storage in fruit submitted to CA with 0.5% of O$_2$. A slight increase in transcript accumulation was detected in fruit submitted to CA with 1.5% of O$_2$, although in fruit treated with 1-MCP, the increase was smaller. Higher levels of MdACO1 transcripts were observed in fruit stored at CS (Figure 3). The higher expression of MdACO1 at the removal from CS suggests that the condition is not effective to reduce ethylene production to basal levels, which can be associated to the intense loss of quality of the fruit stored at room temperature (Table 2). After 7 days, the accumulation of MdACO1 transcripts increased in untreated fruit kept at CA. The increase was slighter in 1-MCP treated fruit (Figure 3). The presence of MdACO1 transcripts observed in fruit treated with the hormone inhibitor may be derived from the synthesis of new ethylene receptors, recovering the responsiveness to the hormone. The transcriptional profile of MdACO1 suggests that the gene is potentially a suitable biomarker for fruit quality, condition and time of apple storage.

The expression of MdEIN2 was variable for different storage conditions and expression of MdEIN3 was low in all conditions tested, discarding the use of these genes as biomarkers. Immediately upon fruit removal from cold storage, significant differences in fruit FF were observed (Table 2). These changes are due to 1-MCP treatment and the conditions used in CA. The most drastic reduction in FF was observed in fruit kept under regular atmospheric conditions (CS). However, 7 days after storage, the period of time for the fruit to reach the consumer markets, FF was not maintained for fruit kept under CA, regardless the 1-MCP treatment or O$_2$ concentration. The effect of 1-MCP on the retention of FF was only observed for fruit kept under CS (Table 2).

The expression profile of MdPG1 was similar to that of MdACO1, clearly demonstrating that the upregulation by ethylene is retained even after long periods of storage. The high expression levels of MdPG1 at the removal from CS may account for the reduction in fruit firmness (Table 2) and reduced crunchiness, observed under these conditions. Similarly, the profile found in fruit stored under CA at the removal from storage and 7 days afterwards, explains the FF observed under these conditions. The transcriptional behavior of MdPG1 after long periods of storage indicates that the gene is a potent biomarker for quality, conservation technologies and storage time in apples.

The expression of MdAF1 was low at removal from cold storage and throughout all investigated conditions. Seven days after storage, an increase in its transcription levels was observed. In contrast, MdAF3 was highly expressed in all tested conditions, including at the removal from cold storage. The high levels of transcription after long term cold storage indicate that other factors, besides ethylene, also contributed to the regulation of MdAF3 expression. The expression profiles observed for MdAF1 and MdAF3 rule out the possibility of using these genes as quality and storage time biomarkers in apple.

Upon the removal from storage, the expression of MdEXPA4 was low for the majority of conditions tested. Seven days afterwards the transcription increased for all investigated storage conditions.
conditions, with higher levels found in 1-MCP treated fruit. These results suggest that the low temperatures negatively affect the transcription of the gene, and that the expression of MdEXPA4 is higher for green fruit, indicating that the gene product functions at early stages of fruit ripening. Moreover, the ethylene independent transcriptional regulation of the gene is also confirmed. Its expression profile demonstrates that MdEXPA4 is not a suitable biomarker for fruit quality and storage time.

Immediately upon removal from storage and seven days afterwards, untreated fruit kept under CS exhibited the lowest values of SSC (Table 2). The low contents of soluble solids in fruit submitted to CS may be associated to the later ripening stages of the fruit, since at this point the sugars have been degraded to generate energy for other cellular processes and for the formation of intermediary compounds. In contrast, the low soluble solids content in fruit treated with 1-MCP may be associated to ripening delay, due to the inhibition of ethylene action caused by storage conditions. The gene MdSUSY3 was slightly expressed after long term storage. The profile suggests that the regulation of MdSUSY3 is independent of ethylene action and that the enzyme coded by the gene does not have a crucial role in sugar metabolism in apples after harvest. Thus, MdSUSY3 is not potentially useful as a biomarker for apple postharvest conservation.

The investigation of the expression profile of MdAAAT2, a key gene in esters biosynthesis, revealed low expression levels for all tested conditions, suggesting that the gene is not suitable as a biomarker for postharvest apples. The accumulation of MdADH transcripts in apples was low upon removal from storage. Seven days later, the profile remained unchanged, suggesting that the gene is not indicated to be used as a biomarker for ripening and long-term storage.

Biomarkers may have different applications in fruit postharvest. In laboratories that do not have capacity for acquiring and maintaining chromatographs, the ethylene synthesis during storage can be monitored by MdACO1 biomarker expression analysis. Similarly, the synthesis of esters in fruits submitted to different storage technologies may be accompanied by MdAAAT2 biomarker expression analysis. Changes in stored fruit pulp firmness may be checked by analysis of MdACO1 and MdPG1 biomarkers.

Conclusions

The results of the current study indicate that the genes MdACO1, MdPG1, MdAF3 and MdAAAT2 are suitable biomarkers associated to ripening due to their function in ethylene biosynthesis, flesh firmness loss and esters biosynthesis, important quality attributes for apples. In contrast, after long term storage, ethylene synthesis is associated to quality loss and the genes MdACO1 and MdPG1 can be used as biomarkers of quality, storage technology and time for apples. MdEXP4 is highlighted as a biomarker for development.

Acknowledgements

The authors would like to thank Coordination for Improvement of Higher Education Personnel (CAPES) and The National Council for Scientific and Technological Development (CNPq) for scholarships and The Brazilian Agricultural Research Corporation (EMBRAPA) for research funding.

Table 2. Physicochemical properties of ‘Gala’ apples clone ‘Baigent’ treated and untreated with 1-MCP, submitted to cold storage for 9 month under distinct conditions

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1-MCP Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RT</td>
<td>CA 0.5</td>
</tr>
<tr>
<td>FF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 day</td>
<td>84.9 aA</td>
<td>43.2 bB</td>
</tr>
<tr>
<td>7 days</td>
<td>80.4 aA</td>
<td>41.6 bB</td>
</tr>
<tr>
<td>SSC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 day</td>
<td>12.5 bcA</td>
<td>1.2 cB</td>
</tr>
<tr>
<td>7 days</td>
<td>14.1 aA</td>
<td>12.6 bB</td>
</tr>
</tbody>
</table>

Means followed by the same lower case letter in line are not statistically different according to Tukey’s test (p≤0.05) comparing the storage conditions within each treatment (with or without 1-MCP). Means followed by the same capital letter in line are not statistically different according to t test (p≤0.05) comparing each time (0 and 7 days) within each treatment (with or without 1-MCP). RT: room temperature; CS: cold storage: 0ºC ±0.5ºC, 90% UR ± 5%.

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References


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