Effect of low oxygen, temperature and 1-methylcyclopropene on the expression of genes regulating ethylene biosynthesis and perception during ripening in apple

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Abstract

Ethylene initiates and controls ripening in climacteric fruit which is developmentally regulated. During this process, ethylene production generates a strong signaling process inducing/suppressing various target genes that are associated with several attributes of fruit ripening. In apple, low temperature, low oxygen and 1-methylcyclopropene (1-MCP) treatments have been used to increase shelf life. In the present study, effort has been made to understand the molecular basis of the increase in shelf life under the influence of temperature, low oxygen and 1-MCP in Granny Smith apple. The apple fruit were exposed to these treatments either individually or in combination and levels of ethylene as well as transcript accumulation of the genes responsible for ethylene biosynthesis and ethylene receptors were measured. A tight regulation of the ethylene production was observed through differential expression of \textit{MdACS1} and \textit{MdERS1} genes. The ethylene levels were highly dependent on temperature, oxygen concentration and 1-MCP and effects of each were not only additive but associated with the expression of \textit{MdACS1} and \textit{MdERS1}.

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1. Introduction

Ethylene plays an important role in many plant processes including seed germination, organ senescence, stress responses and fruit ripening (Nath et al., 2006). Depending on the massive increase in respiration or ethylene production during ripening, fruit are classified as climacteric and non-climacteric (Biale and Young, 1981). At the onset of ripening, climacteric fruit exhibit a peak in respiration followed by a burst in ethylene production. In non-climacteric fruit, no such burst in ethylene production and respiration is observed. Though a basal level of ethylene production is present even in non-climacteric fruit, ripening in these fruits is ethylene independent. Based on the nature and amount of ethylene produced during plant growth and development including fruit ripening, the concept of system-1 and system-2 ethylene was introduced (McMurchie et al., 1972). System-1 ethylene is produced at low level and contributes to the basal levels produced by the plant during various stages of growth and development and is auto-inhibitory (Oetiker and Yang, 1995). System-2 ethylene production is at a high level, and operates during ripening of climacteric fruits and senescence of some flowers and is auto-stimulatory (Oetiker and Yang, 1995). The transition from system-1 to system-2 ethylene production is an important step during fruit ripening and is developmentally regulated. It is now established that genes responsible for ethylene biosynthesis and signal transduction are stimulated during a climacteric burst of ethylene production and may be tightly associated during the transition from system-1 to system-2 (Nakatsuka et al., 1998).

The ethylene biosynthesis pathway is well characterised in higher plants with ACC synthase and ACC oxidase playing key
roles in this pathway (Yang and Hoffman, 1984). Both enzymes are encoded by multi-gene families and differential expression of their members has been reported during fruit ripening (Zarembinski and Theologis, 1994; Barry et al., 2000). In tomato, eight ACS genes have been identified among which LeACS2 and LeACS4 are highly expressed during tomato fruit ripening (Barry et al., 2000). Of the three ACC oxidases in tomato, LeACO1 expresses during fruit ripening while other two play role in other aspects of plant development (Alexander and Grierson, 2002). Ethylene produced by plants is perceived by a family of receptors, ethylene receptors, which have similarity to histidine kinases and are negative regulators of ethylene signal transduction (Guo and Ecker, 2004). These receptors also belong to multi-gene family and their differential expression during ripening has also been observed in various climacteric fruits (Nath et al., 2006). After ethylene receptors, signal passes to downstream components through MAP kinase cascade and induces expression of ethylene responsive factors (ERFs). These ERFs modulates expression of ethylene responsive genes through binding to ethylene responsive elements in promoters of the genes (Johnson and Ecker, 1998).

Apple is a climacteric fruit and use of controlled atmosphere such as low oxygen and low temperature storage as well as treatment with 1-methylcyclopropene (1-MCP), an ethylene perception inhibitor, are effective for delaying fruit ripening (Fan et al., 1999; Rupasinghe et al., 2000; Watkins et al., 2000; DeEll et al., 2002). Since controlled atmosphere storage and treatment with 1-MCP significantly delays ripening of apple fruit by prolonging the time for ethylene burst, we suspected that these treatments may be regulating ethylene biosynthesis and perception at transcriptional level. To confirm this, we stored Granny Smith (GS) apples, an export variety which is susceptible to superficial scald formation (Rudell et al., 2005), in either low oxygen or treated with 1-MCP before storing at different temperatures for various lengths of time. Ethylene production and transcript accumulation of different genes related to ethylene biosynthesis and perception were monitored in various treatments and at different time in order to elucidate regulatory role of these genes during fruit ripening.

2. Materials and methods

2.1. Plant material and treatments

Mature apple (Malus domestica, Var. Granny Smith) fruit, which were not producing detectable ethylene, were harvested from the orchard of the University of Maryland in Western Maryland, MD, USA. Fruits were transported to the University of Maryland, College Park and kept at 1 °C in air with 80% humidity. For the air and low oxygen treatments, fifteen to twenty fruits were placed in 20 L desiccators and connected to air cylinder (Air) or low oxygen cylinder (1.5% O2) at a flow rate of 30–40 ml/min. For 1-MCP treatment, fifteen to twenty fruits were placed in 20 L desiccators and exposed to 10 μL/L 1-methylcyclopropene (EthylBloc from Biotechnologies for Horticulture Inc, Walterboro, SC, USA) for 18–20 h. 1-MCP was generated in the desiccators by placing required inert powder in the Eppendorf tube and adding water through rubber septum using a syringe. After 1-MCP treatment the desiccators were connected to air cylinder with the above flow rate. Depending on the experiments done the above desiccators sets were kept at 18 °C, 7 °C or 1 °C as mentioned in the figure legends. Immature green GS apple fruits kept in air at 18 °C were also subjected to ethylene (10 ppm) for 24 h and 48 h to study influence of exogenous ethylene. Fruits were removed from the desiccators at different time points, peeled and the cortex tissue was cut in small pieces and frozen in liquid N2. The frozen tissue was ground to a fine powder and stored at −70 °C until used.

2.2. Measurement of ethylene production

Two ml gas sample was withdrawn from continuous airflow from 20 L desiccators containing fifteen to twenty apples and injected into the gas chromatograph (HP, 6890 series) to measure ethylene every alternate day. The average of three readings from each desiccators was taken as measure of ethylene production for each set. Data are expressed as means±SD (standard deviation) of at least 3–4 replicates.

2.3. Total RNA isolation, cloning and sequencing of cDNA

Total RNA from apple fruit was isolated as described by Asif et al. (2006). RTPCR was performed using RTPCR kit (Invitrogen Inc., USA) as per manufacturer’s recommendations. Briefly, 2 μg total RNA was reverse transcribed by MuMLV Reverse Transcriptase at 42 °C using oligo(dT) primer. One tenth of the reaction was used for PCR product using degenerate or gene specific primers. The PCR products were cloned using TA Topo cloning kit (Invitrogen Inc., USA). To isolate full-length cDNA of the genes 5'- and 3'-RACE was performed using gene specific primers and respective kits (Invitrogen Inc., USA). The primers used for isolation of partial cDNA and 5'- and 3'-RACE are listed in Supplementary Table 1. Nucleotide sequence of each cDNA was established using M13 universal and reverse primers. Sequencing was carried out on an automated DNA sequencing system (ABI 377A, Applied Biosystems Inc., USA) using the dye terminator cycle sequencing kit. Sequence comparisons against databases were performed using BLAST and BLASTX algorithms (Altschul et al., 1990) at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov).

2.4. Northern blot analysis

Total RNA was resolved on 1.2% formaldehyde denaturing agarose gel and transferred to Hybond XL membrane (Amersham Biosciences, Buckinghamshire, NA, USA) according to the manufacturer’s protocol. The blot was prehybridized overnight at 42 °C in a mixture containing 50% formaldehyde, 1% SDS, 5X SSC and 5X Denhardts solution. Denatured 32P dCTP radiolabelled probes were added to the same but fresh hybridization solution. The probes were prepared to a specific activity of approximately 108 cpm μg−1 using Invitrogen RadPrime labeling kit, and at least 1 × 106 cpm were added per ml hybridization solution. The hybridization was carried out at 42 °C for 14–16 h. The washing of the blot was carried out in SSC plus 0.1% SDS. The final wash of the blots was done at
65 °C for 15 min in 0.1X SSC and 0.1% SDS and then exposed to Kodak XOMAT X-Ray film at −70 °C for 1 week.

2.5. Cloning of ERS1 promoter

Total DNA from young apple leaf was isolated according to Dellaporta et al. (1983) and a genome walker library was constructed using Universal genome walker kit (Clonetech). For the isolation of ERS1 proximal promoter gene specific primers from the 5′ region of ERS1 close to translational start site was designed and used for PCR amplification using the genome walker library as template. The amplified fragment was cloned in plasmid pCR2.1-TOPO (Invitrogen, USA) and sequenced. The search for conserved regulatory regions with other known sequences was carried using http://www.dna.affrc.go.jp/htdocs/PLACE/ a database of motifs found in cis-acting regulatory DNA elements of vascular plants (Higo et al., 1999).

2.6. Statistical analysis

Each experiment was carried out under completely randomized design with three replications repeated at least thrice. The data were analyzed by student’s unpaired t-test, and the treatment mean values were compared at $P \leq 0.05–0.001$.

3. Results

3.1. Effect of temperature, 1-MCP and low oxygen on storage of GS apple fruits

Ethylene production was monitored in different sets of experiments every alternate day. At the time of harvest GS apples did not produce any detectable ethylene (Fig. 1). The untreated fruit connected to air supply started producing detectable ethylene at day 5 at 18 °C (0.63±0.06 nl C2H4/g/h), day 12 at 7 °C (0.515±0.08 nl C2H4/g/h) and day 17 at 1 °C (0.67±0.05 nl C2H4/g/h). Fruits connected to 1.5% O2 supply started producing ethylene after 37 days (0.178±0.01 nl C2H4/g/h) at 18 °C, 60 days (0.19±0.02 nl C2H4/g/h) at 7 °C and 70 days (0.2±0.01 nl C2H4/g/h) at 1 °C respectively. 1-MCP treated fruits started producing detectable ethylene after 73, and 104 days at 18 °C and 7 °C respectively. 1-MCP treated fruits stored at 1 °C did not produce detectable ethylene even 196 days post harvest.

3.2. Expression pattern of ethylene biosynthesis and perception related genes

Expression pattern of ethylene biosynthesis and signal transduction genes was analyzed in fruits 35 days post harvest and kept at 1 °C under various conditions (Fig. 2). The expression of MdETR1, MdACO, MdACS3 and MdADH was observed in air, 1-MCP and 1.5% low oxygen treatments. However, the expression of MdACS1 and MdERS1 could be observed only fruits which were kept in air and had started producing ethylene. No detectable transcript for MdACS1 and MdRES1 was observed in fruits treated with 1-MCP or those connected to or low oxygen. No detectable transcript for MdACS2 is observed in our study. MdADH was taken in the experiment to verify that apples were sensing low oxygen as alcohol dehydrogenase is treated as marker for hypoxia. The

![Fig. 1. Ethylene production by GS apples stored under different treatments. Ethylene was measured every day from continuous airflow from desiccators containing apples using gas chromatography. The detectable ethylene produced for the first day after storage was taken as day for ethylene production in different treatments. Average of three readings was taken as measure of ethylene for each set. Data are expressed as means±SD of at least 3–4 replicates. ** and *** significantly different from air at $P \leq 0.01$ and 0.001, respectively, according to Student’s unpaired t-test.](image)

![Fig. 2. Accumulation of transcripts for different genes after 35 days post harvest under different treatments stored at 1 °C. C, control fruits connected to continuous air flow; L, fruits under continuous 1.5% O2 air flow; M, fruits pretreated with 1-MCP and connected to continuous air flow. Northern blot analysis was carried out using 30 µg total RNA samples electrophoresed and probed with respective radiolabelled probes. Ethidium bromide staining of gel is shown below the blot as loading control.](image)
increased expression of *MdADH* suggests that apples stored at low oxygen were sensing hypoxia.

3.3. Expression of *MdACS1* and *MdERS1* under long term storage

Ethylene production by apple fruits stored at 1 °C and connected either air, 1-MCP pretreated and connected to air or connected to low O2 is shown in Fig. 3. Stages I, II, III and IV have been designated as day post harvest ethylene production by fruits connected to air. Stage I represent 8 days post harvest when fruit kept in air did not produce any ethylene. Fruit connected to air started producing ethylene after 15 days post harvest which increased continuously and reached to 1.55 nl C2H4/g/h in 35 days post harvest (stage II) and 5.98 nl C2H4/g/h in 100 days post harvest (stage III). Ethylene production in fruit kept in air started decreasing after 100 days post harvest and reached to 2.25 nl C2H4/g/h after 196 days post harvest (stage IV). Low O2 storage of fruit delayed ethylene production and a detectable amount was observed only after 70 days post harvest which continuously increased to 2.25 nl C2H4/g/h after 196 days post harvest. 1-MCP pretreatment to fruits connected to air completely blocked ethylene production. However, when 1-MCP pretreated fruits were transferred to 18 °C, ethylene production increased rapidly and reached 18.3 nl C2H4/g/h within 32 days (data not shown).

Expression of differentially expressing genes, *MdACS1* and *MdERS1*, was studied through northern blot analysis at different time points during storage (stages I to IV) of the apple fruits (Fig. 3). The expression of *MdACS1* and *MdERS1* could be first observed only in air treated fruit at stage II. By stage III fruit treated with low O2 also showed the accumulation of *MdACS1* and *MdERS1* transcript. By the time the fruits reached stage IV, the air treated fruits were removed due to over ripening and severe scald formation. At stage IV, *MdERS1* transcript was observed, however no expression of *MdACS1* was observed in 1-MCP treated fruits. Transfer of 1-MCP pretreated fruits connected to air flow from 1 °C to 18 °C initiated ethylene

![Fig. 3](image-url)

**Fig. 3.** Ethylene production (A) and accumulation of *MdACS1* and *MdERS1* transcripts (B) at different stages of ripening during the storage of apple fruits under different conditions. Different stages of fruit ripening have been defined and described in the text. In (A) ■, ▲ and × represent fruits connected to continuous air flow, fruits under continuous 1.5% O2 air flow and fruits pretreated with 1-MCP and connected to continuous air flow respectively. ***significantly different at different time points from air at P ≤ 0.001 according to Student’s unpaired t-test. In (B) I, II, III and IV are different stages from (A) used to isolate RNA from different samples. C, control fruits connected to continuous air flow; L, fruits under continuous 1.5% O2 air flow; M, fruits pretreated with 1-MCP and connected to continuous air flow. Northern blot analysis was carried out using 30 µg total RNA samples electrophoresed and probed with respective radiolabelled probes. Ethidium bromide staining of gel is shown below the blot as loading control.
production which reached 18.3 \( \text{C}_2\text{H}_4/\text{g}/\text{h} \) with in 32 days. These fruits accumulated \( \text{MdACS1} \) and \( \text{MdERS1} \) transcripts (data not shown).

3.4. Regulation of \( \text{MdACS1} \) and \( \text{MdERS1} \) by ethylene

Air treated GS fruit stored at 1 °C that started producing ethylene (1.38 nl \( \text{C}_2\text{H}_4/\text{g}/\text{h} \)) were transferred to 18 °C. The ethylene production increased to 38.4 nl \( \text{C}_2\text{H}_4/\text{g}/\text{h} \) within 24 h (Fig. 4A). However, when some of these fruits were treated with 1-MCP, the ethylene production decreased to 3.8 nl \( \text{C}_2\text{H}_4/\text{g}/\text{h} \) within 24 h of the treatment. Air and 1-MCP treated fruits were harvested at this stage (S1) and 5 days later (S2). Transcripts of both \( \text{MdACS1} \) and \( \text{MdERS1} \) were abundant in fruits kept in air. The transcript of \( \text{MdACS1} \) was absent in the fruits treated with 1-MCP at S1 which appeared 5 days later, at S2, when the fruit were producing 12.1 nl \( \text{C}_2\text{H}_4/\text{g}/\text{h} \) ethylene. Transcripts of \( \text{MdERS1} \) were present at both stages of 1-MCP treated fruit, though lower than the controls.

Immature green GS apple fruits kept in air at 18 °C were exposed to ethylene for 24 h and 48 h and the status of transcript accumulation of \( \text{MdACS1} \) and \( \text{MdERS1} \) were monitored (Fig. 5). Neither air nor ethylene treated fruits produced ethylene till 11 days post treatment. After 11 days, fruits treated with ethylene for 24 and 48 h showed an increase in ethylene production i.e., 0.029 nl \( \text{C}_2\text{H}_4/\text{g}/\text{h} \) and 0.341 nl \( \text{C}_2\text{H}_4/\text{g}/\text{h} \) respectively. The control fruit and the fruit treated for 24 h also did not produce ethylene till 20 days, by which time they were developmentally competent enough to switch to ethylene production. Expression of \( \text{MdACS1} \) was observed only in fruits treated with ethylene for 48 h whereas \( \text{MdERS1} \) expression was observed in fruits treated with ethylene for both 24 and 48 h.

3.5. Promoter analysis of \( \text{ACS1} \) and \( \text{ERS1} \)

Expression analyses of \( \text{MdACS1} \) and \( \text{MdERS1} \) gene suggest that both genes are regulated by the levels of ethylene produced which might be due to a common regulatory mechanism for the expression of both the genes. To determine if promoters of both genes posses some common \( \text{cis} \)-acting elements, we cloned 797 bp proximal promoter of \( \text{MdERS1} \) gene (EMBL accession no. AY359467) and the sequence of the \( \text{MdACS1} \) promoter was downloaded from the genebank (EMBL accession no...
improved the quality and appearance of the fruits as compared to controlled complete scald development and substantially reduced ethylene production during post harvest storage and 1-MCP treatment in different cultivars of apples (Wang et al., 2007). However, the ethylene production in different cultivars varies considerably and since there were no reports of expression of these genes from GS apples, we studied the expression of these genes in various treatments. There are 3 ACC synthase genes (MdACS1, MdACS2 and MdACS3) which have been reported from apple with differential gene expression during ripening (Dong et al., 1991; Rosenfield et al., 1996). We studied the expression of these genes and transcripts of MdACS1 and MdACS3 could be detected in our study. Similar to our study, constitutive expression of MdACS3 was also observed in Golden Delicious and Fuji apples (Sunako et al., 1999). In our study, expression of MdACS1 was shown to be markedly affected by the application of 1-MCP and low O2 treatments (Fig. 2). The expression of MdACS1 has also been shown to be reduced by the application of 1-MCP, however, the intensity of reduction varies from cultivar to cultivar (Cin et al., 2005; Tatsuki et al., 2007). In the present study, the expression of MdACO1 did not change after 1-MCP or low oxygen treatments. These are in accordance with the observation of Tatsuki et al. (2007) in Fuji and Orin apples, where 1-MCP treatment did not affect the expression of MdACO1.

In our study, expression of MdERS1 was down-regulated by 1-MCP and low oxygen treatments whereas MdETR1 expression was not affected by these treatments. In other apple cultivars, 1-MCP treatment also decreased MdERS1 expression (Cin et al., 2006; Tatsuki et al., 2007). This suggests a role for this gene in ethylene production during ripening. When GS apples that were producing ethylene were treated with 1-MCP, ethylene production was significantly reduced (Fig. 4). This was accompanied by a decrease in the abundance of MdERS1 and MdACS1 transcripts, further supporting the notion that their expression is regulated by ethylene fluctuations. During ripening, transcripts of MdERS1 accumulated first, followed by MdACS1 transcripts (Figs. 3 and 5).

It could be inferred from these experiments that when the production of ethylene shifts from system-1 to system-2 to initiate ripening, the expression of MdERS1 increases. This affects the downstream processes and triggers induction of ethylene responsive genes, including ethylene biosynthesis genes like MdACS1 which in turn affect the production of ethylene during ripening.

The promoter analyses of MdACS1 and MdERS1 suggest that both the promoters contain ethylene responsive element (ERE) similar to tomato E4 (Montgomery et al., 1993) and signal transduction and ethylene biosynthesis genes during post harvest storage and 1-MCP treatment in different cultivars of apples.

### Table 1

<table>
<thead>
<tr>
<th>cis-acting element</th>
<th>Consensus sequence</th>
<th>Position with respect to ATG in ACS1 promoter</th>
<th>Position with respect to ATG in ERS1 promoter</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERELEE4</td>
<td>AWTTCAA</td>
<td>−1480 to −1487 to −309 to −317</td>
<td>Ethylene responsive element of tomato E4 and carnation</td>
<td></td>
</tr>
<tr>
<td>CURECORECR</td>
<td>GTAC</td>
<td>−1328 to −1324 to −860 to −864</td>
<td>Core of a copper response element; involved in oxygen-response of the genes</td>
<td></td>
</tr>
<tr>
<td>WBOXNTERF3</td>
<td>TGACT</td>
<td>−228 to −233 to −700 to −705</td>
<td>“W box” found in the promoter region of a transcriptional repressor ERF3 gene in tobacco</td>
<td></td>
</tr>
</tbody>
</table>

AY062129). Both the promoters were analysed in silico and the common cis-acting elements are shown in Table 1. Interestingly, both the promoters contain ethylene responsive element (ERE) similar to tomato E4 (Montgomery et al., 1993) and carnation GST1 (Izhaki et al., 1994) genes. Apart from ERE, both the genes contain core element of a CuRE (copper-response element) found in Cyc6 and Cpx1 genes in Chlamydomonas for the oxygen-response (Quinn et al., 2000).

### 4. Discussion

Granny Smith apples are prone to scald formation during post harvest storage leading to huge losses. Growers have been treating apples with the antioxidant diphenylamine (DPA) to combat this problem (Rudell et al., 2006). It has also been shown that low oxygen stress followed by storage under 1.0 KPa O2 could be a valid alternative to DPA against superficial scald formation in GS apples (Zanella, 2003). 1-MCP treatments also controlled complete scald development and substantially improved the quality and appearance of the fruits as compared to normal air and low O2 treatments (Tatsuki et al., 2007). All these strategies for post harvest storage of fruits are mainly concerned with either inhibition of ethylene production, perception, or ethylene scrubbing or dilution. We show here that a combination of low temperature with 1-MCP treatments and low oxygen treatment significantly decreases ethylene production and increases the post harvest life of these fruits.

1-MCP treatments have been carried out at different temperatures (1–20 °C), concentrations (from 20 nl l⁻¹ to 40 µl⁻¹), durations (4–24 h) and ripening stages at harvest of different fruits (Abdi et al., 1998; Dong et al., 2001a,b, 2002; Fan et al., 2002; Gong et al., 2002; Blankenship and Dole, 2003; Martinez-Romero et al., 2003; Valero et al., 2003; Salvador et al., 2004). However, its treatment in combination with low temperature thereby increasing the efficiency of 1-MCP.

There have been many reports on the expression of ethylene signal transduction and ethylene biosynthesis genes during post harvest storage and 1-MCP treatment in different cultivars of apples (Montgomery et al., 1993). However, the ethylene production in different cultivars varies considerably and since there were no reports of expression of these genes from GS apples, we studied the expression of these genes in various treatments. There are 3 ACC synthase genes (MdACS1, MdACS2 and MdACS3) which have been reported from apple with differential gene expression during ripening (Dong et al., 1991; Rosenfield et al., 1996). We studied the expression of these genes and transcripts of MdACS1 and MdACS3 could be detected in our study. Similar to our study, constitutive expression of MdACS3 was also observed in Golden Delicious and Fuji apples (Sunako et al., 1999). In our study, expression of MdACS1 was shown to be markedly affected by the application of 1-MCP and low O2 treatments (Fig. 2). The expression of MdACS1 has also been shown to be reduced by the application of 1-MCP, however, the intensity of reduction varies from cultivar to cultivar (Cin et al., 2005; Tatsuki et al., 2007). In the present study, the expression of MdACO1 did not change after 1-MCP or low oxygen treatments. These are in accordance with the observation of Tatsuki et al. (2007) in Fuji and Orin apples, where 1-MCP treatment did not affect the expression of MdACO1.

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carnation GST1 (Itzhaki et al., 1994) genes. Apart from ERE, both the genes contain core element of a CuRE (copper-response element) found in Cyc6 and Cpx1 genes in Chlamydomonas for the oxygen-response (Quinn et al., 2000). The W box, found in the promoter region of a transcriptional repressor ERF3 gene in tobacco is also present in both the genes at different locations. This is involved in activation of ERF3 gene by wounding (Nishiiuchi et al., 2004) and activation of genes for biosynthesis of ethylene. Presence of these common elements in the promoter region of MdACS1 and MdERS1 genes and our data on expression analysis using 1-MCP and low O2 suggest that a common regulatory mechanism may be operating for both the genes.

In this study, effort was made to understand the molecular basis of the increase in shelf life under the influence of temperature, low oxygen and 1-MCP in GS apple. The apple fruit were stored in different conditions either individually or in combination and levels of ethylene as well as transcript accumulation of the genes responsible for ethylene biosynthesis and ethylene receptors were analysed. The ethylene production was highly dependent on temperature, oxygen concentration and ethylene receptors were analysed. The ethylene production and 1-MCP and low O2 suggest that a common regulatory mechanism may be operating for both the genes.

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Appendix A. Supplementary data


References


