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Isolation and expression analysis of ethylene receptor genes from melon (*Cucumis melo*) fruit

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Abstract: Ethylene receptors are involved in ethylene sensing and signal transduction. This study was conducted to isolate genes encoding ethylene receptors from melon fruit and to determine their expression during postharvest fruit ripening. The amplification of melon fruit cDNA with degenerate primers revealed two partial cDNAs. Upon 3' and 5' RACE analysis of the cDNAs, two full length genes designated *Cm-ETR1* and *Cm-ETR2* were obtained. Cloning and sequencing of the genes showed that *Cm-ETR1* and *Cm-ETR2* genes were 2320 and 2537 bp in length, respectively. *Cm-ETR1* had a predicted open reading frame (ORF) of 2223 nucleotides encoding a protein of 740 amino acids. *Cm-ETR2* contained a predicted ORF of 2301 nucleotides encoding a protein of 766 amino acids. Structure and sequence analyses demonstrated that both genes had conserved domains found in ethylene receptor genes from other species, and were highly similar in terms of both nucleotide and amino acid sequences to the ethylene receptor sequences isolated from other species. The expression analyses of the genes revealed that the transcript levels of both receptor genes significantly increased during postharvest storage. The isolated receptor genes could be used to manipulate ethylene effects to delay the development of ethylene related physiological disorders and enhance the shelf life of melon fruit.

Key words: Ethylene, gene cloning, melon, receptor

1. Introduction

The plant hormone ethylene is sensed by receptors (Klee and Tieman, 2002). The expression of ethylene-induced genes is induced through the transduction of the ethylene signal from receptors to the corresponding transcription factors (Giovannoni, 2004). Ethylene signaling is a multistep pathway in which ethylene receptors function at the first step and play a crucial role in the regulation of ethylene responses (Giovannoni, 2004).

Ethylene receptors have been isolated from different plant species such as melon (Sato-Nara et al., 1999; Takahashi et al., 2002), tomato (Tieman and Klee, 1999), peach (Bassett et al., 2002), and pear (El-Sharkawy et al., 2003). The receptor proteins share a similar structural organization consisting of an N-terminal hydrophobic domain that contains three or four transmembrane segments and the ethylene binding sites, a GAF domain, and a histidine kinase domain. Moreover, the ETR-type receptors also have a receiver domain at the C-terminus involved in transmitting the ethylene signal.

Melon is a fruit showing climacteric ripening behavior. In climacteric fruit species, ethylene is effective in many physiological events during postharvest ripening. It directly affects factors influencing the shelf life of a fruit including softening, wooliness, watersoaking, and related deteriorations (Karakurt and Huber, 2002; Karakurt and Huber, 2004; Mao et al., 2004; Lima et al., 2005). Ethylene influences fruit ripening through involvement in chlorophyll degradation, increasing respiration rate, carotenoid synthesis, autocatalytic ethylene production, and the expression of genes that leads to the enhancement in the activities of enzymes responsible for starch to sugar conversion and cell wall degradation (Theologis, 1993; Wilkinson et al., 1997). This has been demonstrated through the use of ethylene antagonists and the inhibitors of ethylene synthesis, which have significantly enhanced the shelf life of fruit (Ergun et al., 2005). Thus, the manipulation of receptor genes could be a good alternative to control ethylene synthesis and response, delay ripening, and prevent ethylene related physiological disorders in fruits.

This study was performed to isolate and characterize ethylene receptor genes from melon fruit and determine their expression during postharvest ripening.

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2. Materials and methods

2.1. Plant material and RNA isolation

The melons (*Cucumis melo* L. cv ananas) used in the study were purchased from a local grower (Isparta, Turkey). Fruits were harvested at the commercial ripening stage (Ergun et al., 2005) and immediately transported to the laboratory at the College of Agriculture, Süleyman Demirel University, Isparta, Turkey. The fruits were washed, surface sterilized with 200 µL/L sodium hypochlorite solution, and stored at 20 °C for 10 days. Samples of fruit were taken from storage after 0, 1, 2, 4, 6, 8, and 10 days, frozen in liquid nitrogen, and used for Northern blot analysis (Karakurt and Huber, 2004). Fifteen melons were treated with 50 µL/L ethylene at 20 °C for 12 h to induce the synthesis of ethylene receptor genes. Samples were then taken from the placental tissue and used for RNA extraction. Total RNA was isolated as previously described (Strommer et al., 1993) with modifications (Karakurt and Huber, 2004). The concentration and quality of RNA were determined with a spectrophotometer (PG instruments) and agarose gel electrophoresis (Karakurt and Huber, 2004).

2.2. cDNA synthesis

The total RNA was treated with DNase in order to eliminate any residual DNA contamination using a commercial kit (Ambion). Then the DNase-treated RNA samples were converted to cDNA with an 'Advantage RT-PCR' kit (Clontech).

2.3. Isolation of receptor gene fragments

Degenerate primers (24–27 bp) were designed with the primer BLAST program using ethylene receptor gene sequences of *Arabidopsis thaliana*, *Cucumis melo*, *Solanum esculentum*, and *Citrullus vulgaris* (Accession numbers: AF1379, AF16250, U21952, U38666, U63291, L24119, U41103) and were synthesized (Metis Biyoteknoloji, Ankara, Turkey). Primers used in PCR reactions to isolate ethylene receptor genes were given in the supplemental file (Supplemental 1; on the journal's website).

PCR amplifications were performed with the degenerate primers and melon cDNA (El-Sharkawy et al., 2003). PCR samples consisted of 5 µL of cDNA, 2 µL of degenerate primers, 5 µL of 10X PCR buffer, 1 µL of dNTPmix (10 µM), 1 µL of Taq polymerase, and 34 µL of distilled water. PCR conditions were: denaturation at 95 °C for 1 min 30 s, annealing at 58 °C for 2 min, and extension at 72 °C for 1 min for 5 cycles; denaturation at 95 °C for 30 s, annealing at 58 °C for 1 min, and extension at 72 °C for 30 s for 15 cycles; denaturation at 95 °C for 30 s, annealing at 58 °C for 1 min, and extension at 72 °C for 2 min for 20 cycles; and then final extension at 72 °C for 7 min.

PCR amplification products were run on 1.2% agarose gel and two amplification products with molecular sizes of 1250 and 950 bp were obtained. These cDNA fragments were cut from the gel with a sterile razor blade and extracted from the gel using a NucleoTrap gel extraction kit (Clontech). The cDNAs were then cloned with a pGEMT-Easy cloning system (Promega). The recombinant colonies were grown overnight at 37 °C and their plasmids were isolated with a Wizard Plus SV Minipreps DNA Purification System (Promega) and sequenced (İontek, İstanbul, Turkey). The sequences were compared to those deposited in the NCBI GenBank with the BLAST program, and were confirmed to be ethylene receptor sequences (Altschul et al., 1997). Gene-specific primers were designed from these sequences and synthesized (Metis Biyoteknoloji). These primers were used in RACE analysis in order to obtain the full length sequences of the genes. RACE analysis was conducted using a SMART RACE cDNA amplification kit following the manufacturer's instructions (Clontech). Finally, complete full-length cDNAs were obtained using gene-specific primers prepared from both ends of the corresponding cDNA sequences determined above.

Multiple sequence alignment was performed using the Clustal W program and the phylogenetic tree was constructed using the neighbor-joining (NJ) method. The programs ExPasy (http://www.expasy.org/tools), PSORT (http://psort.hgc.jp/form.html), and SignalP (http://www.cbs.dtu.dk/services/SignalP) were used for the prediction of isoelectric point (pI), molecular weight, and signal peptides of the deduced proteins. The Conserved Domain Search (www.ncbi.nlm.nih.gov) program was used to determine the conserved motifs.

2.4. The expression analysis of receptor genes

Twenty micrograms of total RNA extracted as described above was used in Northern blot analysis to determine the expression of receptor genes during ripening at 20 °C (Karakurt and Huber, 2004). The receptor gene probes were labeled with DIG using a DIG High Prime labeling kit following the manufacturer's instructions (Roche). The results were analyzed with a gel imaging system (Kodak) and converted to numerical values. The expression levels of each transcript in the control fruit (day 0) were set to an arbitrary value of 1.0, to which all other time points were normalized to obtain an induction value (fold induction) (Karakurt and Huber, 2004). Quantitative gene expression data were analyzed according to a completely randomized design with three replications using the GLM program of the Statistical Analysis System (SAS, 1997).

3. Results

PCR of melon cDNA with degenerate primers resulted in the amplification of two cDNA fragments 1250 and 950 bp in size. The amplified fragments were cloned into pGEM-T easy cloning vectors and sequenced. The nucleotide and amino acid sequences of the two cDNA fragments demonstrated significant homologies to those

of ethylene receptor sequences isolated from other species. Primers were designed from these fragments and used in RACE analysis to obtain their full length sequences. The fragments amplified with 3' and 5' RACE analysis were cloned and sequenced. After their confirmation to be receptor sequences by sequence comparisons with BLAST, primers were designed from both ends of the sequences and used in a PCR to amplify the full length gene sequences. Then the full length sequences of these two genes were again cloned and sequenced. After the sequence analysis of the genes, they were designated *Cm-ETR1* and *Cm-ETR2*. The full length nucleotide sequences of these genes were submitted to the NCBI GenBank and given the accession numbers KJ150694 and KJ150695.

The sequence analysis showed that *Cm-ETR1* and *Cm-ETR2* consisted of 2320 and 2537 bp, respectively (Table 1). *Cm-ETR1* contained an open reading frame (ORF) of 2223 nucleotides encoding a predicted protein of 740 amino acids (Table 1). The gene also had 5' and 3' noncoding and a poly (A) tail consisting of 40, 49, and 8 nucleotides, respectively (Table 1). The gene called *Cm-ETR2* was composed of an ORF of 2301 nucleotides encoding a protein of 766 amino acids (Table 1). The 5' and 3' noncoding and poly (A) tail of the gene contained 18, 209, and 9 nucleotides, respectively (Table 1). At the nucleotide level, *Cm-ETR1* demonstrated 99%, 98%, and 97% homology to *Cucumis melo* var. *Cantalupensis Cm-ETR1, Cucumis melo* var. *reticulatus Cm-ETR1*, and *Cucumis sativus Cs-ETR1* genes, respectively (Table 2). The gene also showed 99%, 98%, and 86% homology to *Cucumis melo* var. *cantalupensis*, *Cucumis sativus*, and *Prunus persica* ETR1 amino acid sequences in terms of predicted ORF amino acid sequence, respectively (Table 3). Nucleotide-based homology search results showed that the *Cm-ETR2* gene showed 99%, 98%, and 98% homology at the nucleotide level to the ETR2 nucleotide sequences of *Cucumis melo* var. *cantalupensis CM-ETR2*, *Citrullus lanatus Cl-ETR2*, and *Cucumis sativus CS-ETR2* (Table 2). Likewise, the predicted amino acid sequence of CL-ETR2 demonstrated 99%, 99%, and 98% similarity to the ETR2-like protein sequences of *Cucumis melo* var. *cantalupensis*, *Cucumis sativus*, and *Citrullus lanatus* (Table 3).

Multiple alignments of full-length predicted Cm-ETR proteins with other reported ETR sequences confirmed the presence of conserved motifs and structural similarities that are commonly associated with ethylene

Table 1. The nucleotide and amino acid compositions of the ethylene receptor genes isolated from melon fruit.

Gene	Nucleotide (bp)	Amino acid	5' UTR (bp)	Coding region (bp)	3' UTR (bp)	Poli (A) tail (bp)
Cm-ETR1	2320	740	40	2223	49	8
Cm-ETR2	2537	766	18	2301	209	9

Table 2. Nucleotide-based homology search results of melon ETR genes.

Gene	Species	Homology (%)	E value
Cm-ETR1			
Cucumis melo var. *cantalupensis Cm-ETR1*	*Cucumis melo*	99	0.0
Cucumis melo var. *reticulatus Cm-ETR1*	*Cucumis melo*	98	0.0
Cucumis sativus Cs-ETR1	*Cucumis sativus*	97	0.0
Coffea canephora Cc-ETR1	*Coffea canephora*	82	0.0
Prunus persica Pp-ETR1	*Prunus persica*	80	0.0
Cm-ETR2			
Cucumis melo var. *Cantalupensis Cm-ETR2*	*Cucumis melo*	99	0.0
Citrullus lanatus Cl-ETR2	*Citrullus lanatus*	98	0.0
Cucumis sativus Cs-ETR2	*Cucumis sativus*	98	0.0
Pyrus pyrifolia Pp-ETR2	*Pyrus pyrifolia*	78	0.0
Pyrus comminus putative ethylene receptor	*Pyrus comminus*	77	0.0

Table 3. Homology search results of melon receptor proteins.

Protein	Species	Homology (%)	E value
Cm-ETR1			
Cucumis melo var. *cantalupensis* Cm-ETR1	*Cucumis melo*	99	0.0
Cucumis sativus Cs-ETR1	*Cucumis sativus*	98	0.0
Prunus persica Pp-ETR1	*Prunus persica*	86	0.0
Arabidopsis thaliana At-ETR1	*Arabidopsis thaliana*	82	0.0
Brassica oleracea Bo-ETR1	*Brassica oleracea*	81	0.0
Cm-ETR2			
Cucumis melo var. *Cantalupensis* Cm-ETR2	*Cucumis melo*	99	0.0
Cucumis sativus Cs-ETR2	*Cucumis sativus*	99	0.0
Citrullus lanatus Cl-ETR2	*Citrullus lanatus*	98	0.0
Prunus persica Pp-ETR2	*Prunus persica*	79	0.0
Pyrus comminus putative ethylene receptor	*Pyrus comminus*	78	0.0

receptors (Supplementals 2 and 3; on the journal's website; Figures 1 and 2). Cm-ETR1 predicted protein contained GAF, histidine kinase, HATPase C superfamily (histidine kinase-like ATPases), and signal receiving domains (Figure 1). Moreover, the gene had in its histidine kinase domain an ATP binding site, a Mg binding site, and a phosphorylation site; and in its signal receiving domain phosphorylation, dimerization, and intermolecular recognition sites. Transmembrane domain analysis of the gene showed that it contained three transmembrane helices (Figure 1). Additionally, the amino end of the protein had highly conserved amino acid sequences. The protein

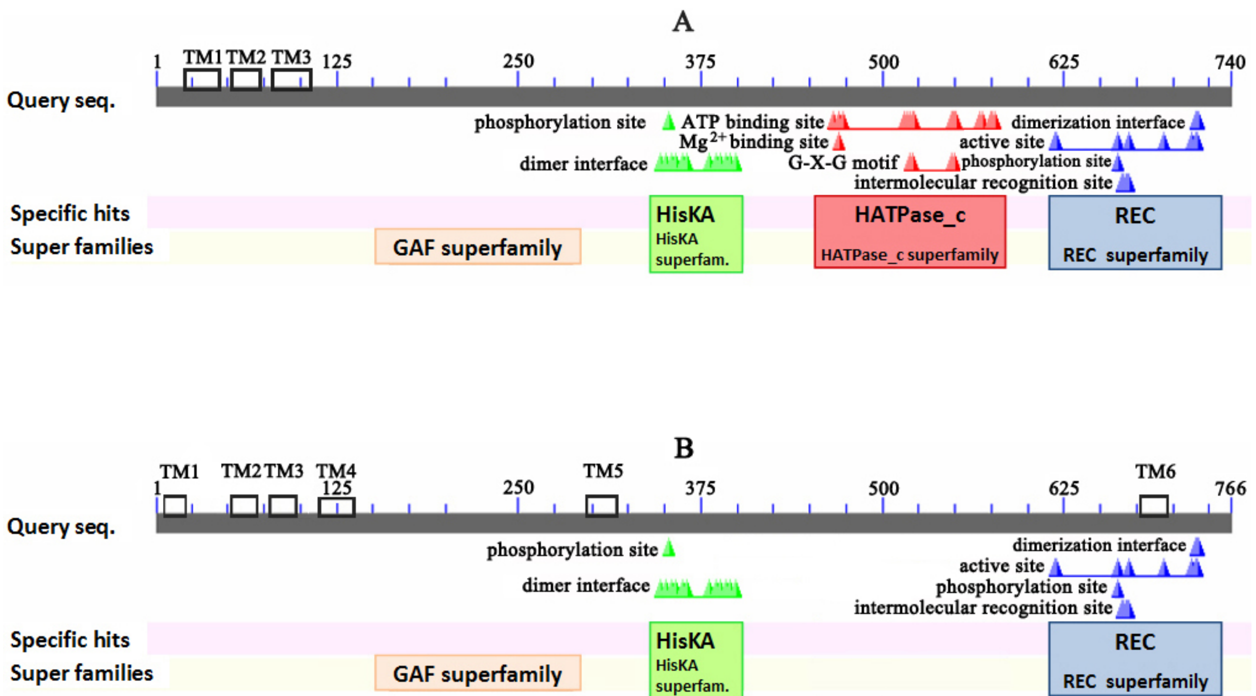

Figure 1. Domain organizations of *Cm-ETR1* (A) and *Cm-ETR2* (B) receptor proteins.
TM: Transmembrane domain

encoded by the *Cm-ETR2* gene had a GAF domain, a histidine kinase domain, and a receiver domain. While the histidine kinase domain possessed a phosphorylation site, the receiver domain had phosphorylation, dimerization, and intermolecular recognition sites (Figure 1). Moreover, there were six transmembrane helices in *CL-ETR2* (Figure 1). It is noted that Mg and ATP binding sites and the HTPase-C-like domain found in ETR1-like genes were not available in the *CL-ETR2* gene. Therefore, the isolated genes structurally resemble their *Arabidopsis* homologs. Moreover, cluster analysis demonstrated that *Cucumis melo* Cm-ETR1 and *Cucumis sativus* ETR1 proteins showed more similarity to each other and were in the same group, while *Cucumis melo* ETR2 protein showed high homology to *Cucumis sativus* ETR2 proteins (Figure 2).

PSORT and SignalP analyses showed that Cm-ETR1 did not contain a signal peptide and were localized in plasma membrane, but Cm-ETR2 contained a signal sequence in its amino terminus and were localized with its carboxyl end outside and amino end inside the cytosol. Cm-ETR1 predicted hydrophobic protein had a molecular weight of approximately 83 kDa and a pI of 7.64, and contained 80 negatively and 81 positively charged amino acids. On the other hand, Cm-ETR2 predicted hydrophobic protein had a molecular weight of approximately 85 kDa and a pI of 7.86, and possessed 79 negatively and 81 positively charged amino acids.

3.1. The expression of receptor genes

To determine the expression levels of receptor genes during postharvest storage, melons were stored at 20 °C for 10 days, and fruit samples were taken at different stages of the storage and used for Northern blot analysis. The results showed a significant variation among various storage periods in the accumulation of transcripts (Figure 3; Supplemental 4; on the journal's website). The mRNA levels of both receptor genes started to increase from the

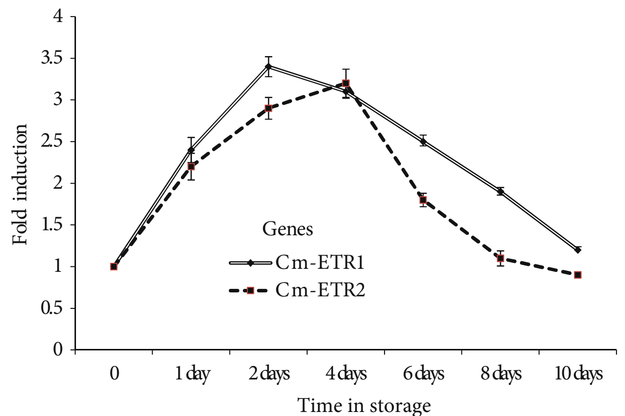

Figure 3. The changes (fold induction as compared to the control) in the mRNA transcript levels of two isolated ethylene receptor genes during storage at 20 °C. Data show the average of three replications and standard deviation.

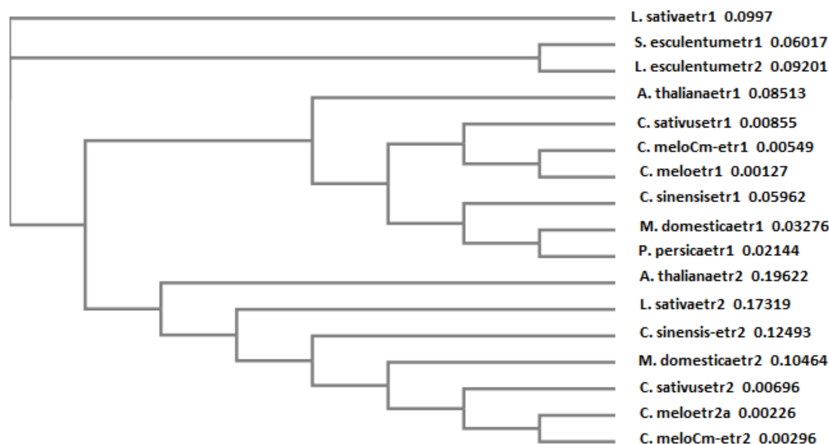

Figure 2. Phylogenetic tree of ETR1-like and ETR2-like ethylene receptors isolated from different plant species. The amino acid sequences were compared using the CLUSTAL Omega multiple alignment program, and the phylogenetic tree was generated using the neighbor-joining (NJ) method. The lengths of the branches are indicated on the right. Accession numbers in GenBank sequence databases are as follows: *AtETR1* (NM_105305) and *AtETR2* (NM_113216) in *Arabidopsis thaliana*; Ls-*ETR1* (AF350321) and Ls-*ETR2* (AF350322) in *Lactuca sativa*; *CsETR1* (NM_001280633) and *CsETR2* (AB026500) in *Cucumis sativus*; *CMeETR1* (AF054806), *CmETR1* (KJ150694), *CMeETR2a* (AB294513), and *CmETR2* (KJ150695) in *Cucumis melo*; *LeETR2* (AY600436) and S*eETR1* (AF043084) in *Lycopersicon esculentum*; *CsETR1* (GQ339592) and *CsETR2* (GQ339593) in *Citrus sinensis*; *MdETR1* (AF032448) and *MdETR2* (DQ847145) in *Malus domestica*; and *PpETR1* (AF124527) in *Prunus persica*.

early hours of storage and these increases continued up to the second day of storage in *Cm-ETR1* and up to day four of storage in *Cm-ETR2*. In general, the increase in *Cm-ETR1* transcripts was much higher than that of *Cm-ETR2*. For example, on the second day of storage, the transcript level of *Cm-ETR1* increased 3.4-fold as compared to the control (day 0). However, the increase in the mRNA level of *Cm-ETR2* was 2.9-fold and on the fourth day of storage 3.2-fold. The mRNA levels of *Cm-ETR1* and *Cm-ETR2* started to decrease on the second and fourth days of storage, respectively.

4. Discussion

Ethylene, a phytohormone, coordinates the plant's physiological responses to physiological events such as senescence, fruit ripening, and abscission, and environmental stresses including water deficit, mechanical wounding, and pathogen infection (Lelievre et al., 1997). Ethylene is received by proteins called ethylene receptors. Ethylene receptors form the first step of the ethylene signal response pathway and are the negative regulators of the pathway (Kieber et al., 1993). This study was conducted to isolate and characterize ethylene receptor genes from melon fruit, and determine their expression during postharvest ripening. At the end of the study, the full length nucleotide sequences of two ethylene receptor genes designated as *Cm-ETR1* and *Cm-ETR2* were isolated and characterized from melon fruit. The isolated genes showed significant homologies to the ethylene receptor genes previously isolated from various plant species such as watermelon, peach, tomato, and *Arabidopsis*. The genes showed 70%–99% homology both at the nucleotide and amino acid levels to the receptor genes isolated from *Cucumis melo*, *Cucumis sativus*, *Pyrus communis*, *Prunus persica*, *Prunus salicina*, *Prunus domestica*, *Zizyphus jujuba*, *Lycopersicon esculentum*, and *Arabidopsis thaliana* (Yamasaki et al., 2000; Wang et al., 2006; El-Sharkawy et al., 2007).

The two genes possessed an ethylene binding domain that uses Cu as cofactor and required for high affinity ethylene binding (Rodriguez et al., 1999) a conserved histidine kinase domain and a GAF domain (Wang et al., 2006). Only *Cm-ETR1* had the ethylene response regulator domain. A similar finding was reported from ETR1 genes previously characterized from species such as watermelon, cucumber, pear, peach, and plum (Guo and Ecker, 2004; El-Sharkawy et al., 2007). Moreover, three membrane binding sites reported for ETR1 genes isolated from other species were determined in the *Cm-ETR1* gene of melon. However, unlike ETR2 of *Arabidopsis*, which had four membrane binding sites, six potential membrane-binding sites were observed in melon *Cm-ETR2*. *Cm-ETR1* and *Cm-ETR2* genes also had a conserved aspartate amino acid found in the autophosphorylated receiver domain of bacterial two component receptors and a GAF domain. The aspartic amino acid in the receiver domain of the protein functions as the phosphorus binding site to conserved histidine in the ETR1 receiver sequences (Gamble et al., 1998). The sequence analysis of predicted proteins of the isolated genes (*Cm-ETR1* and *Cm-ETR2*) showed that they possessed all amino acids required for receptor function, suggesting their participation in ethylene response and signal transduction (Rodriguez et al., 1999; Wang et al., 2006). Moreover, the comparison of the sequences of isolated genes with those of ETR1 and ETR2-like sequences showed that the amino acids at the N-terminus were conserved.

The isolated genes also shared similar expression trends during postharvest storage. The mRNA transcript levels of both genes showed significant increases during ripening in storage, possibly resulting from the increase in ethylene production of melon fruit. Although we did not measure the ethylene production of melon fruit during storage, increases in ethylene production during ripening have been reported for different melon cultivars (Ergun et al., 2005; Pech et al., 2008; Li et al., 2011). Moreover, it has been reported that there was a correlation between the increase in ethylene production and the increase in the transcript levels of *ETR1* and *ETR2* genes in cucumber and melon (Sato-Nara et al., 1999; Yamasaki et al., 2000; Cin et al., 2006). The increase in mRNA levels of ethylene receptors in ripening melon fruit is a natural response against increased ethylene biosynthesis to maintain the homeostatic balance and a mechanism to regulate ethylene responses (Klee et al., 2003). The expressions of ethylene receptors associate with the function and thus the capacity of the plant tissue to respond to ethylene. The fruit of the *cucurbitaceae* family such as melon and watermelon also develop watersoaking, a physiological disorder upon ethylene exposure (Karakurt and Huber, 2002; Ergun et al., 2005). The application of ethylene to melon fruit cv ananas resulted in the development of the watersoaking disorder after 9 days of storage. The ethylene treated fruit also showed 5- and 6-fold increases in mRNA transcript levels of *Cm-ETR1* and *Cm-ETR2*, respectively, after 24 h of storage (data not shown), suggesting that ethylene receptors contribute significantly to the development of the disorder. Although the functions of *Cm-ETR1* and *Cm-ETR2* genes have not been determined, their homology to the ethylene receptor genes from other species suggests that they serve similar functions. Further studies are required in order to fully determine the roles of isolated genes in fruit ripening and the development of physiological disorders.

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Supplemental 1. Primers used in PCR reactions.

Primer	Usage	Sequence
ETR1-F	PCR for gene fragments	5'-gagacgggg[atc]ag[ag]catgt[agct]ag[ag]atg-3'
ETR1-R	PCR for gene fragments	5'-catggg[ac]gttctcatttcatg[ag]ttcat-3'
ETR2-F	PCR for gene fragments	5'-cag aat tgt gcg gtt tgg atg ccg-3'
ETR2-R	PCR for gene fragments	5'-cac aac ttt aac aat ctc aat ctc ctg-3'
ETR1-F	PCR for full-length gene	5'-ctatttgtccatttaacatccgct-3'
ETR1-R	PCR for full-length gene	5'-agattgtccctcaacgcacatgta-3'
ETR2-F	PCR for full-length gene	5'-ggttcggcttcccccggatacaat-3'
ETR2-R	PCR for full-length gene	5'-taccatatgttatcaacagggttc-3'

Supplemental 2. Multiple amino acid sequence alignment of Cm-ETR1 and other receptor proteins.

```
Cmeloetr1           ---------------MENCYCIEPQWPADELLMKYQYISDFFIALAYFSIPLELIYFVKK
Csativusetr1        ---------------METCYCIEPQWPADELLMKYQYISDFFIALAYFSIPLELIYFVKK
Mdomesticaetr1      ---------------MLACNCIEPQWPADELLMKYQYISDFFIALAYFSIPLELIYFVKK
Ppersicaetr1        ---------------MEACNCIEPQWPADELLMKYQYISDFFIALAYFSIPLELIYFVKK
Athalianaetr1       ---------------MEVCNCIEPQWPADELLMKYQYISDFFIAIAYFSIPLELIYFVKK
Csinensisetr1       ---------------MESCNCIEPQWPADELLMKYQYISDFFIALAYFSIPLELIYFVKK
Sesculentumetr1     MGSLLRMNRLLSSIVESCNCIIDPQLPADDLLMKYQYISDFFIALAYFSIPVELIYFVKK
Lsativaetr1         ---------------MDSCNCIEPQWPADELLMKYQYISDFFIALAYFSIPLELIYFVKK
                                         *:** ***:**************:******:********

Cmeloetr1           SAVFPYRWVLVQFGAFIVLCGATHLINLWTFTMHSRTVAVVMTTAKVLTAVVSCATALML
Csativusetr1        SAVFPYRWVLVQFGAFIVLCGATHLINLWTFTMHSRTVAVVMTTAKVLTAVVSCATALML
Mdomesticaetr1      SAVFPYRWVLVQFGAFIVLCGATHLINLWTFSIHSRTVAMVMTTAKVLTAVVSCATALML
Ppersicaetr1        SAVFPYRWVLVQFGAFIVLCGATHLINLWTFSMHSRTVAIVMTTAKVLTAVVSCATALML
Athalianaetr1       SAVFPYRWVLVQFGAFIVLCGATHLINLWTFTTHSRTVALVMTTAKVLTAVVSCATALML
Csinensisetr1       SAVFPYRWVLVQFGAFIVLCGATHLINLWTFNMHSRTVAIVMTTAKVLTAVVSCATALML
Sesculentumetr1     SAVFPYRWVLVQFGAFIVLCGATHLINLWTFNMHTRNVAIVMTTAKALTALVSCITALML
Lsativaetr1         SAVFPYRWVLVQFGAFIVLCGATHLINLWTFNAHTRTVAIVMTTAKVLTAAVSCATALML
                    *******************************. *:*.**:******.*** *** *****

Cmeloetr1           VHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKT
Csativusetr1        VHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKT
Mdomesticaetr1      VHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKT
Ppersicaetr1        VHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKT
Athalianaetr1       VHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKT
Csinensisetr1       VHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRHVRMLTHEIRSTLDRHTILKT
Sesculentumetr1     VHIIPDLLSVKTRELFLKKKAAQLDREMGIIRTQEETGRHVRMLTHEIRSTLDRHTILKT
Lsativaetr1         VHIIPDLLSVKTRELFLKNKAAQLDREMGIIRTQEETGRHVRMLTHEIRSTLNRHTILKT
                    ******************:***:******:**********************:*******

Cmeloetr1           TLVEMGRTLALEECALWMPTRTGLELQLSYTLQQQNPVGYTVPINLPVISQVFSSNRALK
Csativusetr1        TLVELGRTLALEECALWMPTRTGLELQLSYTLHQQNPVGYTVPINLPVISQVFSSNRAVK
Mdomesticaetr1      TLVELGRTLALEECALWMPTRTGLELQLSYTLRQQNPVGYTVPIHLPVINQVFSSNRAVK
Ppersicaetr1        TLVELGRTLALEECALWMPTRTGLELQLSYTLRQQNPVGYTVPIHLPVINQVFSSNRALK
Athalianaetr1       TLVELGRTLALEECALWMPTRTGLELQLSYTLRHQHPVEYTVPIQLPVINQVFGTSRAVK
Csinensisetr1       TLVELGRTLALEECALWMPTRTGLELQLSYTLRQQNPVGYTVPIQLPVINQVFSSNHAVK
Sesculentumetr1     TLVELGRTLALEECALWMPTRTGLELQLSYTLRHQNPVGLTVPIQLPVINQVFGTNHVVK
Lsativaetr1         TLVELGRTLGLEECALWMPTRSGLELQLSYTLRHQNPVGFTVPIQSPAINQVFSTNRAVK
                    ****:****.***********:**********::*:**  ****: *.*.***.:.:.:*

Cmeloetr1           ISPNSPVASLRPRAGRYVAGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSA
Csativusetr1        ISPNSPVASLRPRAGRYVAGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSA
Mdomesticaetr1      ISANSPVAKLRQLAGRHIPGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSA
Ppersicaetr1        ISPNSPVARMRPLAGKHMPGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSA
Athalianaetr1       ISPNSPVARLRPVSGKYMLGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSA
Csinensisetr1       ISPNCPVARLRPLAGKYMPGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSA
Sesculentumetr1     ISPNSPVARLRPAG-KYMPGEVVAVRVPLLHLSNFQINDWPELSTKRYALMVLMLPSDSA
Lsativaetr1         ISPTSPVARLRPASGKYMLGEVVAVRVPLLHLNNFQIYDWPELSTKRYALMVLMLPSDSA
                    **...*** :*  . ::: *************.**** **********************

Cmeloetr1           RQWRVHELELVEVVADQVAVALSHAAILEESMRARDRLMEQNVALDLARREADTAIRARN
Csativusetr1        RQWRVHELELVEVVADQVAVALSHAAILEESMRARDPLMEQNVALDLARREAETANHARN
Mdomesticaetr1      RQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNIALDLARREAETAIRARN
Ppersicaetr1        RQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNIALDLARREAETAIRARN
Athalianaetr1       RQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAETAIRARN
Csinensisetr1       RQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMQQNIALDSARREAETAIRARN
Sesculentumetr1     RQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAEMAVRARN
Lsativaetr1         RQWHVHELELVEVVADQVAVALSHAAILEESMRARDLLMEQNVALDLARREAETAVRARN
                    ***:******************************** **:**:*** *****: * :***
```

*Conserved amino acids
.:Similar amino acids

Supplemental 2. (Continued).

```
Cmeloetr1           DFLAVMNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLINDVLDLSRL
Csativusetr1        DFLAVMNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLINDVLDLSRL
Mdomesticaetr1      DFLAVMNHEMRTPMHAIIALSSLLQETELTAEQRLMVETILRSSNLLATLINDVLDLSRL
Ppersicaetr1        DFLAVMNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSHLLATLINDVLDLSRL
Athalianaetr1       DFLAVMNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLMNDVLDLSRL
Csinensisetr1       DFLAVMNHEMRTPMHAIIALSSLLQETELTPEQRLMVETILKSSNLLATLINDVLDLSRL
Sesculentumetr1     DFLAVMNHEMRTPMHAIIALSSLLQETDLTPEQRLMVETILKSSNLLATLINDVLDLSRL
Lsativaetr1         DFLAVMNHEMRTPMHAIIALSSLLQETDLTPEQRLMVETILKSSNLLATLINDVLDLSRL
                    ***************************:**.**********:**:*****:*********

Cmeloetr1           EDGSLQLDIGTFNLHAVFKEVLNLIKPVTLVKKLSLTLHLGPDLPVFAVGDEKRLMQAIL
Csativusetr1        EDGSLQLDIGTFNLHAVFKEVLNLIKPVTLVKKLSLTLHLGLDLPVFAVGDEKRLMQAIL
Mdomesticaetr1      EDGSLQLEIATFNLHSVFREVHNMIKPVASIKRLSVTLNIAADLPMYAIGDEKRLMQTIL
Ppersicaetr1        EDGSLQLEIATFNLHSVFREVHNLIKPVASVKKLSVSLNLAADLPVQAVGDEKRLMQIVL
Athalianaetr1       EDGSLQLELGTFNLHTLFREVLNLIKPIAVVKKLPITLNLAPDLPEFVVGDEKRLMQIIL
Csinensisetr1       EDGSLQLQIGTFNLHAVFREVLNLIKPIASVKKLLVALNLAPDLPEYAVGDEKRLMQTLL
Sesculentumetr1     EDGSLQLDIGTFNLHALFREVHSLIKPIASVKKLFVTLSLSSDLPEYVIGDEKRLMQILL
Lsativaetr1         EDGSLELDTTTFNLHALFKEVLNLIRPVASVKRLFVTLSLSSDLPEYAVGDEKRLMQIIL
                    *****:*:  *****::*:** .:*:*:: :*:* ::* :. ***  .:******** :*

Cmeloetr1           NVVGNAVKFSKEGSISISAIVAKSETFREIRVPDFHPVPSDRHFYLRVQVKDTGSGISPQ
Csativusetr1        NVVGNAVKFSKEGSISISAIVAKAETFREIRVPDFHPVPSDSHFYLRVQVKDTGSGISPQ
Mdomesticaetr1      NVVGNAVKFSKEGSISITAFVAKSESLRDFRAPDFFPVQSDNHFYLRVQVKDSGSGINPQ
Ppersicaetr1        NVVGNAVKFSKEGSISITAFVAKSESLRDFRAPEFFPAQSDNHFYLRVQVKDSGSGINPQ
Athalianaetr1       NIVGNAVKFSKQGSISVTALVTKS----DTRAADFFVVPTGSHFYLRVKVKDSGAGINPQ
Csinensisetr1       NVVGNAVKFTKEGNISITGFVAKSESLRDSRAPEFFPVPIENHFYLRVQVRDSGSGISPQ
Sesculentumetr1     NVVGNAVKFSKEGNVSISAFVAKSDSLRDPRAPEFFAVPSENHFYLRVQIKDTGIGITPQ
Lsativaetr1         NIIGNAVKFSKEGSISISAIMAKPDSLRDPRAPDFFPLLSDNNFFLRVQVKDTGMGISQQ
                    *::******:*:*.:*::.:::*.    : *..:*.      :*:***:::*:* **. *

Cmeloetr1           DIPKLFTKFAQTTVGPR-NSGGSGLGLAICKRFVNLMEGHIWLESEGLGKGCTATFIVKL
Csativusetr1        DIPKLFTKFAQTTVGPR-NSCGSGLGLAICKRFVNLMEGHIWLESEGLGKGCTATFIVKL
Mdomesticaetr1      DIPKLFTKFAQTQALATRNSGGSGLGLAICKRFVNLMEGHIWIESEGLGKGCTATFIVKL
Ppersicaetr1        DIPKLFTKFAQTQSLATRNSGGSGLGLAICKRFVNLMEGHIWIESEGPGKGCTAIFIVKL
Athalianaetr1       DIPKIFTKFAQTQSLATRSSGGSGLGLAISKRFVNLMEGNIWIESDGLGKGCTAIFDVKL
Csinensisetr1       DIPNLFTKFAQNQAIALRNSSGSGLGLAICKRFVNLMEGHIWIESEGLGKGCTAIFIVKL
Sesculentumetr1     DIPNLFSKFTQSQALATTNSGGTGLGLAICKRFVNLMEGHIWIESEGLGKGSTAIFIIKL
Lsativaetr1         DMPKLFTKFAESQSPATRNPGGSGLGLAICKRFVNLMEGNIWIESEGLGKGSTAIFFVKL
                    *:*::*:**::.   .  .. *:******.*********:**:**:* ***.** * :**

Cmeloetr1           GIADQSNESKLPYTSKIHENSIHTSFPGLKVLVMDDNGVSRSVTKGLLVHLGCEVTTAGS
Csativusetr1        GIAEQSNESKLPFTSKIHENSIHTSFPGLKVLVMDDNGVSRSVTKGLLVHLGCEVTTAGS
Mdomesticaetr1      GFPERSNESKLPFAPKLQANHVQTNFPGLKVLVMDDNGVSRSVTKGLLAHLGCDVTAVSL
Ppersicaetr1        GFAERSNESKLPFLTKVQANHVQTNFPGLKVLVMDDN---GSVTKGLLVHLGCDVTTVSS
Athalianaetr1       GISERSNESKQSGIPKVPAIPRHSNFTGLKVLVMDENGVSRMVTKGLLVHLGCEVTTVSS
Csinensisetr1       GIPEHSNDSNLSFIPKMPVHGQTN-FPGLKVLVMDENGVSRSVTKGLLLHLGCDVMTVSS
Sesculentumetr1     GIPGRANESKLPFVTKLPANHTQMSFQGLKVLVMDENGVSRMVTKGLLTHLGCDVTTVGS
Lsativaetr1         GFPSRLNGSRLP-HMRVPAKLGQTKFPGLKVVVVDDNGVSRTATKGLLVHLGCDVTTVSS
                    *:. : * *. .   ::        * ****:*:*:*     .***** ****:*  :..

Cmeloetr1           IEEFLRVVSQEHKVVFMDICTPGVDGYELAIRIREKFAKC--HERPFMVVLTGNSDKVTK
Csativusetr1        IEEFLRVVSQEHKVVFMDICTPGVDGYELAIRIREKFAKC--HERPFMVVLTGNSDKVTK
Mdomesticaetr1      IDELLHVISQEHKVVFMDVSMPGIDGYELAVRIHEKFTKR--HERPVLVALTGSIDKITK
Ppersicaetr1        IDEFLHVISQEHKVVFMDVCMPGIDGYELAVRIHEKFTKR--HERPVLVALTGNIDKMTK
Athalianaetr1       NEECLRVVSHEHKVVFMDVCMPGVENYQIALRIHEKFTKQR-HQRPLLVALSGNTDKSTK
Csinensisetr1       VEECFQVVSHEHQVVFMDVCVPGIDGYEVAVHIHDKFTRR--HERPLIVALTGSTDNLTK
Sesculentumetr1     RDECLRVVTHEHKVVIMDVSMQGIDCYEVAVVIHERFGKR--HGRPLIVALTGNTDRVTK
Lsativaetr1         GEECLQAITKDSYKVVFIDVSLSSDAYNVARLVHEKLPKRHEKPPPLIVGLTGNTDKAMK
                     :* ::.::::   *.:     . : *::*  ::::: :   :  *.:* *:*. *.  *
```

*Conserved amino acids

.:Similar amino acids

Supplemental 2. (Continued).

```
Cmeloetr1            ESCLRAGMDGLILKPVSIDKMRSVLSELIERRVLFETS
Csativusetr1         ESCLRAGMDGLILKPVSIDKMRSVLSELIERRVLFETS
Mdomesticaetr1       ENCMRVGVDGVILKPVSVDKMRSVLSELLEHRVLFEAM
Ppersicaetr1         ENCMRVGMDGVILKPVSVDKMRSVLSELLEHRVLFEAM
Athalianaetr1        EKCMSFGLDGVLLKPVSLDNIRDVLSDLLEPRVLYEGM
Csinensisetr1        DNCMRVGMDGVILKPVSLEKMRSVLSDLLEHRVLFESM
Sesculentumetr1      ENCMRVGMDGVILKPVSVYKMRSVLSELLEHGVVLES-
Lsativaetr1          ESLLRAGMDGLVLKPVSVEKMRLALSELLEHNT-----
                     :. :  *:**::*****: ::* .**:*:*  .
```

*Conserved amino acids
.:Similar amino acids

Supplemental 3. Multiple amino acid sequence alignment of Cm-ETR2 and other receptor proteins.

```
Cmeloetr2           MLKALPSGFLILLLLASVSAAD-----NGFPRCNCDDEGS-LWSIDSILECQRVSDFLIA
Csativusetr2        MLKALPSGFLILLLLASVSAAD-----NGFPRCNCDDEGS-LWSIDSILECQRVSDFLIA
Mdomesticaetr2      MLKALASSLSISLLLFCVSASD-----NGFPRCNCDDDGS-WWSIESILECQRVSDFLIA
Csinensisetr2       MLRALAVGLVVSSLLISVSVVDGD---NNFPRCNCDDDAS-SWSIESILETQKVSDFLIA
Lsativaetr2         MSKTLASLGLISLLLVGVLAVDDDGSENGFGGCNCEVEG--FFGYRNIMETQRVSDFLIA
Athalianaetr2       MVKEIASWLLILSMVVFVSPVLAING-GGYPRCNCEDEGNSFWSTENILETQRVSDFLIA
Lesculentumetr2     ------------------------------MDCNCFDPLL--PADELLMKYQYISDFFIA
                                                     ***         .    ::: * :***:**

Cmeloetr2           VAYFSIPIELLYFVSCSNVP-FKWVLFQFIAFIVLCGLTHLLNGWTYGPHSFQLMLALTV
Csativusetr2        VAYFSIPIELLYFVSCSNVP-FKWVLFQFIAFIVLCGLTHLLNGWTYGPHSFQLMLALTV
Mdomesticaetr2      VAYFSIPIELLYFVSCSNVP-FKWVLFQFIAFIVLCGLTHLLNGWTYGPHPFQLMLALTV
Csinensisetr2       VAYFSIPVELLYFISCSNVP-FKWVLIQFIAFIVLCGLTHLLNGWTYGPHSFQLMLSLTV
Lsativaetr2         VAYFSIPIELLYFVSCSNVP-FKWVLFQFIAFIVLCGMTHLLNGWTYEPHPFQLMLALTI
Athalianaetr2       VAYFSIPIELLYFVSCSNVP-FKWVLFEFIAFIVLCGMTHLLHGWTYSAHPFRLMMAFTV
Lesculentumetr2     VAYFSIPIELVYFVQKSAVFPYRWVLVQFGAFIVLCGATHLINLWTSTPHTRTVAMVMTT
                    *******:**:**:.  * *  ::***.:* ******* ***:: **   .*.  : : :*

Cmeloetr2           FKILTALVSCATAITLITLIPLLLKVKVREFMLKKKTWDLGREVGMILKQKEAGLHVRML
Csativusetr2        FKILTALVSCATAITLITLIPLLLKVKVREFMLKEKTWDLGREVGMILKQKEAGLHVRML
Mdomesticaetr2      FKILTALVSCATAITLITLIPLLLKVKVREFMLKKKTWDLGREVGIIMRQKEAGMHVRML
Csinensisetr2       FKILTALVSCATSITLITLIPLLLKVKVREFMLKKKAWDLGREVGIIMKQKEAGVHVRML
Lsativaetr2         FKFLTALVSFATAITLVTLIPLLLKVKVREFMLRKKTWDLGVEMGMIKKQKEAGWHVRML
Athalianaetr2       FKMLTALVSCATAITLITLIPLLLKVKVREFMLKKKAHELGREVGLILIKKETGFHVRML
Lesculentumetr2     AKFSTAAVSCATAVMLVHIIPDLLSVKTRELFLKNKAAELDREMGLIRTQEETGRYVRML
                     *: ** ** **:: *: :** **.**.**::*::*: :*. *:*:*   ::*:* :****

Cmeloetr2           TQEIRKSLDRHTILYTTMFELSETLGLHYCAVWMPNESKTLMNLTHELKDRSFSNGYNVS
Csativusetr2        TQEIRKSLDRHTILYTTMFELSETLGLHYCAVWMPNESKTLMNLTHELKDRSFSNGYNVF
Mdomesticaetr2      TQEIRKSLDRHTILSTTLFELSETLGLHYCAVWMPNEIKTEMILTHELKGRNYSHAYNFS
Csinensisetr2       TQEIRKSLDRHTILYTTLVELSNTLGLQNCAVWMPNEIKTEMNLTHQLNGRNYS-DMCSS
Lsativaetr2         TQEIRKSLDRHTILYTTLDKLSEILDLQNCAIWMPDNTKTVMNLTHQLKG----GQSSTI
Athalianaetr2       TQEIRKSLDRHTILYTTLVELSKTLGLQNCAVWMPNDGGTEMDLTHELRGRGGYGGCSVS
Lesculentumetr2     THEIRSTLDRHTILKTTLVELGRALQLEECALWMPTRTGVELQLSYTLHHQN---PVGFT
                    *:***.:******* **: :*.. * *. **:***     . : *:: *.

Cmeloetr2           IPISDSDVIKIKGSDGVNVLGPNSALVVANCGESDERGPAAAIRMPMLRVSNFKGG-TPE
Csativusetr2        IPISDSDVIKIKGSDGVNVLGPNSALVVANCGESDERGPAAAIRMPMLRVSNFKGG-TPE
Mdomesticaetr2      IPISDPDVAHIKGSDGVSILRPDSALVHASG-DSGEPGPVAAIRMPMLRVSNFKGG-TPE
Csinensisetr2       IPITDQDVVRIKGSDGVNILGPDSELAAASSGESVESGPVAAIRMPMLRVSNFKGG-TPE
Lsativaetr2         IPIQNPEVQEIKQSEVVKLLDHESELGTLSSGGSEPPGAVAAIRMPMLRVSDFKGG-TPE
Athalianaetr2       MEDLDVVRIRESDEVNVLSVDSSIARASGGGGDVSEIGAVAAIRMPMLRVSDFNG-----
Lesculentumetr2     VPIQLPVINQVFSANCAVKISPNSAVARLRPTRKYIPGEVVAVRVPLLHLSNFQTNDWPE
                    :         .       .  :  .              *  ..*:*:*:*::*:*:

Cmeloetr2           IVPTYYAILVLVLPGGQPRSWNKQELEIIKVVADQVAVALSHAALLEESQLMRDKLAEQN
Csativusetr2        IVPTYYAILVLVLPGGQPRSWNNQELEIIKVVADQVAVALSHAALLEESQLMRDKLAEQN
Mdomesticaetr2      VIQACYAILVLVLPGGQPRCWSSQDLEIIKVVADQVAVALSHAAVLEESQLMREKLAEQN
Csinensisetr2       LVSACYAILVLVLPNEQFRTWSNQELEIVKVVADQVAVALSHAAVLEESQHMREKLEEQN
Lsativaetr2         MIQACYAILVLVLPGGQIRSWTGPELEIVKVVADQVAVALSHAAVLEESQLMRDKLAEQN
Athalianaetr2       --ELSYAILVCVLPGGTPRDWTYQEIEIVKVVADQVTVALDHAAVLEESQLMREKLAEQN
Lesculentumetr2     LSPKSYALMVLMLPSNSARQWHVHELELVDVVADQVAVALSHAAILEESMRARDLLIEQN
                         **::* :**.    * *    ::*::.******:***.***:****    *: * ***
Cmeloetr2           RDLQQAKENALMASQARNSFQKVMSDGMRRPMHSIMGLLSMLQNENMNDDQRIILDAMVR
Csativusetr2        RDLQQAKENALMASQARNSFQKVMSDGMRRPMHSIMGLLSMLQNENMNDDQRIILDAMVR
Mdomesticaetr2      RALQQAKMKAMMASHARNAFQKVMSDGMRRPMHSILGLLSLMQDNTLDNDQRVIVDAMVR
Csinensisetr2       RALQQAQKDALMASQARNAFQKVMSNGMRRPMHSILGLLSIMQDVNLNSDQRMIVETMMK
Lsativaetr2         RALQQAKHDAMRASQARNLFQTVMSKSLRKPMHSIVGLLSLIQDDNLMNQQKVLIDSMVK
Athalianaetr2       RALQMAKRDALRASQARNAFQKTMSEGMRRPMHSILGLLSMIQDEKLSDEQKMIVDTMVK
Lesculentumetr2     VALDLARREAETAVRARNDFLGVMNHEMRTPMHAVVALSSLLQESELIPEQRLMVETILK
                      *: *: .*  * :*** *  .*.. :* ***:::.* *::*:  :  :*::::::::
```

*Conserved amino acids

.:Similar amino acids

Supplemental 3. (Continued).

```
Cmeloetr2            TGNVVSTLIDDVMEDPIKDSARFPLELEMRSFRLH-SMKEAACLAKCLCAYKGFGFAFEV
Csativusetr2         TGNVVSTQIDDVMEHPIKDSARFPLELEMRSFRLHSMIKEAACLAKCLCAYKGFGFAFEV
Mdomesticaetr2       TSNVLSTLINDVMDNSAKESGRFPLEMRS--FGLHATIKEAACLAKCLCVFRGFDFAIDV
Csinensisetr2        SSNVLSTLISDVMDNSPKDSGRFPLEIRS--FRLHAMIKEAACLARCLSIYRGFGFSIEV
Lsativaetr2          TSNVLSMLIDDVMDDSSKE--RFPLEMRS--FRLHSLIKEAAHLAKCLCVYKGYEFVMDV
Athalianaetr2        TGNVMSNLVGDSMD---VPDGRFGTEMKP--FSLHRTIHEAACMARCLCLCNGIRFLVDA
Lesculentumetr2      SSNLLATLINDVLDLSRLEDGSLQLDVGT--FNLHALFREVLNLIKPVAAVKKLFVTLSL
                     :.*:::  :.* ::        :  ::    * **  ::*.  : : :.  .   . ..

Cmeloetr2            QRSLPDHVMGDERRVFQVLLHMVGSLLND-INQGGGYALFRVVAESGSQGRNDQRWGNWR
Csativusetr2         QRSLPDHVMGDERRVFQVLLHMVGSLLND-INQGGGYALFRVVAESGSQGRNDQRWGNWR
Mdomesticaetr2       DKSLPDHVMGDERRVFQVILHMVGSLLNG-NGVGG-LVMFRVASEKGSQGRSDQRWAAWR
Csinensisetr2        DRSLPDHVMGDERRVFQVILHMVGSLLN--CNSRRGTVLFRVVSENGSQDRNDQKWATWR
Lsativaetr2          DKSLPDNVMGDERRVFQVILHMVGYLLNR-GNGGGGMVLR-ILKESGSYGRNDQRWASWR
Athalianaetr2        EKSLPDNVVGDERRVFQVILHIVGSLVKPRKRQEGSSLMFKVLKERGSLDRSDHRWAAWR
Lesculentumetr2      SSDFPEVAIGDEKRLMQILLNVVGNAVKFSKEGSVSVSAVNAKSESLIDPRAPEFFPVQS
                     . .:*: .:***:*::*::*::**  ::                *     *  . :

Cmeloetr2            Q--SSSDGDAFIRFEVGINKSNSQSEGSIPNVVSGDRR-----YASDGAEERLSFTICKK
Csativusetr2         Q--NSSDGDAFIRFEVGINKSNSQSEGSIPNMVSGDRR-----YASDGAEERLSFTICKK
Mdomesticaetr2       H--SSSDGDICVRFEIGISNSGSQSEVTIPAVQLVGRR-----YAGEGVDEGLSFTICKK
Csinensisetr2        Q--SSVDGDVHIRFEILLNEVGSQPEVSTSVAQLGIRR-----IVNEGIEDRMSFSVCKK
Lsativaetr2          S--NSGDGYVSVKFEIGINDHDTKLER-----SFADER-----IRSGGVEQSLSFGMCRK
Athalianaetr2        SPASSADGDVYIRFEMNVENDDSSSQSFASVSSRDQEVGDVRFSGGYGLGQDLSFGVCKK
Lesculentumetr2      E------NHFYLRVQVKDTGSGINPQDFPKLFCKFAQNQE--PATKNSAGTGLGLAICKR
                            .   ::.::     . . :          .          .    :.: :*::

Cmeloetr2            LVKLMQGNIWVIPNPQGFTRSMALVLRFQLRPSIAVAMPEPGESSEH---PHSNSIFRGL
Csativusetr2         LVKLMQGNIWVIPNPQGFTRSMALVLRFQLRPSIAVAMPEPGESSEH---PHSNSIFRGL
Mdomesticaetr2       LVQMMQGNIWAVPNPQGFAQSMALVLRFQPRLSIAIAISEPGESSEH---PHSNSLFKGL
Csinensisetr2        LVQLMQGNIWMVPSSHGFAQSMGLVLRFQLRPSIAIPISESGESSEY---SHSNSLLRGL
Lsativaetr2          LVEMMQGKIWVVPNPVGFDQAMSLILRFQLRPSIVIGISEAGESSDHN--PLSNSIFRNL
Athalianaetr2        VVQLIHGNISVVPGSDGSPETMSLLLRFRRRPSISVHGSSESPAPDHHAHPHSNSLLRGL
Lesculentumetr2      FVNLMEGHIWIESEGVGKGSTAIFIVKLGIPGRLNESKLPFTAGLPAN---HMQMTFQGL
                     .*:::.*:*   .   *   :    :::::   :         .         :  ::.*

Cmeloetr2            QVILADADDMNRAVTRKMLEKLGCNVTAVSSGYECLTVMA---PAG---SSIQVVLLDLH
Csativusetr2         QVILADADDMNRAVTRKMLEKLGCNVTAVSSGFECLTVMA---PAG---SSIQVVLLDLH
Mdomesticaetr2       QVLLTDDDDVNRVVTRKMLEKLGCIVTAVSSGFECLSTIG---TIGPAGSSFQFVFLDLH
Csinensisetr2        QVLLADGDDMNRVVTRKLLEKLGCSVSAVSTGYECLSAMGPTA-------VFQLVILDLQ
Lsativaetr2          QVLLADEDDMNRAVTRKQLEKLGCIVSTVASGSDCIMALN------QPVSSYQIILLDLH
Athalianaetr2        QVLLVDTNDSNRAVTRKLLEKLGCDVTAVSSGFDCLTAIAP--GSSSPSTSFQVVVLDLQ
Lesculentumetr2      KVLVMDDNGFSRMVTKSLLVHLGCDVTTIGSGDECLRILTR---------EHKVLIMDAS
                     :*:: * :. .* **:. * :*** *:::.:* :*:  :             :.:.:*

Cmeloetr2            MPELDGFEVTTRIRKFRSQNYR--PVIIALTASAGEDWERCVQIGMNGVIRKPVQLQGIA
Csativusetr2         MPELDGFEVTTRIRKFRSQNYR--PVIIALTASAGEDWERCVQIGMNGVIRKPVQLQGIA
Mdomesticaetr2       MPELDGFEVAIRIRKFRSRTWP--LIIGVTASADEDVWDRCMQTGINGVIRKPVLLQGIA
Csinensisetr2        MPELDGFEVALKIRKFRSRSWP--LIVALTASGDEDVWEKCMQVGMNGVIRKPVLLQGIA
Lsativaetr2          MSDVDGFEVAARIRKSRSRNWP--LIVALTASGDADVWERCLQMGINGVIQKPVVLQGIS
Athalianaetr2        MAEMDGYEVAMRIR---SRSWP--LIVATTVSLDEEMWDKCAQIGINGVVRKPVVLRAME
Lesculentumetr2      ITGMNCYDVAVSVHEKFGKRLERPLIVALTGNTDQVTKENCLRVGMDGVILKPVSIDKMR
                     :. :: ::*:  ::   .:      ::           :.* : *::**: *** :  :

Cmeloetr2            HELRRALLQASKVV-
Csativusetr2         HELRRALLQASKVV-
Mdomesticaetr2       NELRRVLLQANKGMT
Csinensisetr2        NELRRVLLHANKVV-
Lsativaetr2          DELRRVMVHTNKVH-
Athalianaetr2        SELRRVLLQADQLL-
Lesculentumetr2      SVLSGLLEHGTVL--
                       *   : :
```

*Conserved amino acids

.:Similar amino acids

Supplemental 4. mRNA transcripts of *Cm-ETR1* (A) and *Cm-ETR2* (B) genes in melon fruit during storage at 20 °C. Twenty micrograms of total RNA from fruit stored for 0 (0), 1 (1), 2 (2), 4 (4), 6 (6), 8 (8), and 10 days (10) were used for Northern blot analysis.