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Expression of CMe-ACS1 and Ethylene Receptor Genes in Melon F1 Progenies under Cold Storage Condition

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ABSTRACT

Introducing new melon types able to endure cold storage and cold transportation is among the major goals of breeders. Therefore, providing a better understanding of the fruit physiological traits and postharvest associated genes expression can help to select the superior type for cold storage condition. This experiment was carried out to investigate the postharvest behavior of various melon lines and their F1 breeds based on fruit characteristics and their relation to the expression of *CMe-ACS1*, *CM-ETR1* and *CM-ETR2* genes during cold storage. For this purpose, six melon inbred lines were cross-pollinated to form a full diallel F1 population. Thus, the studied population constituted 6 parental lines, 15 direct hybrids, and 15 reciprocal F1 hybrids. After fruits harvest and during one month of storage, fruits firmness loss, color changes, and weight loss were evaluated. A significant positive correlation coefficient was found between *CMe-ACS1* gene expression and ACC content with two distinct clusters based on this relation. Gene expression profiles referred to the presence of gradual and continuous senescence behaviors in the studied types, which was reflected in their physiological postharvest performance. G1 cluster types expressed the studied receptor genes at lower rates compared to the other groups. The G1 corresponded with P3 and P4 groups that were characterized by low physiological changes and thus better postharvest performance during cold storage. This result highlights the importance of Persian inodorus melons, generally, and the line 'Khatouni', specifically, in melon breeding programs for postharvest purposes.

Keywords: Persian melon, Postharvest behavior, Senescence behaviors.

INTRODUCTION

Many studies were conducted to investigate the genetic regulation of ethylene and its response in melon. So far, eight Aminocyclopropane-1-Carboxylate Synthase (ACS) genes responsible for the synthesis of 1-Aminocyclopropane-1-Carboxylic aAcid (ACC) have been reported in melon (Yano and Ezura, 2016). Among these genes, *CMe-ACS1* and *CMe-ACO1* are considered the most important in the process of ripening and climacteric behavior. *CMe-ACS1* was reported as the wound-induced gene transcribed in mesocarp (Yamamoto *et al.*, 1995), the expression level of which also increased in climacteric fruit types after the burst of ethylene, suggesting a positive-feedback mechanism affecting the regulation of this gene's expression (Owino *et al.*, 2007; Yamamoto *et al.*, 1995; Yano and Ezura, 2016). *CMe-ACO1* expression has also been reported during fruit ripening (Yano and Ezura, 2016), and the antisense-suppression experiment of the *CMe-ACO1* gene resulted in a 97–99% reduction in ethylene production compared to the wild type along with the retardation of various ethylene-dependent ripening processes (Ayub *et al.*, 1996). However, *CMe-ACS1* was highly expressed in climacteric melon types compared to its expression in non-

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climacteric melon types with no significant differences in *CMe-ACO1* expression between the two types, which confirms the importance of *CMe-ACS1* when classifying for climacteric characteristics (Saladié *et al.,* 2015; Shiomi *et al.,* 1999).

It was reported that there are at least three receptor genes of ethylene in melon. *CM-ERS1* and *CM-ETR1* belong to receptor subfamily I, and the receptor *CM-ETR2* belonging to receptor subfamily II (Owino *et al.,* 2007). Although the expression of *CM-ERS1* increases during the first period of fruit development, it decreases during the final stages of fruit development. *CM-ETR1* and *CM-ETR2* expression increases after full fruit enlargement and with the implementation of exogenous ethylene, but it decreases after 1-Methylcyclopropene (1-MCP) treatment (Owino *et al.,* 2007; Yano and Ezura, 2016).

Various Iranian melon types were the case of Hatami *et al.* (2016) postharvest study, especially those of Inodorus and Dudaim groups. However, these Iranian melon types were not studied for their postharvest-related gene expression. Additionally, it was reported that storing these Iranian melon types under 5°C resulted in the best postharvest behavior and a longer storage life compared to higher storage temperature (13°C) (Hatami *et al.,* 2016). Furthermore, introducing new types of melon derived from Iranian melon with higher storability potentials is highly important due to the high demand for Iranian melon in the regional markets.

Since there is an increased demand for new melon types with improved storability under cold storage conditions to endure long cold shipping phases to overseas markets, the aim of this study was to provide a better understanding of postharvest behavior of these melon types and their F1 progeny during cold storage based on fruit characteristics and their relation to the expression of *CMe-ACS1, CM-ETR1,* and *CM-ETR2* genes.

MATERIALS AND METHODS

Plant Materials and Experimental Design

The experiment was conducted at the Department of Horticultural Sciences (University of Tehran) in Karaj, Iran, during 2016-2017. The genotypes chosen for the crosses consisted of six inbred lines 'Khatouni', 'Abadan', 'J1025', '1026', 'Izabel from Spain', and one dudaim as 'Kermanshah', selected for their diverse morphological and agronomic characteristics that are mentioned in Table 1.

All possible combinations of crosses between these genotypes (including reciprocals) were conducted and the seeds needed for F1 progeny were gathered and stored. Thirty hybrids along with seeds from the 6 parents were sown in cell trays in the greenhouse, and the seedlings were transplanted to the field in a randomized complete block design in duplicates (two blocks for each hybrid or parental line) with a row distance of 1.5 m and a distance of 1 m between each plant. Considering the unknown nature and ripening characteristics of the newly developed F1 hybrids, deciding the right time to harvest was crucial. Fruits were harvested according to the recommendations of Hatami *et al.* (2016): melon fruits were tagged on the day of anthesis and harvested when the development of abscission layers was clear (approximately day 30), while those which were found not to develop an abscission layer were harvested at day 42 (the best harvest time for non-climacteric inbred lines of this study). Harvested fruits were transported directly to the cold storage and stored at 4°C for one month.

Evaluation of Fruits

All harvested fruit was weighed using an electronic scale, and the fruit color was recorded using the colorimetric CIE LAB

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Table 1. Morphological and agronomic characterizations of the genotypes used for the crosses. [a]

Accession name (With abbreviation)	Group and origin	Climacteric behavior	Fruit shape	Rind	Mesocarp	Volatiles	Origin
Khatouni (Kh)	Inodorus,	Non-climacteric	Oval elongated	Yellow – Shallow netted	Greenish white	Odorless	Khorasan (Southern east of Iran)
Abadan (A)	Cantaloupensis * Inodorus derived inbred line	Non-climacteric	Oval – Round	Dark yellow – Without net	White	Odorous (Medium)	Abadan (Southern part of Iran)
J1025 (J)	Cantaloupensis	Non-climacteric	Oval – Round	Green – Netted	Green	Odorless	Alborz (Northern-west of Iran)
1026 (1026)	Cantaloupensis	Climacteric	Crooked elongated	Orange with green areas – Without net	Orange	Odorous (Medium)	Fars (Southern-west part of Iran)
Izabel (Z)	Cantaloupensis	Climacteric	Round flatted	Creamy – Shallow netted	Pale orange	Odorless	Spain
Kermanshah (K)	Dudaim	Climacteric	Round flatted	Dark yellow with orange areas – Without net	White	Odorous (High)	Kermanshah (Western part of Iran)

method and Minolta Chroma Meter (Konica Minolta) both at day 0 and on the day of stored fruit processing. Rind color was expressed in CIE-lab scale as Lightness (L*), Chroma (C*) and Hue angle (h°) using a chromameter (Konica Minolta, Japan). Flesh firmness was measured using a hand penetrometer equipped with an 8-mm plunger tip at three areas after removing the skin: at the stem end, equator and blossom end for each fruit. The previous tests were conducted on fruit at four different evaluation times, day 0 (day of harvest) and after 10, 20, and 30 days of cold storage with two fruit from each block at each inspection. Weight and firmness losses were calculated as the difference percentage between the analysis day and day 0, while color changes were calculated using the same system proposed by Mohi-Alden *et al.* (2021).

Gene Expression Analysis

Samples from two fruit of each replicate were taken from the mesocarp at days 0, 10, 20, and 30 (2 g each), flash-frozen in liquid nitrogen, and stored at -80°C until RNA extraction. Samples of each biological replicate of each hybrid and parent were ground using a mortar and pestle with liquid nitrogen. Approximately 0.1 g of the fine powder was directly homogenized with pBIOZOL Plant Total RNA Extraction Reagent (Bioer). RNA samples were evaluated for both quantity and the presence of contaminants using a NanoDrop™ 2000 spectrophotometer (Thermo Scientific). Furthermore, RNA integrity was assessed using TAE agarose gel electrophoresis with Mini-Sub® Cell GT Horizontal Electrophoresis (BioRad). The reverse transcription step was carried out after RNase-free DNase (TaKaRa) treatment to eliminate genomic DNA contamination.

Reverse transcription was carried out using PrimeScript first-strand cDNA synthesis kit (TaKaRa). RNA (500 ng) was used in each 10 µl cDNA reaction, and cDNA was synthesized using Bio-Rad C1000 Touch cycler.

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Primers were designed for *CME-ACS1* (NM_001297535.1), *CM-ETR1* (NM_001297524.1), and *CM-ETR2* (NM_001297539) using Primer-BLAST and the corresponding mRNA templates with *RPS15* as a reference gene (Kong *et al.*, 2016) (XM_008440358.2) (Supplementary Table S1). 5x HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) (Solis BioDyne) was used for qPCR assay on the ABI StepOne™ Real-time PCR system (Applied Biosystems). After collecting Cq data for relative gene expression of each sample, data were normalized according to Pfaffl (2001) method.

Aminocyclopropane-1-Carboxylate (ACC) Quantification

ACC was quantified according to Bulens *et al.* (2011). Flash-frozen mesocarp samples (2 g) were powdered using a mortar and pestle with liquid nitrogen. Then, 4 mL of 5% sulfosalicylic acid were added to the powdered sample in a 15 mL falcon tube, and the formed homogenate was left in darkness at 4°C for 30 minutes and briefly vortexed occasionally during this time. Falcon tubes were centrifuged at 4°C and 3,090×g for 10 minutes, the supernatant was transferred to a fresh tube, and stored at -80°C until quantification. The stored sulfosalicylic acid extract (0.7 ml) was transferred to a new 10 mL tube, 0.4 mL of 10 mM $HgCl_2$ was added, and the tube was directly airtight sealed with a proper septum. A mixture of NaOCl (5% v/v): NaOH (6M) (2:1, 0.1 mL) was then added to the tube using a syringe equipped with a needle through the rubber seal. The formed mixture was left on ice for 4 min, and briefly vortexed at the end. 0.1 mL of the gaseous phase was collected with a syringe through the septum and injected into Agilent, 7890-A Gas Chromatography instrument equipped with Flame Ionization Detector (FID) and a CPSill-88-Varian (30 m×320 µm×0.25 µm) column to quantify the generated ethylene followed by calculating the amount of ACC transformed to ethylene.

Statistical Analysis and Plotting

The correlation between *CMe-ACS1* expression levels and ACC content was carried out using GraphPad Prism 7 software, and the cluster analysis based on this relation was carried out using IBM SPSS 24 software. Ward linkage cluster analysis with Euclidean distance was performed for postharvest performance traits (weight loss, color changes, and firmness loss) in addition to genes expression profiles separately using Heatmapper software (Babicki *et al.*, 2016). The logarithmic base gene expression correlation was carried out using IBM SPSS 24 software.

RESULTS

Fruit Evaluation

Mean values for each evaluated trait were obtained (Supplementary Table S2), and those related to postharvest behavior (firmness loss, weight loss, and rind color changes) were used in ward linkage cluster analysis, which resulted in four distinguished clusters (P1, P2, P3, and P4) of hybrids and parents (Figure 1) and (Figure 2-A). Members of P1 were characterized by more pronounced firmness losses, weight losses and color changes throughout storage period (Poor postharvest performance) compared to other clusters, such as hybrid K1026 (Figure 1). Moderate postharvest changes of the studied traits were observed in P2 cluster (Moderate postharvest performance), such as hybrid 1026Z (Figure 1). The members of P3 showed low to moderate changes of the studied postharvest traits throughout cold storage (Good postharvest performance), such as hybrid JA (Figure 1). In addition, the members of P4 cluster were characterized by overall low changes in the four traits

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Figure 1. Storage changes patterns represented by a member of each group suggested by gene expression level and cluster analysis. The yellow line above each fruit represents 1 cm in length. F1 hybrids are referred to by combining the abbreviation of both parents; the first part of each combination refers to the female parent.

(Excellent postharvest performance), such as hybrid ZJ (Figure 1).

Gene Expression Patterns in Hybrids and Parents

Genes expression profile clustering showed four distinctive clusters (G1, G2, G3, and G4) using ward linkage clustering method (Figure 2-B). Members of the first cluster (G1) had an overall low *CMe-ACS1*, *CM-ETR1* and *CM-ETR2* expression levels. G2 cluster members had a gene expression profile similar to G1; however, higher *CMe-ACS1* expression levels were recorded mainly at the beginning of storage (day 0) for four out of the six members (KZ, ZK, K, and K1026). Members of G3 cluster showed relatively low *CMe-ACS1*expression levels with relatively high expression levels for receptors genes (CM-ETR1 and CM-ETR2). An overall relatively high expression level characterized G4 members for the three genes.

Correlation between *CMe-ACS1* Expression and ACC Content

A significant positive correlation coefficient (R^2= 0.388) was found between the $\log_{10}$ of *CMe-ACS1* gene expression and $\log_{10}$ of ACC content (Figure 3). On the other hand, the study of regression and clustering between these two factors showed two distinct clusters. Cluster I with a high ACC content and *CMe-ACS1* expression for the center, while cluster II had a center with a lower ACC content and *CMe-ACS1* expression than the other cluster center (Figure 3).

Correlation between Gene Expression Profiles:

CMe-ACS1 gene expression at day 0 showed significant negative correlations with expression levels of both *CM-ETR1* and *CM-ETR2* after 10 days in cold storage. Contrarily, significant positive correlations

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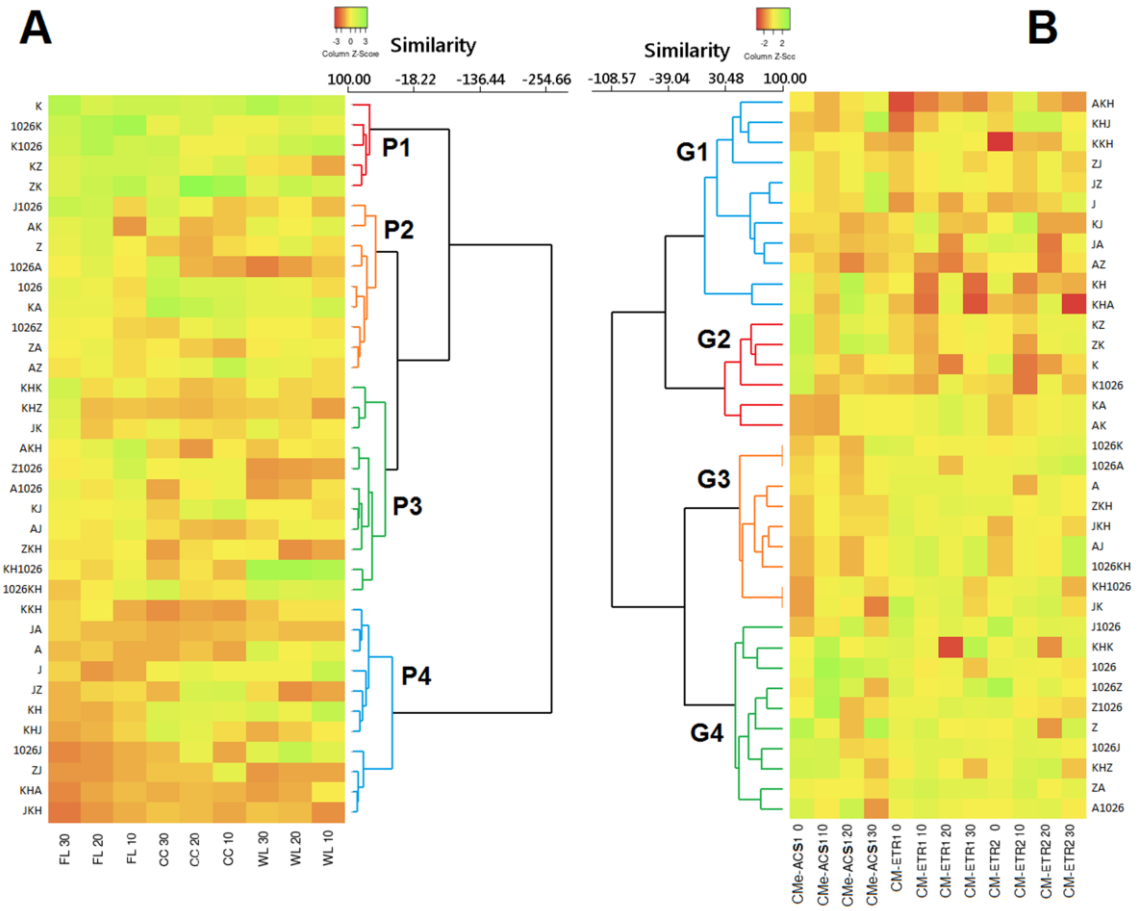

Figure 2. Ward linkage cluster analysis of postharvest performance related traits (A) and the studied genes (CMe-ACS1, CM-ETR1 and CM-ETR2) expression profile (B) during cold storage period (days 0, 10, 20 and 30) in parents and F1 hybrids used in the experiment. 1026, A (Abadan), J (J1025), K (Kermanshah), KH (Khatouni) and Z (Izabel) are the parents used in this experiment. Combination between letters refers to F1 hybrids and the first part of each combination refers to the female parent. FL: Firmness Loss, CC: Color Changes, WL: Weight Loss. The number next to each gene or physiological trait refers to the day of storage phase when the evaluation was carried out (at 0, 10, 20, and 30 days).

were found between expression levels of *CM-ETR1* and *CM-ETR2* at day 0 with those of *CMe-ACS1* after 10 days of cold storage. The correlation between *CMe-ACS1* and *CM-ETR1* expression levels after 10 days was also significantly positive (Table 2).

On the other hand, there was a significant correlation between *CM-ETR1* and *CM-ETR2* during each storage period. Interestingly, this correlation increased throughout the storage period to reach 0.8 after 30 days (Table 2).

DISCUSSION

CMe-ACS1 and ACC Correlation

The correlation (R^2) between gene expression and protein abundance in multicellular eukaryotes has been reported to range between 0.27 and 0.46 (de Sousa *et al.*, 2009, Vogel and Marcotte, 2012). This observation means that only 27-46% of active protein content can be predicted by the abundance of mRNA transcript of this protein, while the remaining portion is

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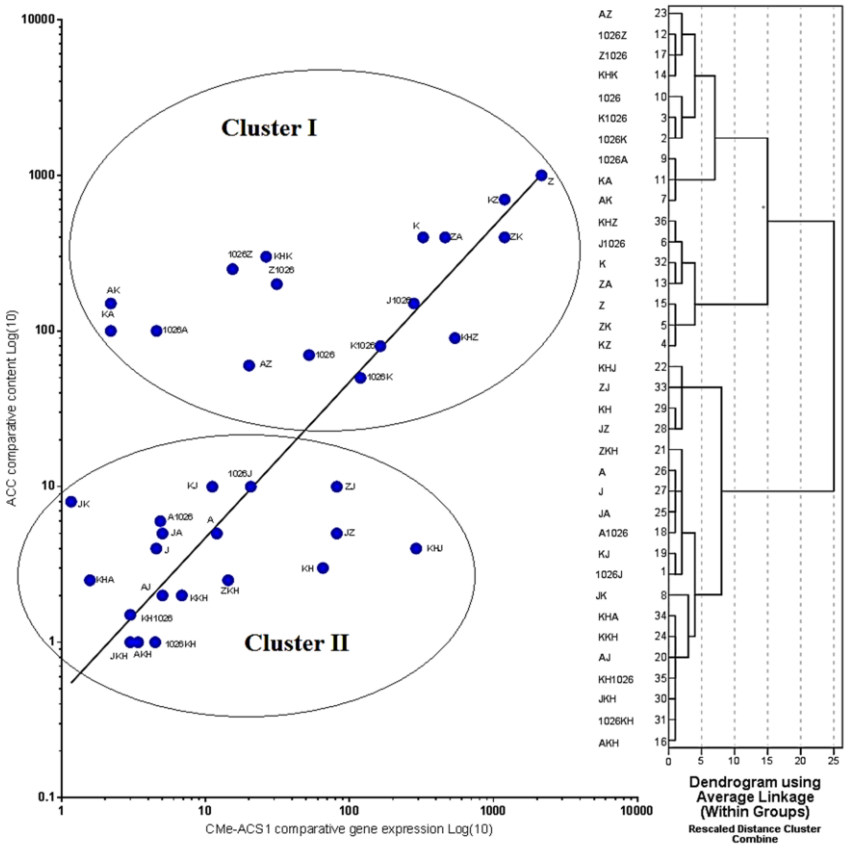

Figure 3. Regression and cluster analysis of *CMe-ACS1* gene expression levels and corresponding ACC content in hybrids and parents. Cluster analysis shows two distinctive clusters: the higher cluster correlated with faster senescence, while the lower cluster is correlated with slower senescence. 1026, A (Abadan), J (J1025), K (Kermanshah), KH (Khatouni) and Z (Izabel) are the parents used in this experiment. F1 hybrids are referred to by combining the abbreviation of both parents; the first part of each combination refers to the female parent.

related to other post-transcriptional factors. In this study, the significant positive correlation between *CMe-ACS1* expression levels and ACC content (R^2= 0.388) refers to the significant role of this gene as a key player in ACC synthesis and thereafter in ethylene synthesis (Owino *et al.*, 2007; Saladié *et al.*, 2015; Shiomi *et al.*, 1999). Furthermore, the correlated abundances of ACC and *CMe-ACS1* copies refer to a highly correlated post-transcriptional phase affiliated with this gene.

Cluster Analysis of Gene Expression and Postharvest Performance

The main traits that can be used to evaluate postharvest performance in melon fruit are firmness loss, weight loss, and color changes (Pech *et al.*, 2008; Obando-Ulloa *et al.*, 2009; Alabboud *et al.*, 2020). These aspects were considered in the current study (Supplementary Table S2) and used to construct the clustering dendrogram (clusters P1-P4) (Figure 2-B).

From both dendrograms and heat maps presented in Figure 2, it can be concluded that clustering based on postharvest performance corresponded with clustering based on gene expression profiles. As P1 corresponded with G2, P2 with G4, P3 with G3, and P4 with G1. This correspondence might refer to the high correlation between postharvest performance and the expression of the studied genes. Additionally, clustering based on ACC content and *CME-ACS1* expression levels (Figure 3) seemed to have divided the genotypes into two group with rapid and slower postharvest

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Table 2. Correlation coefficient between CMe-ACS1, CM-ETR1 and CM-ETR2 gene expression during cold storage period (days 0, 10, 20, and 30).[a]

	Correlations											
	ACS1 0	ACS1 10	ACS1 20	ACS1 30	ETR1 0	ETR1 10	ETR1 20	ETR1 30	ETR2 0	ETR2 10	ETR2 20	ETR2 30
ACS1 0	1	0.105	0.283	0.210	-0.186	-0.355*	-0.126	-0.256	0.096	-0.336*	-0.086	-0.165
ACS1 10	0.105	1	0.118	-0.095	0.476**	0.336*	-0.031	0.294	0.403*	0.276	0.054	0.096
ACS1 20	0.283	0.118	1	0.005	0.026	-0.321	0.330*	-0.337*	0.143	-0.223	0.358*	-0.321
ACS1 30	0.210	-0.095	0.005	1	-0.362*	-0.184	-0.137	0.055	-0.170	-0.071	-0.058	0.214
ETR1 0	-0.186	0.476**	0.026	-0.362*	1	0.538**	0.232	0.402*	0.500**	0.154	0.163	0.346*
ETR1 10	-0.355*	0.336*	-0.321	-0.184	0.538**	1	0.229	0.619**	0.066	0.566**	0.109	0.536**
ETR1 20	-0.126	-0.031	0.330*	-0.137	0.232	0.229	1	-0.075	-0.083	-0.050	0.687**	-0.087
ETR1 30	-0.256	0.294	-0.337*	0.055	0.402*	0.619**	-0.075	1	0.157	0.248	-0.004	0.802**
ETR2 0	0.096	0.403*	0.143	-0.170	0.500**	0.066	-0.083	0.157	1	0.157	0.097	0.005
ETR2 10	-0.336*	0.276	-0.223	-0.071	0.154	0.566**	-0.050	0.248	0.157	1	0.069	0.164
ETR2 20	-0.086	0.054	0.358*	-0.058	0.163	0.109	0.687**	-0.004	0.097	0.069	1	-0.055
ETR2 30	-0.165	0.096	-0.321	0.214	0.346*	0.536**	-0.087	0.802**	0.005	0.164	-0.055	1

[a] The number next to each gene refers to the day of storage phase when the evaluation was carried out (at 0, 10, 20, and 30 days).
* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed).

senescence. This classification was mirrored by the performance clustering of P1 and P2 representing the types with fast senescence, while P3 and P4 representing the types with slower senescence.

CM-ETR1 is considered as the only member of subfamily I expressed during melon ripening and postharvest (Owino *et al.*, 2007; Yano and Ezura, 2016). Therefore, the low *CM-ETR1* expression in G2 types accompanied with the high *CMe-ACS1* expression levels can be suggested as the reason behind the poor postharvest performance witnessed in the members of this group (i.e. K, K1026, KZ, ZK, KA, and AK). In this study, line 'Kermanshah' (K) was also found to be a member of G2 with high *CMe-ACS1* expression and ACC content at harvest (Figure 3). Additionally, G2 contained many 'Kermanshah' derivative hybrids with similar gene expression profiles (Figure 2-B), which is in correspondence with the results of Hatami *et al.* (2019) for this type having a climacteric behavior.

Both G3 and G4 members had relatively high levels of *CM-ETR1* and *CM-ETR2* genes expression. However, G3 members were characterized with higher *CMe-ACS1* expression levels compared to G4 members. Similar to previous reports of increased ethylene production following ACC synthesis (Chatzopoulou *et al.*, 2020; Mayobre *et al.*, 2021), the high ACC level was most likely followed by an increased ethylene production in G3 types, which was strong enough to initiate the ethylene response signal and induce the climacteric behavior and the moderate postharvest performance P2; while on the other hand, ACC levels were lower in G4, which resulted in a slower senescence and a good postharvest performance (P3).

No distinctive boundaries were noticed between the P2 and P3 clusters. Although P2 and P3 were divided into two clusters, these two groups were closely related to

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each other than the other clusters (P1 and P4) (Figure 2-A). This agrees with Obando *et al.,* (2007), Saladié *et al.,* (2015) and Yano and Ezura (2016) results, indicating that it is an oversimplification to classify melon fruit ripening and postharvest behavior into only climacteric or non-climacteric. The cluster P4 was characterized with an excellent postharvest performance. These cluster members were mainly segregated within G1 according to their gene expression (Figure 2-B), with low *CM-ETR1* and *CM-ETR2* expression. This observation raises a question about the reason behind the superior postharvest behavior in this group, although the low receptors' expression might suggest a possibility of a third active ethylene receptor to sustain EIN2 phosphorylation and prevent ethylene independent response in this group (Liu *et al.,* 2010). Furthermore, many of G1 cluster types were derivatives of the Persian inodorus line 'Khatouni'. These G1 types corresponded with P3 and P4 groups that were characterized by low physiological changes and thus low post harvest losses, which highlights the importance of Persian inodorus melons generally, and the line 'Khatouni' specifically, in melon breeding programs for postharvest purposes (Alabboud *et al.,* 2020). On the other hand, there might be a group that has a postharvest senescence behavior even in the absence (or at very low levels) of ethylene, which can be considered to have an ethylene independent senescence behavior (P1, G2) mostly belonging to line 'Kermanshah' of dudaim group (Figure 2), which illustrates the importance of further studies on segregated populations of crosses between typical non-climacteric inodorus melons and dudaim melons.

Co-Expression and Gene Regulation

In this study, the observed correlation between *CM-ETR1* and *CM-ETR2* expression levels (Table 2) suggests that there is a case of co-expression for these two genes. It was reported previously that genes that contribute to common biochemical pathways in higher plants have a propensity to be clustered together (Kopcsayová, and Vranová, 2019; Ribeiro *et al.,* 2020; Zhao *et al.,* 2020). This suggests the conclusion that functional similarities of genes involved in the same metabolic pathways or biological processes can activate co-expression due to common regulatory elements (van Wersch and Li, 2019).

CONCLUSIONS

The study of melon fruit senescence during cold storage is of high importance, not only for their economic importance as a locally consumed and exported fruit in the region but also as a model plant to provide a better understanding of senescence mechanism. In this work, the high correlation between ACC content in melon fruit mesocarp and *CMe-ACS1* gene expression highlights the dominance of this gene in ethylene production pathway in fruit during senescence. Among the 36 studied types (6 parental lines and 30 hybrids) and based on senescence and postharvest behavior (firmness loss, weight loss and color changes), four groups were distinguished, ranging from poor to excellent postharvest performance. It was also noticed that lower relative ethylene receptors gene expression (*CM-ETR1 and CM-ETR2*) with low *CMe-ACS1* expression was accompanied with a better postharvest performance such as that in group G1. On the other hand, the high expression of *CMe-ACS1* with lower receptor genes expression correlated with higher losses during storage, such as group G2, which mainly consisted of the dudaim inbred line 'Kermanshah' and its hybrids. Finally, the correlation between both ETR genes expression patterns throughout storage time indicates a status of co-expression in these two genes, which might suggest common regulatory factors.

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Table S1. The properties and efficiency of qPCR primers used in the study.

Gene symbol	Description	Gene ID	NCBI Reference Sequence	Forward primer	Reverse primer	Amplicon size	Amplicon tm	Primer efficiency
CMe-ACS1	1-aminocyclopropane-1-carboxylate synthase 1	LOC103497687	NM_001297535.1	ACACAACATTTGCTCGCTGCT	TCTTCTGTGAACCTTGCATGTCTT	107	82.9	100.8%
CM-ETR1	ethylene receptor 1	LOC103492012	NM_001297524.1	AAGGGATGCACGGCGACTTT	GTCACCGAACGACTAACTCCATT	164	82.16	104.4%
CM-ETR2	ethylene receptor 2	LOC103483624	NM_001297539.1	GCCAGTGATGGAGCTGAGGA	GTTCCCTTGCATCAACTTAACAAGC	72	81.26	121.3%
CM-RPS15	Cytosolic ribosomal protein S15	LOC103483644 MELO3C006471	XM_008440358.2	CGGCAGGAGTCCCGAAGAAG	TGGAGCCTCACGTTTCGCTT	197	80.81	102.4%

Table S2. Post-harvest characteristics of the studied melon genotypes during cold storage period (days 0,10, 20 and 30) in parents and F1 hybrids used in the experiment. 1026, A (Abadan), J (J1025), K (Kermanshah), KH (Khatouni) and Z (Izabel) are the parents used in this experiment. Combination between letters refers to F1 hybrids and the first part of each combination refers to the female parent. FL: Firmness Loss, CC: Color Changes, WL: Weight Loss. The number next to each gene or physiological trait refers to the day of storage phase when the evaluation was carried out (at 0, 10, 20, and 30 days).

Genotype	WL 10	WL20	WL 30	CC 10	CC 20	CC 30	FL 10	FL 20	FL 30
1026	3.57168	10.3274	14.399	6.544	8.15487	10.64489	13.14031	36.69265	54.1587
1026A	6.3083	10.87983	14.21868	6.904215	10.22382	12.21547	15.21547	40.2165	50.45897
1026J	5.331803	15.16077	15.19494	1.274076	6.592598	3.455873	0.907912	1.815824	8.819715
1026K	7.320981	14.13235	15.04159	4.767446	5.636208	10.30026	33.86364	68.40909	65.79545
1026KH	1.428571	6.28328	9.139073	2.470037	2.297656	3.566254	6.732892	14.7351	58.0574
1026Z	2.770638	2.860835	3.234584	1.136094	2.38811	9.897049	16.69231	44.40966	44.58855
A	2.568716	5.132823	9.015062	2.195096	2.137247	2.818787	5.495283	13.67925	34.41038
A1026	1.793558	1.726699	11.1087	4.443231	4.302696	1.952963	17.85216	26.91771	37.88935
AJ	1.779261	2.864596	4.634146	5.008867	6.196413	6.194806	33.33333	35.2657	42.02899
AK	4.413884	9.774564	11.21304	1.919396	3.263103	6.044432	13.01389	40.00567	43.88999
AKH	4.512686	9.551295	9.630027	1.765237	2.605033	5.090656	25.41857	27.24505	44.14003
AZ	2.458073	8.00609	9.824296	2.494062	5.98745	9.48756	10.65292	53.2548	70.5947
J	6.826677	9.015193	12.37389	4.610175	7.001952	6.523621	1.818182	1.636364	33.63636
J1026	3.627175	8.94176	12.01639	6.355523	6.28156	4.345736	10.5487	30.54487	47.23338
JA	5.179132	8.94001	16.10574	1.29327	2.921029	2.632003	0.938086	20.26266	26.82927
JK	3.577644	3.62952	5.165931	4.993813	5.158249	2.078923	25.58659	26.81564	50.61453
JKH	0.990998	4.858257	8.465305	1.25	2.55478	3.789395	1.587302	3.174603	3.492063
JZ	7.195254	9.671597	15.90244	5.421236	7.354702	8.740001	9.191759	9.825674	24.77549
K	6.53652	14.91875	21.62921	7.322568	8.6658	10.2489	36.08696	47.82609	80.2148
K1026	4.875826	12.18995	13.26168	5.028604	8.377543	7.38774	52.90816	67.7551	69.38776
KA	3.531502	11.18431	11.68414	3.425656	1.943814	3.935948	18.27637	50.03715	52.15453
KH	3.907638	5.99455	6.65	3.270745	7.069384	9.107469	11.11111	12.34568	19.10641
KH1026	3.924962	6.940447	14.39393	3.131022	4.055582	7.366539	14.93506	15.82792	53.40909
KHA	3.847533	3.556473	5.52	1.904704	2.60878	2.651644	4.555315	6.290672	8.02603
KHJ	1.687117	1.553398	10.21739	6.856099	7.993177	3.156952	12.6183	23.02839	22.60778
KHK	8.080171	18.3787	22.74413	2.030278	4.518687	3.470488	21.40039	21.47436	40.03945
KHZ	5.843647	11.85297	17.08108	3.634796	4.266921	9.457	25.46429	30.35714	29.10714
KJ	3.62506	8.081754	8.42997	4.011076	0.696998	4.584137	37.0512	42.39775	44.46613
KKH	3.797327	7.519127	8.264566	0.724254	1.490619	0.978943	2.058824	30.98039	32.7451
KZ	5.875281	14.62592	15.12675	10.47756	15.12106	8.787457	43.46209	57.27229	57.65379
Z	3.57168	10.3274	14.399	8.170949	6.604995	6.597369	13.14031	36.69265	37.86192
Z1026	3.546327	8.849853	12.23562	7.227406	7.439456	4.308434	21.89974	31.7942	47.36148
ZA	5.382063	8.71798	14.34033	2.348064	2.091255	8.267968	-3.05556	48.61111	54.51389
ZJ	1.666667	3.333333	4.583333	5.484099	3.095148	3.740158	1.590909	2.954545	13.18182
ZK	1.673867	7.045478	10.4932	6.714625	6.55737	9.489829	34.66667	53.87387	58.55856
ZKH	3.502578	6.450798	12.94971	2.677251	2.660611	5.022033	23.97661	24.5614	67.25146

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بیان ژن‌های CMe-ACS1 و گیرنده اتیلن در نتاج F1 ملون در شرایط نگهداری انبار سرد

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چکیده

معرفی انواع متحمل ملون به نگهداری در شرایط سرد و انتقال یکی از اهداف اصلی اصلاحگران می‌باشد.بنابراین شناخت بهتر از صفات فیزیولوژیکی میوه و بیان ژن‌های مرتبط با آن بعد از برداشت، به انتخاب انواع برتر برای نگهداری در شرایط دمای سرد انبار کمک می‌کند. این آزمایش جهت بررسی رفتار پس از برداشت لاین‌های متفاوت ملون بهمراه نتاج F1 بر اساس صفات میوه و بیان ژن‌های *CMe-ACS1, CM-ETR1, CM-ETR2* در انبار سرد صورت گرفت. برا ی این منظور شش لاین اینبرد ملون برای ایجاد جمعیت F1 تلاقی دای‌آلل کامل داده شدند. بنابراین جمعیت‌ها از ۶ والد اینبرد، ۱۵ هیبرید مستقیم (تلاقی رفت) و ۱۵ هیبرید تلاقی برگشتی تشکیل شده بود. بعد از برداشت میوه‌ها و نگهداری آنها به مدت یک ماه در انبار سرد، صفات فیزینولوژیکی کاهش سفتی، تغییر رنگ و از دست دادن وزن ارزیابی شدند. همبستگی مثبت معنی‌دار بین بیان ژن *CMe-ACS1* و میزان ACC مشاهده شد و دو گروه مجزا بر اساس این ارتباط در کلاستر بدست آمد. بررسی بیان ژن‌های مورد مطالعه نشان داد که یک روند تدریجی و پیوسته در فرایند پیری وجود داشته که در ویژگی‌های فیزیولوژیکی پس از برداشت هم منعکس شده است.در خوشه بندی، گروه G1 کمترین بیان ژن‌های گیرنده اتیلن را در میان سایر گروه‌ها نشان دادند.گروه G1 با گروه‌های P3 و p4 مرتبط بودند که کمترین تغییرات فیزیولوژیک را داشتند و کارایی بهتری در شرایط نگهداری دمای سرد بروز

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دادند که اهمیت ملون‌های ایرانی بویژه والد خاتونی را در برنامه اصلاحی ملون‌ها برای اهداف پس از برداشت، نشان می‌دهد.

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