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# UV-C radiation modifies the ripening and accumulation of ethylene response factor (ERF) transcripts in tomato fruit

Joseana Severo<sup>a,\*</sup>, Aline Tiecher<sup>b</sup>, Jullien Pirrello<sup>c</sup>, Farid Regad<sup>c</sup>, Alain Latché<sup>c</sup>, Jean-Claude Pech<sup>c</sup>, Mondher Bouzayen<sup>c</sup>, Cesar Valmor Rombaldi<sup>d</sup>

<sup>a</sup> IFF, Instituto Federal Farroupilha, Eixo de Produção Alimentícia, Rua Fábio João Andolhe, 1100, Bairro Floresta, CEP 98590-000, Campus Santo Augusto, Santo Augusto, RS, Brazil

<sup>b</sup> UNIPAMPA, Universidade Federal do Pampa, Campus Itaqui, Rua Luiz de Joaquim de Sá Britto, s/n, Bairro Promorar, CEP 97650-000, Itaqui, RS, Brazil

<sup>c</sup> UMR 990 INRA/INP-ENSAT, Pole de Biotechnologie Végétale, 24 Chemin de Borde Rouge, P.O. Box 107, 31326 Castanet Tolosan Cedex, France

<sup>d</sup> UFPEL/FAEM, Departamento de Ciência e Tecnologia, Campus Universitário, Caixa Postal 354, CEP 90010-900, Pelotas, RS, Brazil

## ABSTRACT

Ultraviolet-C (UV-C) radiation is used as a postharvest treatment to prolong the shelf life of fruit. However, this stressful process may also affect ethylene production and, consequently, the expression of genes encoding ethylene response factors (ERFs). To test this hypothesis, MicroTom tomatoes harvested at the breaker stage were subjected to: 1 – application of  $3.7 \text{ kJ m}^{-2}$  UV-C radiation, 2 – application of  $2 \mu\text{L L}^{-1}$  1-methylcyclopropene (1-MCP) followed by UV-C radiation; and 3 – without 1-MCP or UV-C (control treatment). After treatment all fruit were stored for 12 d at  $21 \pm 2^\circ\text{C}$  and  $80 \pm 5\%$  relative humidity (RH). Although UV-C radiation increased ACC oxidase transcripts and stimulated ethylene production, the ripening evolution was delayed. Fruit treated with UV-C showed lower accumulation of lycopene,  $\beta$ -carotene, lutein + zeaxanthin and  $\delta$ -tocopherol; but retained higher levels of chlorogenic acid, *p*-coumaric acid and quercetin after 6 d. Additionally, UV-C treated fruit had higher contents of polyamines (putrescine and spermidine). Among the 14 ERFs studied, 11 (*Sl-ERF A.1*, *Sl-ERF A.3*, *Sl-ERF B.1*, *Sl-ERF B.2*, *Sl-ERF B.3*, *Sl-ERF C.6*, *Sl-ERF D.1*, *Sl-ERF D.3*, *Sl-ERF E.1*, *Sl-ERF E.5*, *Sl-ERF G.2*) exhibited increased transcript accumulation, 2 ERFs (*Sl-ERF E.2* and *Sl-ERF E.4*) showed decreased transcript accumulation and only 1 ERF (*Sl-ERF E.3*) was not significantly affected by UV-C treatment. As expected, the transcript profiles of 1-MCP and/or UV-C-treated tomatoes demonstrate that ethylene plays an important role in the expression of ERFs. The delay in fruit ripening may be caused by the activation of ERFs that could act as regulators of metabolic pathways during ripening. However, this hypothesis needs to be better tested. In conclusion, a relationship has been established between UV-C treatment and ripening delay, correlated to changes in 13 ERF transcripts evaluated during postharvest treatment.

## Keywords:

Abiotic stress

Ripening

Senescence

*Solanum lycopersicum*

1-Methylcyclopropene

Ultraviolet C

## 1. Introduction

UV-C radiation (100–280 nm) is a treatment with germicidal capabilities that has been used to prevent postharvest rot in fruits and vegetables (Stevens et al., 1998; Liu et al., 2011; Syamaladevi et al., 2014). Because it is a stressor, UV-C can also accelerate ethylene production and therefore activate the expression of ethylene response factor (ERFs) genes. Altering the expression of ERF, either through hormonal induction or abiotic stress, can induce secondary metabolic pathways; these pathways may

activate pathogenesis-related (PR) genes related to the synthesis of phytoalexins, phenols and terpenoids (Maharaj et al., 1999; Charles et al., 2008a,b; Liu et al., 2011; Pombo et al., 2011). Pombo et al. (2011) reported that UV-C treatment of strawberries helps prevent rot not only by direct inoculum reduction, but also by activating genes encoding enzymes involved in plant defense. The beneficial effects of the application of UV-C can vary between species, cultivars and time of application. Bu et al. (2013) previously reported that UV-C maintained the firmness of Cherry tomatoes (*Solanum lycopersicum* L. cv. Zhenzhu1.), with decreased expression of cell wall degrading enzymes. In comparison, Tiecher et al. (2013) observed delay in fruit maturation without a commensurate prolongation of tomato firmness (*S. lycopersicum* cv. Flavortop). Obande et al. (2011) reported maintained the

\* Corresponding author. Tel.: +55 53 32757258; fax: +55 53 32757258.  
E-mail address: joseana.severo@iffarroupilha.edu.br (J. Severo).

firmness of preharvest UV-C treatment of tomatoes (*S. lycopersicum* L. cv. Mill.) with varying results depending on the applied dose.

It is widely known that the phytohormone ethylene controls many events related to growth and development in plants, and is expressed in response to abiotic and biotic stressors (Cara and Giovannoni, 2008; Bapat et al., 2010). 1-Methylcyclopropane (1-MCP) is a potent inhibitor of ethylene perception, which has been used successfully in studies to understand the action of ethylene in ripening process and consequently the expression of related genes (Hoeberichts et al., 2002; Opiyo and Ying, 2005).

Ethylene is formed from the amino acid methionine by S-adenosyl-L-methionine (AdoMet) and 1-carboxylic acid-1-aminocyclopropane. The enzymes that catalyze the conversion of AdoMet to ACC and ACC to ethylene are ACC synthase (ACS) and ACC oxidase (ACO), respectively. During ripening of climacteric fruit, this biosynthesis pathway is autocatalytically regulated by ethylene (Barry et al., 1996; Cara and Giovannoni, 2008). In response to ethylene, the expression profile of several transcription factors may be altered, which results in the activation of pathways that induce or delay senescence (Ohme-Takagi and Shinshi, 1995; Chen et al., 2008; Erkan et al., 2008; Liu et al., 2009, 2011).

After synthesis, ethylene is recognized by receptors (ETRs) located in the membrane of the endoplasmic reticulum. A signaling cascade which includes positive and negative regulators, modulates the expression of ERF, which are subsequently responsible for changes in the metabolic pathways involved in ripening and plant defense (Barry et al., 1996; Bapat et al., 2010). This process culminates in biochemical and physiological responses such as chlorophyll degradation, carotenoid accumulation, softening, and changes in tomato aroma and flavor. In addition, there are changes in the levels of L-ascorbic acid, tocopherols and phenolic compounds (Stevens et al., 1998; Cara and Giovannoni, 2008). The ERFs belong to the AP2/ERF family of transcription factors that are characterized by the presence of a DNA binding domain called AP2/ERF, which is present exclusively in plants. This family of transcription factors has a 58-59 amino acid conserved domain (ERF binding domain) that can bind to two cis-elements: (i) *GCC-box*, which is present in the promoter region of PR-genes that confer a response to ethylene, and (ii) *C-repeat (CRT)/dehydration-responsive element (DRE)*, which is involved in the expression of genes related to dehydration and response to low temperatures (Singh et al., 2002; Xu et al., 2008, 2011). Whereas some of these transcription factors bind to only one of these cis elements (Gu et al., 2002; Singh et al., 2002), others may modulate responses to stress tolerance through interactions with both (*GCC-box* and *DRE*) cis elements (Huang et al., 2004; Zhang et al., 2004; Xu et al., 2007, 2011).

Since the first ERF binding domain was identified in four tobacco proteins (Ohme-Takagi and Shinshi, 1995), new ERF genes have been identified in other plant tissues (Zhou et al., 1997; Tournier et al., 2003; Wang et al., 2007; Xu et al., 2007; Zhang et al., 2010; Yin et al., 2012; Girardi et al., 2013). Several studies have sought to relate the influence of biotic and abiotic stressors to the expression of these transcription factors (Singh et al., 2002; Gutterson and Reuber, 2004; Xu et al., 2007, 2011; Yin et al., 2012). In general, studies that have modified ERF expression in plants have demonstrated an increased tolerance to salinity (Huang et al., 2004; Wang et al., 2004; Zhang et al., 2004; Pan et al., 2010), drought (Chen et al., 2008; Zhang et al., 2010), temperature (Chen et al., 2008; Zhang and Huang et al., 2010) and/or pathogen infection (He et al., 2001; Pan et al., 2010). Yin et al. (2012) showed that 13 ERFs sequences are differentially expressed during postharvest abiotic stresses (low temperature, high temperature, high CO<sub>2</sub> and high water loss) in Kiwifruit. Liu et al. (2011), using microarray techniques, determined that UV-C irradiation induced

the expression of defense response genes (such as PR related proteins,  $\beta$ -1,3-glucanase and chitinase), signal transduction genes (such as ethylene related genes, IAA receptor protein and calmodulin) and protein metabolism genes. At the same time, some genes related to cell wall disassembly (such as expansin, pectinesterase and endo- $\beta$ -1,4-D-glucanase), photosynthesis (such as chlorophyll a/b binding protein precursor) and lipid metabolism (such as lipoxygenase) seem to be suppressed in the tomato fruit after UV-C radiation.

The tomato is one model for the study of the relationships between stress, hormonal responses and fruit quality. Tomatoes are a good model because their structural genomics are well-known, their transcriptome and proteome databases are relatively rich, and because they are a species of great economic importance (Cara and Giovannoni, 2008; Bapat et al., 2010; Barsan et al., 2010).

The goal of this research was to understand how UV-C affects the transcriptional profiles of *ACO1* and *ERFs* as well as levels of the major secondary metabolites in tomatoes. The application of 1-MCP prior to UV-C treatment was used to distinguish if the effect of UV-C treatment on gene expression was mainly dependent on ethylene.

## 2. Material and methods

### 2.1. Plant material

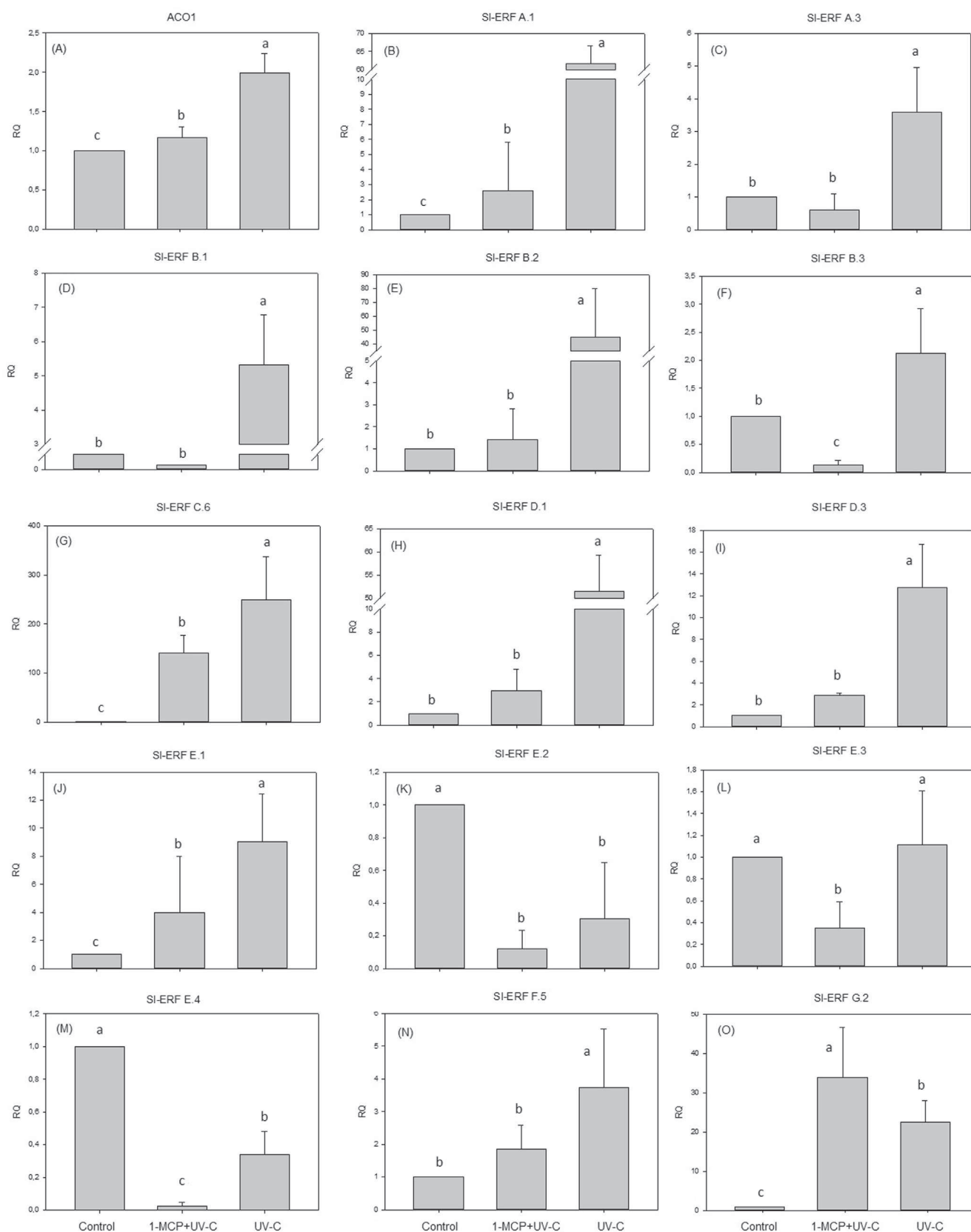
Tomato plants (*S. lycopersicum* Mill., "MicroTom") were cultivated in pots with peat substrate (Klasmann-Deilmann, R.H. P. 15). Growing conditions were: a 14:10 h light/dark cycle with temperatures of 25 °C during the day and 20 °C overnight, 70% relative humidity (RH) and a light intensity of 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Tomato fruit were harvested at the breaker stage of the ripening process and transported at room temperature (RT) for treatment. The average time between harvest and treatment was 30 min.

### 2.2. UV-C treatment

For UV-C treatment, the harvested tomatoes were packed in trays and placed under UV-C lamps (TUV G30T8, 30 W, Philips). Four lamps were placed at a distance of 30 cm from the fruit, providing a UV-C dose of 3.7  $\text{kJ m}^{-2}$  as measured by a digital radiometer (Model MRUR-203, Instrutherm®). To achieve the total dose, 4 min of exposure were required on each of the four sides of the fruit, totaling 16 min of treatment. To isolate the effect of ethylene, a treatment of 1-MCP was applied to the fruit in the 1-MCP+UV-C group at a concentration of 2  $\mu\text{LL}^{-1}$  before UV-C treatment. These conditions were previously optimized by Tiecher et al. (2013). Thus, the experimental design contained the following treatments: 1 – UV-C: fruit were harvested and treated with UV-C at 3.7  $\text{kJ m}^{-2}$  and stored at RT (20  $\pm$  3 °C and 80  $\pm$  5% RH) for 12 d. 2 – 1-MCP+UV-C: fruit were harvested and treated with 1-MCP at 2  $\mu\text{LL}^{-1}$  for 12 h, followed by treatment with UV-C as described above and stored at RT for 12 d. 3 – Control (untreated fruit): fruit were harvested and immediately placed at RT for 12 d.

### 2.3. RNA extraction, cDNA synthesis and real time PCR (qPCR)

The exocarps of the harvested tomato fruit were used to study the transcriptional expression of *ACO1* and *ERF* genes by quantitative PCR (qPCR). The samples described in Section 2.2 were collected after 6 h of storage. Total RNA was extracted using Pure Link™ reagent (Invitrogen®) according to the manufacturer's instructions. The quality and concentration of RNA extracts were evaluated using an Agilent 2100 Bioanalyzer® (Agilent Technologies, CA), in which only RNA samples that had RIN (RNA integrity) values greater than 6 were used for cDNA synthesis. For RT-PCR,



**Fig. 1.** Effects of UV-C treatment on relative accumulation of *ACO1* (A) and *ERF* (B–O) gene transcripts in “MicroTom” tomato fruit after 6h of storage. The relative quantification of transcripts (RQ) is relative to control fruit and normalized with  $\beta$ -actin transcripts. Vertical bars represent the standard deviation.

2 µg of RNA extract was treated with DNase (Qiagen, Valencia, CA, USA). Reverse transcription of mRNA was completed using the Omniscript Reverse Transcription kit (Qiagen, Valencia, CA, USA), resulting in a total volume of 20 µL. For qPCR, 2 µL of cDNA was added to 25 µL of reaction agent - SYBR GREEN PCR Master Mix (PE-Applied Biosystems, Foster City, CA, USA), and an ABI7900ht sequence-detection system was used. The *Sl-ACO1* gene (Barry et al., 1996) and 14 *ERF* genes (*Sl-ERF A.1* – Pirrello et al., 2012; *Sl-ERF A.3* – Zhou et al., 1997; *Sl-ERF B.1* – Pirrello et al., 2012; *Sl-ERF B.2* – Pirrello et al., 2012; *Sl-ERF B.3* – Tournier et al., 2003; *Sl-ERF C.6* – Zhou et al., 1997; *Sl-ERF D.1* – Pirrello et al., 2012; *Sl-ERF D.3* – Pirrello et al., 2012; *Sl-ERF E.1* – Tournier et al., 2003; *Sl-ERF E.2* – Zhang et al., 2004; *Sl-ERF E.3* – Wang et al., 2004; *Sl-ERF E.4* – Pirrello et al., 2012; *Sl-ERF F.5* – Tournier et al., 2003; *Sl-ERF G.2* – Zhou et al., 1997) were used. Primers were used at a concentration of 50 nM, and the qPCR conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, 40 cycles at 95 °C for 15 s, 60 °C for 1 min, 1 cycle of 95 °C for 15 s and 1 cycle of 60 °C for 15 s. Analyses were performed in triplicate on plates with a capacity of 384 reactions. The Ct (threshold cycle) values were calculated for each sample. The relative quantification (RQ) was calculated with the method proposed by Livak and Schmittgen (2001), using  $\beta$ -actin (Pirrello et al., 2006) as an internal standard (non affected by 1-MCP + UV-C, UV-C, fruit growth and development) and control fruit for calibration.

#### 2.4. Ethylene production and fruit color

Fruit ethylene production was quantified by gas chromatography 1 h, 6 h, and 12 h, and daily (up to 12 d) after the UV-C application. The fruit in each group was placed in a 100 mL screw-cap glass vial. After 30 min of incubation, 1 mL of headspace was collected to determine the rate of ethylene production, and the results were expressed in ng kg<sup>-1</sup> s<sup>-1</sup>.

The color of all each of the six fruit in each group was measured daily (up to 12 d) on 4 sides with a colorimeter (Minolta CR-300 TM), and the results were expressed as the hue angle “H” [ $H = \tan^{-1}(b/a)$  when  $a > 0$  and  $b > 0$  or  $h = 180 + \tan^{-1}(b/a)$  when  $a < 0$  and  $b > 0$ ].

#### 2.5. Levels of lycopene, $\beta$ -carotene, lutein and zeaxanthin

Extraction techniques and chromatographic analysis were performed following methods described by Rodriguez-Amaya (2001) followed by saponification of the ether extract. Levels of lycopene,  $\beta$ -carotene, lutein and zeaxanthin were quantified using a high performance liquid chromatography (HPLC) system from Shimadzu equipped with an automatic injector, UV-vis detector at 450 nm, a RP-18CLC-ODS (5 mm, 4.6 mm  $\times$  150 mm, Shimadzu) reverse-phase column and CLC-GODS (5 mm, 2 mm  $\times$  4 mm, Supelco) guard column. Separation was performed using a gradient elution system with methanol (solvent A), acetonitrile (solvent B) and ethyl acetate (solvent C) as the mobile phase at a flow rate of 16.7 µL s<sup>-1</sup> (i.e. 1 mL min<sup>-1</sup>). The initial phase consisted of 30% A and 70% B; after 10 min the composition was changed to 10% A, 80% B and 10% C; after 35 min the composition was changed again to 5% A, 80% B and 15% C; the initial composition was repeated at 40 min and maintained for 2.5 min to rebalance the system. The peaks were identified by comparison with the retention times of standards and quantified by comparison with external calibration curves for lycopene,  $\beta$ -carotene, lutein and zeaxanthin (Sigma-Aldrich®) standards. The HPLC results are expressed as mg kg<sup>-1</sup> of fresh material.

#### 2.6. $\delta$ -tocopherol levels

Tocopherol extraction was performed as described by Rodriguez-Amaya (2001), using a method similar to that used for carotenoid extraction. The tocopherols were separated and quantified using HPLC in a manner identical to that described in item 2.5. The separation was performed by a gradient elution system with a mobile phase of: methanol (solvent A), isopropanol (solvent B), and acetonitrile (solvent C) at a flow rate of 16.7 µL s<sup>-1</sup> (i.e. 1 mL min<sup>-1</sup>). The gradient began with a ratio (A/B/C) of 40:50:10 (v/v/v), which was changed linearly to 65:30:5 over 10 min, then decreased to 40:50:10 over another 2 min and held constant for 15 min. The peak was identified by comparison with the retention time of the standard and quantified by comparison with an external calibration curve for  $\delta$ -tocopherol (Sigma-Aldrich®). Results on a fresh weight basis are expressed as mg kg<sup>-1</sup>.

#### 2.7. Levels of *p*-hydroxybenzoic acid, *p*-coumaric acid and quercetin

The extraction and identification of individual phenolic compounds was performed following the methods described by Häkkinen et al. (1998). Phenolic compounds were extracted with methanol acidified with 6 M HCl and separated and quantified using an HPLC process identical to that described in item 2.5. The mobile phase consisted of an elution gradient with acetic acid in water (99:1) (solvent A) and methanol (solvent B) at a flow rate of 15 µL s<sup>-1</sup> (i.e. 0.9 mL min<sup>-1</sup>). The starting percentage of 100% A was gradually changed to 60% A and 40% B over a period of 25 min, held constant at this ratio for a further 2 min, gradually changed to 95% A and 5% B at 37 min, held constant for an additional 5 min and then returned to the starting proportion for a total run time of 45 min. The phenolic compounds were identified by comparison with the retention time of standards and quantified based on calibration curves of external standards for *p*-hydroxybenzoic acid, *p*-coumaric acid and quercetin (Sigma-Aldrich®). The results are expressed on a fresh weight basis as mg kg<sup>-1</sup>.

#### 2.8. Polyamine levels

Polyamine extraction and quantification was carried out following Vieira et al. (2007) with minor changes. Polyamines were extracted with trichloroacetic acid (5% in water) and analyzed by HPLC separated in a C18 column (30 cm  $\times$  3.9 mm i.d.  $\times$  10 µm, Waters). Polyamine analyses used an elution gradient program in which mobile phase A was acetate buffer (0.1 M) containing 1-octanesulfonic sodium salt (10 mM), adjusted to pH 4.9 with acetic acid and eluent B was acetonitrile, at a flow rate of 11.7 µL s<sup>-1</sup> (i.e. 0.7 mL min<sup>-1</sup>). After separation, the amines were derivatized with *o*-phthalaldehyde (OPA) and detected fluorometrically at 340 nm excitation and 445 nm emission. Results were expressed on a fresh weight basis as mg kg<sup>-1</sup>.

#### 2.9. Experimental design and statistical analysis

The experimental design was completely randomized, consisting of 3 UV-C treatment groups (control, 1-MCP + UV-C, UV-C) with 3 analytical replicates. Data was verified for normality using Shapiro-Wilks' test and for homoscedasticity using Hartley's test. Results were analyzed using ANOVA, with a  $P \leq 0.05$  considered significant. Post-hoc analysis was performed using Tukey's test ( $p \leq 0.05$ ). SAS software was used for all statistical analysis (Sas Institute, 2002).



### 3. Results

#### 3.1. The effects of UV-C treatment on the transcriptional accumulation of ACO1 and ERF genes

As revealed by the relative accumulation of ACO1 and ERF transcripts, UV-C treatment affected the expression of most genes investigated (Fig. 1). There was an increase in the accumulation of ACO1 gene transcripts when fruits were treated with UV-C (Fig. 1A), and the application of 1-MCP prior to UV-C treatment reduced levels of ACO1 transcripts compared to UV-C treatment alone; however, levels were still above those observed in the control fruit.

Among the 14 ERF genes studied (Fig. 1B–O), 11 ERFs (*SI-ERF A.1*, *SI-ERF A.3*, *SI-ERF B.1*, *SI-ERF B.2*, *SI-ERF B.3*, *SI-ERF C.6*, *SI-ERF D.1*, *SI-ERF D.3*, *SI-ERF E.1*, *SI-ERF F.5*, *SI-ERF G.2*) increased with UV-C treatment, 2 ERFs (*SI-ERF E.2* and *SI-ERF E.4*) had decreased transcript accumulation and 1 ERF (*SI-ERF E.3*) was not affected by UV-C treatment. When the ethylene action inhibitor (1-MCP) was applied prior to UV-C treatment, there was less transcript accumulation compared to UV-C treatment alone for all of the ERFs studied, with the exception of *SI-ERF G.2*.

#### 3.2. The effects of UV-C treatment on ethylene production and color

The fruit subjected to UV-C treatment showed high ethylene production in the first hour after treatment. The evolution of ethylene production in all treatments followed a classic climacteric pattern, with increased ethylene production corresponding to the climacteric peak. However, the maximum climacteric peak was delayed by 1 d with UV-C treatment (Fig. 2A), and 3 to 4 d with the application of 1-MCP prior to UV-C, as compared with control tomatoes (Fig. 2A).

The application of UV-C helped to maintain the green color of the fruit, and 1-MCP+UV-C treatment further inhibited color change, and retained a higher °Hue value (Fig. 2B), despite the

increased ethylene production of this fruit (Fig. 2A). Additionally, better visual appearance in UV-C treated fruit was observed after 12 d of storage (Fig. 2C).

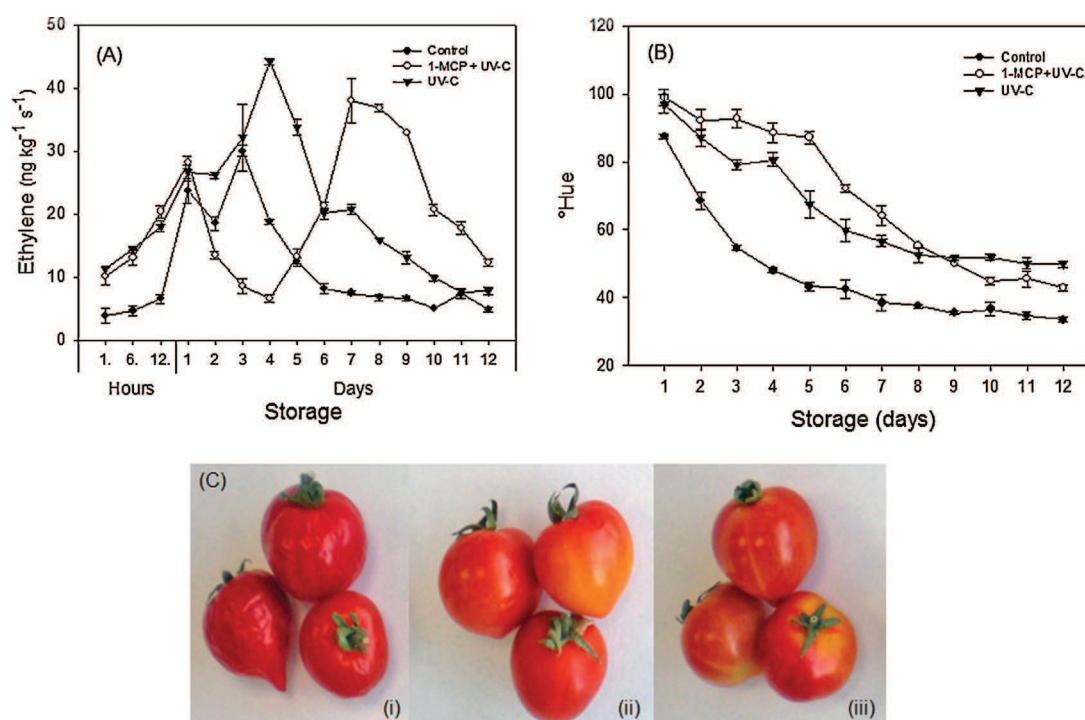
#### 3.3. Effect of UV-C on secondary metabolite levels

The UV-C treatment delayed the ripening evolution, with lower levels of lycopene,  $\beta$ -carotene, lutein and zeaxanthin and  $\delta$ -tocopherol observed, after six days of storage. Slower accumulation was observed when 1-MCP was applied before UV-C treatment (Table 1). The treatment also resulted in higher levels of all measured phenolic compounds (Table 1, chlorogenic acid, p-coumaric acid and quercetin).

Putrescine and spermidine were predominant among the polyamines detected in treated fruit (Table 1). It was clear that the application of UV-C promoted a greater accumulation of putrescine and spermidine after 6 d of treatment. In fruit that was previously subjected to treatment with 1-MCP before UV-C, polyamine levels were lower than in fruit treated only with UV-C but higher than control.

### 4. Discussion

There is a large body of research demonstrating the beneficial effects of UV-C radiation treatment on fruit (Maharaj et al., 1999; González-Aguilar et al., 2007; Charles et al., 2008a,b; Erkan et al., 2008; Liu et al., 2009; Pombo et al., 2011; Stevens et al., 1998; Liu et al., 2011; Tiecher et al., 2013; Maharaj et al., 2014; Syamaladevi et al., 2014). The results observed in this work have confirmed that UV-C treatment stimulates ethylene production, especially in the first few hours after treatment (Fig. 2A, Maharaj et al., 1999). Additionally, UV-C treatment causes an increase in ACO1 gene transcripts (Fig. 1A) that code for the enzyme ACC oxidase, which is active during the last step of ethylene biosynthesis (Barry et al., 1996; Cara and Giovannoni, 2008). This physiological response is consistent with the fact that UV-C is a stressor and that plants



**Fig. 2.** Effects of UV-C treatment on ethylene production (A), °Hue (B) and tomato fruits (C): (i) control, (ii) 1-MCP+UV-C, (iii) UV-C, in “MicroTom” tomato fruits stored for 12 d. Vertical bars represent the standard deviation.

**Table 1**Levels of carotenoids,  $\delta$ -tocopherol, phenolics, and polyamines from exocarps of "MicroTom" tomato fruit 6 d after the UV-C treatment.

	Carotenoids			Tocopherol		Phenolics		Polyamines	
	Lycopene (mg kg <sup>-1</sup> )	$\beta$ -carotene (mg kg <sup>-1</sup> )	Lutein + zeaxanthin (mg kg <sup>-1</sup> )	$\delta$ -tocopherol (mg kg <sup>-1</sup> )	Chlorogenic acid (mg kg <sup>-1</sup> )	p-coumaric acid (mg kg <sup>-1</sup> )	quercetin (mg kg <sup>-1</sup> )	Putrescine (mg kg <sup>-1</sup> )	Spermidine (mg kg <sup>-1</sup> )
Control	69.1a <sup>*</sup>	12a	3.6a	14.64a	192.8c	7.8a	6.8b	5.1c	17.3b
1-MCP + UV-C	20c	6.3b	2.4b	7.43b	256.9b	9.7b	7.9b	57.1b	40.2a
UV-C	26.6b	8.2b	2.5b	9.67b	367.6a	10.1b	19.8a	94.2a	43.1a

<sup>\*</sup> Means with the same lower case letter in the column are not statistically different according to the Tukey's test ( $p \leq 0.05$ ).

generally increase ethylene production under stress, likely by acting on system 2 autocatalytic ethylene (González-Aguilar et al., 2007; Liu et al., 2011; Van de Poel et al., 2012; Tiecher et al., 2013).

UV-C delayed ripening in tomato fruit, in despite of the increase in ethylene production (Fig. 2A) and ACO1 transcriptional expression (Fig. 1A). In fact, in fruit treated with UV-C radiation the development of coloration was slower than in the control fruit, and the treated fruit showed the fewest senescence signals (Fig. 2 B, C). This finding is consistent with Stevens et al. (1998), Maharaj et al. (1999), Liu et al. (2009) and Tiecher et al. (2013) who also found that UV-C treatment led to a reduction in ripening and delayed the onset of red coloration in tomatoes. The effect of UV-C on the development of fruit coloration may be due to its interference with carotenoids (Table 1), which are the predominant pigments in tomatoes (Stevens et al., 1998; Maharaj et al., 1999; Liu et al., 2009). Liu et al. (2011) reported a change in the profile carotenoids genes expressions in tomatoes treated with UV-C. A change in color is one of the most obvious transformations that takes place during tomato fruit ripening and involves the ethylene-dependent transition of chloroplasts to chromoplasts (Opiyo; Ying, 2005; Barsan et al., 2010). Moreover, UV-C treatment may cause changes in other antioxidant pathways, such as the production of antioxidant enzymes (Erkan et al., 2008) and synthesis of phenolic compounds (Charles et al., 2008b) and/or bioactive amines (Stevens et al., 1998; Maharaj et al., 1999; González-Aguilar et al., 2004; Tiecher et al., 2013). These compounds may prevent the degradation of chlorophyll and/or slow carotenoid degradation (Maharaj et al., 1999; Liu et al., 2009; Tiecher et al., 2013).

The effects of UV-C treatment on the levels of compounds derived from plant secondary metabolism, previously reported by several authors (Charles et al., 2008a,b; Erkan et al., 2008; González-Aguilar et al., 2004; Liu et al., 2009; Pombo et al., 2011; Tiecher et al., 2013) were partially confirmed by this work (Table 1). The UV-C slows the accumulation of lycopene and  $\beta$ -carotene in fruit, which explains the lower intensity of the characteristic red color (Fig. 2B). When applying 1-MCP prior to UV-C, this physiological response was strengthened (Fig. 2B). Variations in the fruit profile of these compounds according to differences in variety, ripening stage, growth, and postharvest conditions is widely reported (Charles et al., 2008a,b; Erkan et al., 2008; Liu et al., 2009; Pombo et al., 2011). UV-C radiation induced the accumulation of at least three of the phenolic compounds investigated (Table 1), which is in agreement with Charles et al. (2008b), who also found higher concentrations of phenolic compounds, an accelerated lignification process and the formation of suberin in tomatoes treated with UV-C.

The fact that UV-C radiation stimulated the accumulation of these compounds is interesting not only for prolonging shelf-life, but also for increasing plant defenses, and for increasing potentially bioactive compound levels (Stevens et al., 1998; González-Aguilar et al., 2004; Charles et al., 2008; Erkan et al., 2008; Tiecher et al., 2013). It is plausible that the ERFs influenced by UV-C (Fig. 1) control the biosynthesis of these compounds because the tomato is a climacteric fruit, and ethylene is involved in the control of several of its biosynthetic pathways (Cara and Giovannoni, 2008).

The delay of senescence signals (Fig. 2) may be correlate to the levels of polyamines (Table 1). These results support what has been reported in the peach by González-Aguilar et al., (2004) and by Maharaj et al. (1999) and Tiecher et al. (2013) in tomatoes. These authors suggested that by acting as a stressor, UV-C initiates the synthesis of polyamines that may be involved in the regulation of ripening.

Because ethylene can activate different transcription factors, including regulators of metabolic pathways involved in fruit ripening and those related to the stress response, the



transcriptional expression of ERFs was also evaluated. UV-C was found to have different effects on the expression of these genes (Fig. 1B–O). Ohme-Takagi and Shinshi (1995) characterized the first four ERFs in tobacco demonstrating that they respond differently to ethylene. Chen et al. (2008) reported that in tomatoes ERFs may be differentially regulated during ripening and in response to stress.

Most of the ERFs studied here (*Sl-ERF A.1*, *Sl-ERF A.3*, *Sl-ERF B.1*, *Sl-ERF B.2*, *Sl-ERF B.3*, *Sl-ERF C.6*, *Sl-ERF D.1*, *Sl-ERF D.3*, *Sl-ERF E.1*, *Sl-ERF F.5*, *Sl-ERF G.2*) showed higher transcript accumulation when the tomatoes were treated with UV-C suggests that these genes are strong candidates for explaining the UV-C response, and its relationship to ethylene. The delay in the ripening process, despite the increase in ethylene production, ACO1 level, and ERFs transcription level, could be due to activation of metabolic pathways of antioxidant protection for these ERFs (Liu et al., 2011; Erkan et al., 2008; Tiecher et al., 2013). In general, when 1-MCP was applied prior to UV-C, reduced accumulation of ERFs transcripts was observed, thus confirming that the expression of these transcription factors can be regulated by ethylene (Zhang et al., 2004; Pirrello et al., 2006; Wang et al., 2007). Moreover this data suggests that regulation of ERF transcripts by UV-C is ethylene dependent.

In this work, the classification proposed by Pirrello et al. (2012), who classified tomato ERFs into 8 sub-classes (A, B, C, D, E, F, G, H) was used; however, members of sub-class H were not evaluated. Of the 14 ERFs assessed in the present study, 6 (*Sl-ERF A.1*, *Sl-ERF B.1*, *Sl-ERF B.2*, *Sl-ERF D.1*, *Sl-ERF D.3* and *Sl-ERF E.4*) were isolated and characterized by Pirrello et al. (2012), who was the first to relate these ERFs to other types of plant stress.

Zhou et al. (1997), who studied ERFs *Sl-ERF A.3*, *Sl-ERF C.6* and *Sl-ERF G.2*, (described in his work as *pti4*, *pti5* and *pti6*, respectively), reported the ability of these ERFs to bind specific regions of EREBR's (ethylene-responsive element-binding proteins), also known as the GCC-box of PR-genes, increasing the tolerance of plants to biotic stress. The regulation of these ERF genes through phosphorylation may also influence the interaction of these transcription factors with the GCC-box regions of PR-genes (Gu et al., 2000; Xu et al., 2008, 2011). In the present study, these ERFs were strongly influenced by the abiotic stress generated by UV-C treatment, showing a significant increase in the accumulation of transcripts, especially *Sl-ERF C.6*, which showed an approximately 250-fold increase in expression relative to control fruit. This indicates that the induction of ERFs may contribute to the acquisition of tolerance to adverse conditions (He et al., 2001; Gu et al., 2002; Chen et al., 2008). Liu et al. (2011) also reported a significant increase in the expression of these three genes, especially *Sl-ERF C.6*, which corresponds to *pti5*. By over-expressing *Sl-ERF C.6* in tomatoes, He et al. (2001) reported increased levels of *GluB* and *catalase* gene transcripts, which are associated with resistance to diseases such as *Pseudomonas syringae* pv. *Tomato*. Likewise, Gu et al. (2002), observed that in *Arabidopsis thaliana* plants the ERFs *Sl-ERF A.3*, *Sl-ERF C.6* and *Sl-ERF G.2* interact with the GCC-box regions of PR-genes, resulting in pathogen defense. Chen et al. (2008) reported that water stress and low temperatures reduce the levels of *Sl-ERF A.3* transcripts. However, mechanical damage also increased the expression of this gene, which suggests that there may be different regulatory mechanisms depending on the stimulus.

The increase of transcript accumulation of *Sl-ERF B.3* agrees with the results published by Liu et al. (2011), which showed the relationship of this gene to the ripening tomatoes process and, Liu et al. (2014) that also reported delay of the onset of ripening caused for over-expression of *Sl-ERF B.3-SRDX* (a climacteric dominant repressor reversion).

Results from the present study on *Sl-ERF E.1*, previously characterized by Tournier et al. (2003) as *LeERF2*, reveal the

strong impact of ethylene on ERF expression, in agreement with the results of Pirrello et al. (2006), Liu et al. (2011), Zhang et al. (2009) and Zhang and Huang (2010) who also related the expression of this transcription factor to the hormone ethylene in tomato and tobacco plants.

*Sl-ERF E.2* and *Sl-ERF E.4* showed significantly reduced accumulation of transcripts after UV-C treatment. Zhang et al. (2004), who described *Sl-ERF E.2* as *JERF1*, demonstrated that expression of this ERF in tomatoes was induced by a number of factors: ethylene, methyl jasmonate (MeJA), abscisic acid (ABA) and salt treatment. In rice, plants over-expressing *JERF1* show increased drought tolerance (Zhang et al., 2010). In contrast, the results presented herein suggest that *Sl-ERF E.3* is not significantly involved in the response to UV-C, although Wang et al. (2004) reported that *Sl-ERF E.3* responds to jasmonic acid, ethylene, cold, salt stress and abscisic acid by binding to GCC-box and DRE regions of target genes.

In this study, *Sl-ERF F.5* also showed increased transcription as a result of UV-C treatment. Chen et al. (2008), studying *Sl-ERF F.5* (which they refer to as *LeERF3b*), related the expression of this ERF to stress generated by drought and low temperatures. This ERF possesses a amphiphilic repressor binding domain (EAR) (Xu et al., 2008; Pan et al., 2010; Pirrello et al., 2012). Pan et al. (2010) deleted the EAR of *Sl-ERF F.5* (referred to as *Sl-ERF3* in their study) and observed the induction of PR-gene expression, with increased tolerance to salt stress and reduced lipid peroxidation in *Ralstonia solanacearum*.

Herein, a relationship between UV-C treatment and ripening delay was established, and correlated with changes in 13 ERF transcripts evaluated during postharvest treatment. The ethylene action in response to UV-C treatment was confirmed with 1-MCP application before UV-C. It is clear that although UV-C promotes an increase in ethylene production, the concomitant increases in the ACO1 expression profile, and virtually all of the ERFs evaluated, result in extended fruit preservation. The delay in fruit ripening may be caused by the activation of ERFs that could act as regulators of metabolic pathways during ripening. However, this hypothesis needs to be better tested.

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