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

Joseana Severo [a,*], Aline Tiecher [b], Jullien Pirrello [c], Farid Regad [c], Alain Latché [c], Jean-Claude Pech [c], Mondher Bouzayen [c], Cesar Valmor Rombaldi [d]

[a] IFF, Instituto Federal Farroupilha, Eixo de Produção Alimenticia, Rua Fábio João Andolhe, 1100, Bairro Floresta, CEP 98590-000, Campus Santo Augusto, Santo Augusto, RS, Brazil
[b] UNIPAMPA, Universidade Federal do Pampa, Campus Itaqui, Rua Luiz de Joaquim de Sá Britto, s/n, Bairro Promorar, CEP 97650-000, Itaqui, RS, Brazil
[c] UMR 990 INRA/INP-ENSAT, Pole de Biotechnologie Végétale, 24 Chemin de Borde Rouge, P.O. Box 107, 31326 Castanet Tolosan Cedex, France
[d] UFPel/FAEM, Departamento de Ciência e Tecnologia, Campus Universitário, Caixa Postal 354, CEP 90010-900, Pelotas, RS, Brazil

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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 kJ m^{-2} UV-C radiation, 2 – application of 2 μL 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 ± 2 °C and 80 ± 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, β-carotene, lutein + zeaxanthin and δ-tocopherol; but retained higher levels of chlorogenic acid, ρ-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 F.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.

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).

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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-Methylcyclopropene (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-amino-cyclopropane. 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_2 and high water loss) in Kiwifuit. Liu et al. (2011), using microarray techniques, determined that UV-C irradiation induced the expression of defense response genes (such as PR related proteins, β-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-β-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 $\mu mol\,m^{-2}\,s^{-1}$. 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 $kJ\,m^{-2}$ 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 $\mu L\,L^{-1}$ 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 $kJ\,m^{-2}$ and stored at RT (20 ± 3 °C and 80 ± 5% RH) for 12 d. 2 – 1-MCP + UV-C: fruit were harvested and treated with 1-MCP at 2 $\mu L\,L^{-1}$ 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,