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Impact of UV irradiation in leaves, fruits and suspension-cultured cells of Micro-Tom, tomato

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Abbreviations: ACC= 1-amincocyclopropane-1-carboxylic acid; *ACS1a*= 1-amincocyclopropane-1-carboxylic acid synthase; APX= cytosolic ascorbate peroxidise; CIA= chloroform and isoamyl alcohol; DNase= deoxyribonuclease; PAL= phenylalanine ammonia-lyase; PR= pathogenesis-related; ROS= reactive oxygen species; RT-PCR= reverse transcription polymerase chain reaction; SA= salicylic acid; UV, ultraviolet.

Abstract: The present study aims to understand both positive and negative impacts of ultraviolet (UV) rays in living dwarf tomato plants (*Solanum lycopersicum* L. *cv*. Micro-Tom). This paper examines the impact of UV-C (254 nm) and UV-A (365 nm) on induction of cell death and expression patterns of pathogenesis-related (PR), stress-related and redox-related genes, namely, of 1-amincocyclopropane-1-carboxylic acid synthase (*ACS1a*), cytosolic ascorbate peroxidise (APX), phenylalanine ammonia-lyase (PAL), and pathogenesis-related genes (PR1 and PR-P2), in leaves, fruits (both green and red), and suspension-cultured cells of Micro-Tom. Effects of short exposure to UV-C, but not to UV-A, on induction of cell death (in cell suspension) and development of lesions accompanied by ion leakage (in the leaves) were observed while no morphological change was observed in the UV-treated green and red fruits. UV-dependent induction of PR genes (PR1 and PR-P2) in these samples suggested that UVs can be used for plant defense activation. In addition, expression of ACS1a was shown to be negatively and positively regulated by UV-C and UV-A, respectively. Thus UV-dependent post-harvest controls of fruit maturity and shelf-life are likely applicable (*i.e.* retardation and/or acceleration of maturation).

1. Introduction

Plants are naturally exposed to ultraviolet (UV) rays mostly at UV-A range (wave length, 320-400 nm), especially on sunny days in summer. In contrast, the level of harmful UV-C (wave length >220 nm) and UV-B (wave length, 280-320 nm) reaching the ground surface which potentially damages living organisms including plants could be effectively blocked and minimized by the presence of ozone layer above the stratosphere (Staehelin *et al*., 2001). However, especially in the southern hemisphere, the area of seasonal depletion of the ozone layer often expands outside the Antarctic region, occasionally allowing temporal increases in solar UV-B and relatively long-wave range of UV-C reaching the Earth's surface (Staehelin *et al*., 2001). Therefore, from the global point of view, studies on the plant-damaging impact of UV rays have key importance to both biologists and environmental researchers.

In general, irradiation with a high dose UV-C results in induction of programmed cell death in living plants. In seedlings and protoplasts of *Arabidopsis thaliana*, a dose of UV-C around 10-50 kJ m^{-2} induces an oligonucleosomal DNA fragmentation which is reminiscent of the apoptotic DNA laddering often described in mammalian cells (Danon and Gallois, 1998). In addition, UV-C-dependent cell death development in *Arabidopsis* involves caspase-like activity which is also similar to the events in animal apoptosis (Danon *et al.*, 2004).

On the other hand, a pulse or low dose of UV-C radiation is frequently used for direct removal of germs from fresh produce (Stevens *et al*., 1998). For instance, exposure of fruit tissue slices of zucchini squash to low dose UV-C

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reportedly lowers the microbial activity and prevents deterioration of fruit quality during subsequent storage at low temperatures, while a burst in respiration rate in the treated tissues is induced (Erkan *et al*., 2001). In addition to direct removal of pathogenic microbes from the plant surface by UV, researchers have reported attempts to use UV radiation for so-called 'plant hormesis' by which the susceptibility of plants to pathogens could be minimized and shelf-lives of fresh produce could be extended (Stevens *et al*., 1996, 1998). To date, various fruits and vegetables including sweet potatoes (Stevens *et al*., 1990, 1999), apples (Lu *et al*., 2007), peaches (Stevens *et al*., 1998; Lu *et al*., 2007), and tomatoes (Liu *et al*., 1993) have been tested for UV-mediated disease resistance controls. In most of the cases, resistance against pathogens was induced by UV-C (Stevens *et al*., 1999).

It is likely that treatment with UV rays (including UV-C) builds up the "immunity" against microbial infection in living plants, thus the UV hormesis effects described above may be, at least partially, attributed to stimulation of plants' innate immunity. The innate immune system of plants against pathogenic microbes is known to be elicited in response to recognition of pathogen-derived molecules by plant cells, as reviewed elsewhere (Dangl and Jones, 2001; Chisholm *et al*., 2006; Yoshioka *et al*., 2008). Recognition of such elicitors by the host cells' transmembrane receptors (Jones and Dangl, 2006; Altenbach and Robatzek, 2007) or resistance (R) proteins (Allen *et al*., 2004; Chisholm *et al*., 2006; Dodds *et al*., 2006) reportedly initiates the cellular signaling cascades which finally activate the defense mechanisms.

Micro-Tom, a dwarf cultivar of tomato (*Solanum lycopersicum* L.), originally produced for ornamental purposes, has been proposed as preferred plant material for molecular biological research, mainly because of its compact habitat and short life cycle (Meissner *et al*., 1997; Eyal and Levy, 2002; Marti *et al*., 2006). Recently, AbuQamar *et al*. (2009) suggested that Micro-Tom is a good model for studying the crosstalk between biotic and abiotic stress responses through regulation of gene expression.

In the present study, cellular damage (*viz*. cell death increase in suspension culture and increase in ion leakage at tissue level) and preceding changes in the gene expression patterns, especially those of defence-related, redox-related, DNA maintenance-related and fruit maturation-related genes, are shown to be induced by UV-A and UV-C in the leaves, fruits and suspension-cultured cells of Micro-Tom. The signalling mechanisms contributing to the regulation of UV responses are also discussed by analogy to plant immunity responses. Furthermore, the discussion addresses UV-dependent extension of the storage life of tomato fruits through stimulation of plant immunity mechanism by post-harvest exposure to UVs.

2. Materials and Methods

Plant materials

Following the protocols described elsewhere (Kadono *et al*., 2009), seeds of tomato (*Solanum lycopersicum* L., *cv*. Micro-Tom) were allowed to germinate on a wet paper towel in a transparent plastic container placed in a light-cycle-conditioned incubator (12 h light and 12 h dark at 23°C). Immediatly after germination the resulting plantlets were re-planted in plastic pots filled with a standard soil mixture and watered daily in the light-cycle-controlled incubator for three months. From the adult plants, leaflets, green immature and red ripe fruits (minimum size, 15 mm in diameter) were harvested before and after irradiation with UV lights.

Preparation of cell suspension culture

Again, following protocols described elsewhere (Kadono *et al*., 2009), cell suspension culture derived from Micro-Tom was prepared. Briefly, the leaf slices were taken from a seedling of Micro-Tom grown *in vitro* and placed on a MS agar plate containing 2,4-dichlorophenoxyacetic acid (0.2 μg ml^{-1}) to promote the formation of calli. Suspension culture of cells was initiated by addition of the sliced calli into the MS liquid medium (pH 5.8). The cells suspended in 30 ml of media in 100 ml-conical flasks were kept on gyratory shakers (at 130 rpm) at 23°C in darkness, with occasional sub-cultures by innoculating the fresh media with 3 ml of confluent culture. After about six months of continuous propagation of the cells with constant sub-culturing (initially twice a month and later once a week), a stable cell line was obtained.

For experimental purposes, the culture was pre-conditioned as follows. The confluent culture was used to inoculate the fresh MS liquid medium (3 ml culture to 30 ml medium) and pre-cultured for three days. Then 6.5 ml of each pre-culture was transferred to 100 ml of fresh MS liquid medium (in a 500 ml conical flask) and further cultured for three days. The resultant three-day-old large scale culture (at log-phase) was harvested and used for the experiments.

UV treatments

Intact and/or excised leaves (leaflets and leaf disks), green and red fruits, and suspension-cultured cells of Micro-Tom were irradiated with UV-C (254 nm) or UV-A (365 nm) using a handy UV trans-illuminator (SLUV-4, As-one, Osaka, Japan). The intensities of UV-A and UV-C were monitored with UV meters (UVX-25, UVP Inc., Upland, CA; YK-34UV, Lutoron Electronic Enterprise Co., Ltd., Taipei, Taiwan) and the intensity of UV-A and UV-C applied to the surfaces of plant materials were adjusted to 2.2 mW cm^{-2}, therefore the doses of UV irradiation were controlled by altering the length of irradiation time. Leaflets and fruits were exposed to UV rays immediately after harvest. To prevent water loss from the leafy samples during UV irradiation and incubation, leaflets and leaf discs were floated on ultrapure water in Petri dishes. For treatment of the cultured cells, 1 ml of cell suspension was added to each well on 12-well microplates and used for irradiation with UV rays. Irradiation time varied as indicated in the Results section.

Measurement of ion leakage from leaf discs

Measurements of ion leakage were performed using the discs of leaflets floated on the ultrapure water. Leaf discs were irradiated with UV rays for 30 min and further in-

cubated in darkness for up to 24 h. Each single well on a 12-well microplate was filled with 5 ml of ultrapure water and used to float three leaf discs (diameter, 9 mm) freshly prepared from the leaflets. Using a handheld conductivity meter (CD502A, Custom, Tokyo, Japan), monitoring of the changes in conductivity in the bathing liquid (with 1 h intervals up to 10 h) were carried out following 24 h of post-UV incubation. An increase in conductivity reflects the leakage of ions from the UV-dependently damaged leaves. The extent of ion leakage was expressed as the ratio (percentage) of recorded conductivity to the maximal conductivity obtained after boiling the samples.

Evaluation of cell death

Analysis of induced cell death in suspension culture was carried out as described elsewhere (Iwase *et al*., 2014). Following UV-treatments, 200 µl-aliquots of cell suspension were sampled and transferred into 1.5 ml tubes and statically incubated in darkness (for 2 h unless indicated). Then, 0.1 % (w/v) Evans blue was added to the cell suspension and further incubated for an additional 1 h. Following repeated washes with fresh media, counting of the stained cells was performed under microscopes (SMZ800 and Labophoto, Nikon, Tokyo, Japan; VHX-100, Keyence, Tokyo, Japan) and the level of cell death was quantified. For statistical analysis, three to four different digital images of cells under the microscope (each covering 50-100 cells to be counted) were acquired and analyzed.

RNA isolation following UV irradiation

Isolation of RNA followed by reverse-trancriptase polymerase chain reaction (RT-PCR) was carried out basically as described (Kunihiro *et al*., 2011). Leaflets and fruits were subjected to irradiation with UV rays for 30 min, and were sampled and frozen in liquid N_2 at 0, 1 and 10 h after irradiation. Post-UV incubation was carried out in darkness.

Cell suspensions on 12-well microplates subjected to 15 min of irradiation with UV rays were further incubated for 1 h in the darkness. Cells were then harvested by filtering through 40-µm pore nylon mesh and washed with fresh liquid culture medium. The obtained samples were frozen in liquid N_2.

These frozen samples were ground using a pestle and mortar and transferred to plastic tubes and extracted with the RNA extraction buffer containing 100 mM Tris-HCl (pH 8), 100 mM ethylenediaminetetraacetic acid, 100 mM LiCl, and 1% sodium dodecyl sulfate. Then aliquots of 1:1 mixture of phenol and CIA (chloroform:isoamyl alcohol at 24:1) were added and samples were subjected to centrifugation at 14,000 *g* for 10 min at 4°C. The upper layer collected in separate tubes were further subjected to extraction with aliquots of phenol and CIA and centrifugation. The resultant upper layer was again collected in new tubes and mixed with 1/3 volume of 10 M LiCl and kept at -30°C for 2 h. Following centrifugation, the resultant pellets were collected and re-suspended in 2 M LiCl and spin-collected. The pellet formed was dissolved in TE buffer and added with 1/2 volume of both phenol and CIA. The samples were subjected to 10 min of centrifugation and the upper layer was collected and mixed with the aliquot of CIA, and further centrifuged. For precipitation of RNA, the upper layer was collected and mixed with 1/10 volume of 3 M sodium acetate and 2.5 volume of absolute ethanol, and centrifuged. The pellet washed with 70% ethanol was spin-collected, dried and dissolved in diethylpyrocarbonate-treated water.

RT-PCR

Prior to analysis with RT-PCR, genomic DNA concomitantly present in the total RNA preparations was eliminated with RNase-free Cloned DNase I (Takara Bio Inc., Otsu, Japan). First-strand cDNA synthesis was performed using SuperScript™ III reverse transcriptase (Invitrogen Corporation, CA). The reaction mixtures (20 µl/tube) contained total RNA (2 µg), oligo-$(dT)_{20}$ (2.5 mM), and dNTP mixture (2 mM). The tubes were heated to 65°C for 5 min, cooled and kept at 4°C for 1 min using Program Temp Control System PC-320 (ASTEC, Fukuoka, Japan). To the reaction mixture, 4 µl of 5x First-Strand buffer, 1 µl of 0.1 M dithiothreitol, 1 µl of RNaseOUT recombinant RNase inhibitor and 1 µl of SuperScript™ III reverse transcriptase (200 units μl^{-1}) were added and incubated at 50°C for 60 min. The reaction was terminated by heating at 70°C for 15 min and cooling at 4°C for 15 min. The resultant cDNA solution was used for PCR performed with 60 ng of first-strand cDNA and Takara ExTaq™ (Takara Bio Inc., Otsu, Japan). For each sample, 30 cycles of PCR were performed with denaturing at 94°C for 1 min, annealing for 1 min and elongation at 72°C for 1 min. Sequences of the primers used and anealing temperatures employed are listed in Table 1.

Table 1 - List of primers used for RT-PCR

Genes studied	Accession numbers *	Forward primers	Reverse primers	Tm (°C)
Actin	U60481	cacactgtccctatttacga	gtaataacttgtccatcagg	51.3
ACS1a	U72389	gctttgggttagtttcagctc	gttcatgaactcatgatccaatc	56.5
APX	DQ096286	gagtacctcaaggctgttgacaaatg	gagcctcagcaragtcagcaaag	60.0
PR-P2	X58548	ggagagagttaacaagttgtgtg	gagtagtattaaaagttagctcg	60.0
PR1	DQ159948	cttctcatggtattagcc	ccaccatccgttgttgc	50.3
PAL	M83314	gacacacaagttgaagcatcac	cacatcttggttgtgttgctc	56.5

* Accession numbers were of NCBI GenBank.
Tm= melting temperature used for annealing.

3. Results

Cellular damage

On the UV-C-treated leaves, the damaging impact of UV-C at cellular level could be visualized as spotted lesions appeared (Fig. 1). Such visible damage was not observed on the UV-A-treated leaves. In addition to visible symptoms, UV-C irradiation resulted in a marked increase in ion leakage from leaf disc preparations (Fig. 2). Leakage of ions indicates that the cellular membrane is one of the targets of the damaging impact of UV-C irradiation.

After irradiations with UV rays at 2.2 mW cm^{-2}, suspension-cultured cells of Micro-Tom showed development of cell death depending on the type of UVs, exposure time and the length of post-exposure incubation (Fig. 3). Compared to UV-A, impact of UV-C irradiation was shown to be much more severe, requiring shorter irradiation time and post-irradiation incubation.

UV-responsive gene expressions

The genes examined were 1-amincocyclopropane-1-carboxylic acid (ACC) synthase gene (ACS1a), ascorbate peroxidise gene (APX, cytosolic isoform), pathogenesis-related (PR) genes PR1 and PR-P2, and phenylalanine ammonia-lyase gene (PAL) as possible targets of UV impact, and *actin* gene as non-inducible reference.

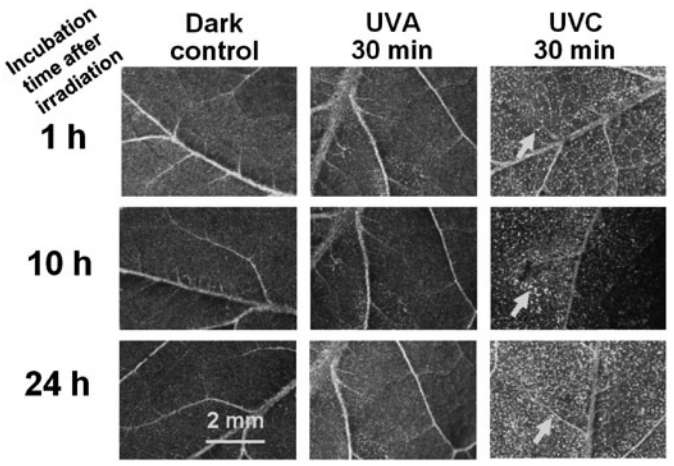

Fig. 1 - Development of UV-induced symptoms (lesions) on the leaves of Micro-Tom after the UV-irradiation. Leaflets were irradiated with UV rays for 30 min and further incubated in darkness on ultrapure water. Lesions on the leaves were observed under microscopes. Typical images from three repeated experiments are shown.

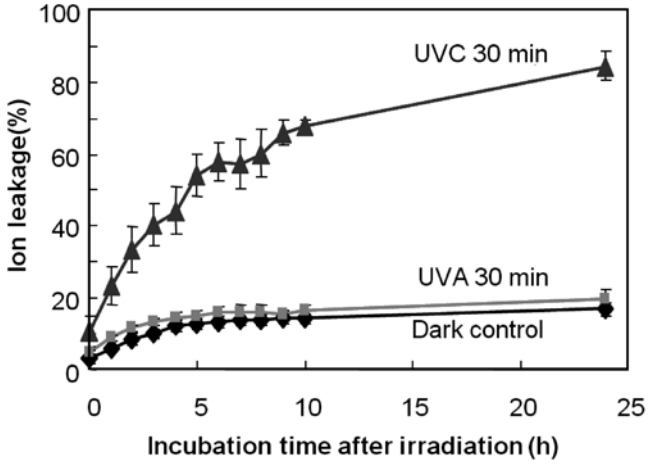

Fig. 2 - Measurement of ion leakage from the UV-irradiated leaf discs of Micro-Tom. Leaf discs were irradiated with UV rays for 30 min and further incubated in darkness on ultrapure water. The percentage of ion leakage was calculated as a ratio to conductivity after boiling of the leaf samples. Each data point and error bar reflect the mean and S.D., respectively (n = 3).

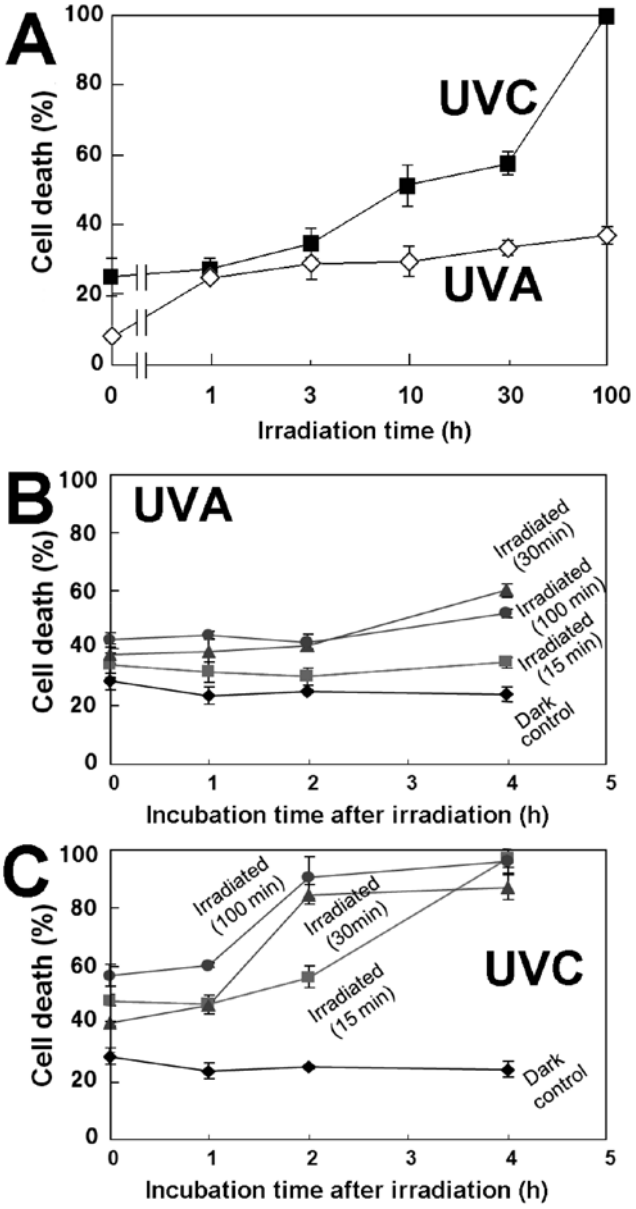

Fig. 3 - UV-induced cell death in suspension cultured cells. (A) Effect of irradiation time on the UV-induced cell death. (B) Progress of cell death after the UV-A-irradiation. (C) Progress of cell death after the UV-C-irradiation. Cell death was judged by Evans blue staining under microscopes. Each data point and error bar reflect the mean and S.D., respectively (n = 3).

In the cell suspension culture of Micro-Tom, expression of APX, PR-P2, PAL, and actin were shown to be maintained at active level even prior to the irradiation with UV rays (Fig. 4 A, dark control). In the leaf samples of Micro-Tom, ACS1a, APX, PAL, PR-1 and actin were shown to be expressed without UV irradiations (Fig. 4 B). Similarly, mature red fruit samples revealed high expressions of ACS1a, APX, PR-1 and *actin*, prior to the irradiation with UV rays (Fig. 5 B). In contrast, in the green fruit samples, only APX and actin were expressed in the dark control (Fig. 5 A).

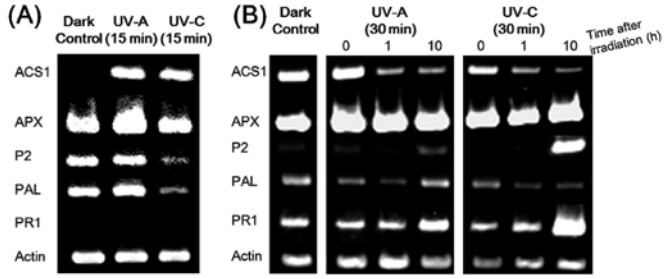

Fig. 4 - RT-PCR analysis of UV-responsive gene expressions in cell suspension and green leaves. Typical RT-PCR profiles of gene expressions in UV-irradiated cell suspension (A) and leaf samples (B) are shown. *Actin* was used as an internal control. Note: comparison of the density of bands on several gels were performed after gathering the images of gels on the black background.

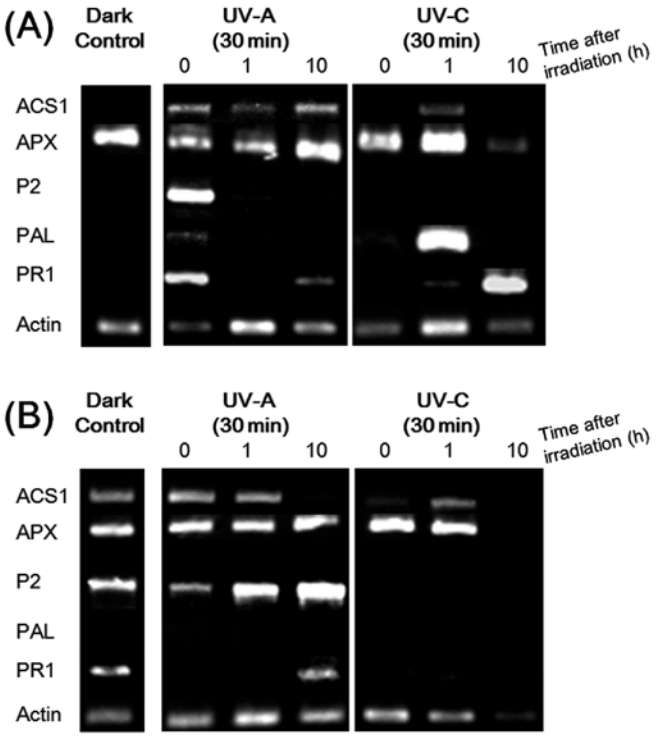

Fig. 5 - RT-PCR analysis of UV-responsive gene expressions in green fruits and red ripe fruits. Typical RT-PCR profiles of gene expressions in UV-irradiated green fruits (A) and red ripe fruits (B) are shown. *Actin* was used as an internal control. Note: comparison of the density of bands on several gels were performed after gathering the images of gels on the black background.

Among the genes tested, ACS1a was the only gene UV-dependently activated in the cell suspension culture. Expression of ACS1a was induced by both UV-A and UV-C (Fig. 4 A). UV-A-dependent activations of PR1 in the green fruits and leaves, of ACS1a in the green fruits and cell suspension culture, and of PAL and PR-P2 in the green fruits were observed (Fig. 4 and 5). UV-C-dependent activation of PR1 in green fruits and leaves, of PR-P2 in the leaves, of PAL in green fruit tissue, and of ACS1a in the green fruits and also in the cell suspension culture were observed (Fig. 4 and 5). Although PR1 was shown to be responsive to both UV-A and UV-C in green fruit tissue, the temporal profiles of induced gene expression largely differed. In green fruits, UV-A and UV-C were shown to be rapid and slow inducers of PR1 expression, respectively (Fig 5).

Since, most of the genes examined were active in the dark control of red fruit samples, drastic activation of gene expression could not be observed, except for the case of enhancement of PR-P2 expression which was originally active in the dark control.

APX was shown to be active in all materials even prior to UV irradiation, thus only the suppressive impacts of UV irradiations could be expected with this gene. Suppression of *APX* expression was observed in the UV-C-treated red fruit samples at 10 h after irradiation. Similarly, following irradiation with UV-C, expressions of PR-P2 and PAL, which were constitutively active in the cell suspension, were eventually suppressed. Expression of ACS1a in the leaves and red fruits was shown to be suppressed by UV-A irradiation. Expression of ACS1a, PR-P2, and PR1, originally active in red fruits, were shown to be suppressed by UV-C treatments.

4. Discussion and Conclusions

Cell death or defense activation?

A number of researchers have documented toxic impacts of UV rays in living plants including the damages to DNA, inhibition of photosynthesis, generation of reactive oxygen species (ROS) (Roldán-Arjona and Ariza, 2009). On the other hand, there have been some attempts to develop the UV irradiation protocol for so-called 'plant hormesis' by which the susceptibility of plants to invading pathogen is minimized and thus shelf-life of fresh produces is likely extended (Lu *et al*., 2007). For example, Kunz *et al*. (2008) demonstrated that UV-C-induced DNA damage in *Arabidopsis* accompanies the dose- and time-dependent development of resistance against *Hyaloperonospora parasitica*. Interestingly, it has been shown that, even in the absence of UV-C, plant nucleotide excision repair mutants displayed the identical type of resistance to *H. parasitica*, suggesting a positive role for UV-C-mediated DNA damaging events in plant immunity activation.

APX, one of the typical antioxidant genes, encodes for the enzyme capable of H_2O_2 elimination, thus protecting

the plants from the damaging impacts of ROS. PR genes (*viz.*, PR-P2 and PR1) and PAL are related to the actions and production of salicylic acid (SA). SA is a hormone-like natural signaling molecule involved in the defense response against infection by pathogens in higher plants. SA acts by stimulating the production of PR proteins through a complex signaling mechanism involving ROS, calcium and protein phosphorylation in the early stages (Kawano *et al*., 1998; Kawano and Bouteau, 2013). In *Solanaceae* plants including tobacco and tomato, the increase in *PAL* expression reportedly results in accumulation of SA (Kawano *et al*., 2004).

Here, the short exposure to UV-C but not to UV-A resulted in an acute increase in cell death in the cell suspension culture (Fig. 3). Development of lesions (visible symptoms), (Fig. 1) accompanied by ion leakage (sign of membrane damage) (Fig. 2) was induced in the UV-C-treated leaf samples. In contrast, no visible damage was observed in the UV-treated green and red fruits. It is noteworthy that UV-A or sub-lethal low dose UV-C was shown to induce the defense-related genes (*PR1* and *PR-P2* genes) in the leaves (Fig. 4 B) and green fruit tissues (Fig. 5 A).

The above data imply that cell death (possibly apoptotic, data not shown) and plant protection mechanisms (expression of antioxidant genes and PR genes) are induced by high and low doses of UV-C, respectively. Therefore, moderate doses of UV irradiation should be applicable to plants to confer tolerance to a variety of abiotic stresses (chiefly, oxidative stress) and innate plant immunity (represented by the induced expression of PR genes).

Possible involvement of SA signaling

Our data are comparable to the work of Marco *et al*. (2008) who reported the ozone-induced gene expression controls in the leaves of three tomato cultivars (*viz.*, Nikita, Alisa Craig and Valenciano). Similar to our data on UV-treated Micro-Tom, expression of *APX* is reportedly suppressed in the ozone-treated Nikita tomato (Marco *et al*., 2008). In addition, the tomato leaves chronically exposed to ozone showed activation of redox-related and defense-related genes such as *PAL* (Marco *et al*., 2008). Thus, ozone-induced gene expression patterns and the UV-induced gene expression patterns may share a common regulatory mechanism.

One of the key pathways that may be common to ozone response and UV response in tomato is the SA signaling pathway. Expressions of PR genes and *EDS1*, known factors functioning upstream of SA-dependent expression of PR genes (Falk *et al*., 1999), were induced by ozone in three tomato cultivars (Marco *et al*., 2008). In the present investigation, activation of *PAL* and PR genes in the UV-treated Micro-Tom leaves (Fig. 4B) was observed, suggesting the possible involvement of a SA signaling path.

Recently, in the culture of *Arabidopsis* cells, we observed that over-expression of bacterial salicylate hydroxylase gene (NahG) and pharmacological reagents targeting either ROS or calcium signaling effectively blocked both the cell death induction by high-dose UV-C and induction of defense-related gene expressions by sub-lethal low-dose UV-C (Hiramatsu *et al*., unpublished results). Requirements for early calcium signaling, proven by aequorin luminescence and the action of calcium targeted pharmacological reagents, and generation of ROS, are commonly observed in various plant models during the responses to SA (Kawano *et al*., 1998; Kawano and Muto, 2000; Kawano and Bouteau, 2013), pathogen-derived molecules (Kadota *et al*., 2004), ozone (Kadono *et al*., 2006, 2010; Tran *et al*., 2013), peroxyacetyl nitrate (Yukihiro *et al*., 2012), toxic metal ions (Lin *et al*., 2005; Kagenishi *et al*., 2011; Kunihiro *et al*., 2011), and UV (Hiramatsu *et al*., unpublished results).

Possible regulation of SA action by UV-C

According to the work by Nawrath *et al*. (2002) using *Arabidopsis thaliana*, expression of EDS5, a member of multidrug and toxin extrusion transporter family, known to be involved in the accumulation of SA and *PR1* transcript, is very low in unstressed plants but strongly induced by attacks by pathogens, treatment with SA, and irradiation with UV-C. EDS5 expression induced by pathogen infection and UV-C exposure largely depends on the pathogen response proteins EDS1, PAD4, and NDR1, suggesting the requirements for the signal transduction pathways commonly employed in UV-C responses and defence responses (Nawrath *et al*., 2002). This defense mechanism-dependently induced *EDS5* transcript reportedly starts accumulating 2 h after exposure to UV-C, and the transcription level likely remains for two days, thus further contributing to the long-lasting SA responses. This view shall be studied in detail in our future experiments.

Possible role of chlorophylls.

Our data indicate that the response in green samples (leaves and immature fruits) and non-green samples (ripe fruits and suspension cultured cells) might differ in their modes of responses to UVs. While PR1 expression, one typical measure of stress responses, was induced by UV-C in green tissues, non-green samples (both the ripe fruits and cell suspensions) showed no induction of PR1 expression (Fig. 4 and 5). It is tempting to speculate that the chlorophyll-related compounds may behave as secondary active signals for mediating the stress responses. One of such candidate compounds may be pheophorbide *a* derived from chlorophyll *a*, which is known to be produced in the ethylene-exposed green tomato fruits (Kawano *et al*., 1999). Recently, it was revealed that pheophorbide *a* plays a key role in both light-dependent and light-independent cell death mechanism in plants (Hirashima *et al*., 2009). This aspect is worth testing in future experiments.

Regulation of ethylene production

ACS1a coding for ACC synthase is a ripening-related gene responsible for production of ethylene precursor, ACC. Since ethylene promotes the ripening of fruits and senescence of leaves, UV-dependent changes in ACS1a expression may drastically affect the life-cycle of the

plants and shelf-life of the fruits. This ethylene biosynthesis-related gene was shown to be activated by both UV-A and UV-C in the cell suspension culture and green fruits, suggesting that UV treatments may be available for post-harvest enhancement of fruit maturity. Interestingly, UV-A and UV-C irradiation to red fruits resulted in gradual lowering of the ACS1a expression level (Fig. 5), suggesting that UVs are possibly applicable for retardation and prevention of ethylene-mediated fruit over-ripening and softening.

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