The role and mode of action of UV-C hormesis in reducing cellular oxidative stress and the consequential chilling injury of banana fruit peel

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Abstract: The reduction of cellular oxidative stress causing chilling injury (CI) of banana fruit peel by UV-C hormesis was investigated. Banana [*Musa* (AAA group, Cavendish subgroup) cv. Cavendish] fruits were treated with UV-C at dosages of 0.02 kJ m^{-2}, 0.03 kJ m^{-2} or 0.04 kJ m^{-2} prior to storage at 5 and 25°C. Symptoms of CI were observed when fruits stored at 5°C and severity was increased with time of storage. However, UV-C treatment reduced both the incidence of CI and its severity compared with controls. UV-C treatment activated phenylalanine ammonia-lyase and resulted in higher levels of total phenolic compounds in comparison with untreated controls. Our results showed that oxidative stress caused by CI in banana resulted from the accumulation of reactive oxygen species (ROS) intermediates such as H_2O_2 and superoxide anion, and at CI-inducing temperatures, a marked increased in H_2O_2 content was observed. However, UV-C treatment led to significantly higher activities of superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and glutathione reductase compared to control fruit during later storage. The activation of antioxidants by UV-C treatment reduced cellular oxidative stress damage, as indicated by lower levels of malondialdehyde and DNA degradation. In addition, heat-shock protein (HSP) 70 gene expression was increased by UV-C treatment, suggesting a possible mode of action of UV-C in the prevention of cellular damage and maintenance of cellular homeostasis. Taken together, these results suggest that the development of CI symptoms in banana fruit are associated with ROS accumulation, and UV-C treatment, by activation of defense mechanisms such as antioxidant enzymes and HSP70 gene expression, can reduce cellular oxidative stress, thus preventing membrane degradation and DNA damage associated with CI.

Keywords: Banana, chilling injury, UV-C, antioxidant enzymes, DNA damage, heat-shock protein

Introduction

Banana (*Musa* sp.) is the most widely grown fruit crop in tropical countries, and it is globally important in international trade (FAO, 2004). However, banana is a delicate and highly perishable fruit that is extremely sensitive to low temperatures, which can cause chilling injury (CI). This sensitivity greatly limits the maintenance of postharvest quality, mainly because of poor handling and storage practices. CI symptoms of banana fruit are usually apparent only in the peel. The most common symptom is browning of the peel vascular tissues throughout the peel surface (Pantastico *et al*., 1967).

It is widely accepted that CI symptoms developing during or after exposure of the plant to low temperature are a consequence of oxidative stress caused by an accumulation of toxic metabolites in the tissue, particularly free radicals and reactive oxygen species (ROS), which themselves may be causally related to alterations in membrane function (Foyer and Noctor, 2003).

The formation and accumulation of ROS, including superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and peroxyl radical ($ROO^{\bullet}$), can result in oxidization of proteins, unsaturated fatty acids, DNA, and RNA, causing cellular damage and eventually cell death (Mittler, 2002). To protect cellular membranes and organelles from the damaging effects of ROS, plants have evolved an efficient antioxidant defense system that can both prevent the accumulation of ROS and repair oxidative damage. These ROS scavenging systems consist of several antioxidant enzymes, such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR), and some non-enzymatic antioxidants, such as ascorbic acid, α-tocopherols, phenolic compounds, and reduced glutathione (Mittler, 2002). In the postharvest period, increased tolerance to oxidative stress of fruits and vegetables has been associated with factors such as activation of water- and lipid-soluble antioxidants, regulation of ROS production and accumulation, and membrane composition (Hodges and Forney, 2000).

The exposure of fruit to several stress conditions can elevate injury caused by inducing defenses mechanisms (Sabehat *et al*., 1998). UV-C hormesis, a recently introduced approach to postharvest strategy,

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involves the application of potentially harmful radiation at low doses to living organisms to induce beneficial stress responses (Shama and Alderson, 2005). The focus of this research has been on the utilization of UV-C to delay postharvest senescence, improve quality, and control decay in various fruits and vegetables (González-Aguilar *et al*., 2007; Maria *et al*., 2008; Mustafa *et al*., 2008). In particular, Vicente *et al*. (2005) reported that a short UV-C treatment can maintain fruit quality and also reduce the CI incidence in bell pepper. However, there have been no reports on how UV-C treatment affects overall ROS and the antioxidant defense system involved in oxidative damage, whether from chilling stress or occurring during banana fruit ripening.

Molecular chaperones are stress proteins, and many of them were originally identified as heat shock proteins (HSPs). HSPs facilitate protein refolding and stabilize polypeptides and membranes, and HSP70 in particular has essential functions in preventing aggregation and assisting refolding of nonnative proteins under stress conditions. Moreover, HSP mRNA expression levels may increase after exposure to low temperature (Wang *et al*., 2004). Although biochemical and physiological mechanisms of chilling stress tolerance are comparatively well understood, the role and mode of action of UV-C hormesis in inducing chilling tolerance are still unclear. Thus, molecular-based approaches investigating plant responses to UV-C hormesis are imperative.

The objective of this study was to investigate the role and mode of action of UV-C hormesis in reducing cellular oxidative stress and the consequential chilling injury of banana fruit peel. We also examined the relationship of changes in antioxidant potential and ROS intermediates to CI symptoms and that between HSP70 expression and reduced cellular oxidative damage.

Materials and Methods

Plant materials and UV-C treatments

Banana (*Musa* (AAA group, Cavendish subgroup) cv. Cavendish) fruits, imported to Japan from the Philippines, were obtained from a wholesale market at the mature green stage. Fingers were selected and separated from the bunch, then sorted to eliminate damaged and shriveled fruit. Selected fruits were randomized and used for the experiments. Fruit were subjected to UV-C at three illumination dosages (0.02, 0.03, and 0.04 kJ m^{-2}) from an EL Series UV lamp (UVP, Model UVS-28, 8W, Upland, California, USA) equipped with a filter to emit only one wavelength, 254 nm, with an intensity of 2 Wm^{-2}

at a distance of 50 cm. Both sides of the banana fruits were illuminated with UV-C each at the nominal illumination duration to obtain irradiation uniformity. The intensity of the UV-C lamp was determined with a Delta OHM photo/radiometer (Model HD2102.2, Padua, Italy). The UV-C illumination was performed at 25°C. After the UV-C treatment, fruits were placed on a plastic tray, covered with a perforated plastic bag, and then stored at 5 and 25°C in the dark.

Chilling injury assessment

CI of stored banana fruit was scored visually as described by Nguyen *et al*. (2003) with slight modification. We used a rating scale from 0 to 5, based on the intensity of peel surface browning: 0 = no CI; 1 = very mild injury; 2 = mild injury; 3 = moderate injury; 4 = severe injury; and 5 = very severe injury.

Measurement of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) content

The rate of superoxide production was measured following the method of Chaitanya and Naithani (1994). Samples of about 3 g were homogenized under a N_2 atmosphere in 10 mL of cold (0−4 °C) 100 mM sodium phosphate buffer (pH 7.5) containing 1 mM diethyldithiocarbamate (to inhibit SOD activity) and 0.5 g polyvinyl polypyrrolidone (PVPP). After centrifugation of the mixture at 22 000 × *g* for 20 min, $O_2^{\bullet-}$ was measured in the supernatant by its capacity to reduce nitroblue tetrazolium (NBT). The assay mixture (total volume 3 mL) consisted of 100 mM sodium phosphate buffer (pH 7.2) containing 1 mM diethyl thiocarbamate, 0.25 mM NBT, and the supernatant. The absorbance of the end product was measured at 540 nm. $O_2^{\bullet-}$ formation was expressed as ΔA 540 min^{-1} mg^{-1} protein.

The H_2O_2 content was measured colorimetrically as described by Mukherjee and Choudhuri (1983) with modification. H_2O_2 was extracted by homogenizing a sample (3 g) in 100 mL of 5% trichloroacetic acid (TCA) and 0.5 g PVPP. The homogenate was centrifuged at 6000 × *g* for 25 min. One milliliter of supernatant was then mixed with 1 mL of 0.1% titanium sulfate in 20% H_2SO_4 (v/v), and the mixture was centrifuged at 6000 × *g* for 15 min. The intensity of the yellow color of the supernatant was measured at 410 nm. The H_2O_2 content was calculated using the extinction coefficient 0.28 μmol L^{-1} cm^{-1}.

Total phenolics and phenylalanine ammonia-lyase (PAL) activity

Total free phenolic content was estimated colorimetrically by the method described by Singleton and Rossi (1965). Briefly, frozen tissue was

homogenized in ethanol, filtered, and centrifuged. Then, phenolic compounds were determined photometrically after reaction with the Folin Ciocalteau reagent. PAL was extracted and assayed as described by Ke and Salveit (1986) with slight modification. Peel tissue (5 g) was homogenized in 20 mL of 50 mM borate buffer (pH 8.5) containing 5 mM 2-mercaptoethanol and 0.5 g PVPP. The homogenate was filtered through three layers of cotton cloth and then centrifuged at $18000 \times g$ for 20 min at 4°C. PAL activity was determined in the supernatant, 0.3 mL of which was added to a reaction mixture containing 0.7 mL of 100 mM L-phenylalanine and 3 mL of 50 mM borate buffer (pH 8.5). After incubation of the mixture at 40°C for 1 h, the reaction was stopped by adding 0.1 mL of 5 mM HCl. PAL activity was measured at room temperature. PAL activity was calculated from the absorbance of the assay mixture at 290 nm, based on the production of cinnamic acid.

Malondialdehyde (MDA) content

The MDA content was assayed by the method of Wang *et al*. (2005) with slight modification. Five grams of banana peel were homogenized in 20 mL of 100 mM sodium phosphate buffer (pH 7.0) containing 0.5 g PVPP. The homogenate was filtered through Miracloth (Calbiochem, San Diego, USA) and then centrifuged at $17\,000 \times g$ for 30 min at 4 °C. Thiobarbituric acid (TBA) reactivity was determined by adding 4 mL of 0.5% (w/v) TBA in 15% (v/v) TCA to 1.5 mL of the supernatant. The reaction solution was held for 20 min in a boiling water bath, 100 °C, cooled quickly, and finally centrifuged at $12\,000 \times g$ for 10 min to clarify the solution. Absorbance was measured at 532 and 600 nm. The level of MDA was calculated with an extinction coefficient of 1.55 nmol L^{-1} m^{-1} and expressed as nmol.g^{-1} FW.

Enzyme extraction and assays, and protein analysis

Five grams of frozen banana peel sample was homogenized in 20 mL of ice-cold extraction buffer and 0.5 g PVPP with a Kinematica tissue grinder (Polytron PT-MR2100, Lucerne, Switzerland). For the SOD, POD, and CAT assays, 100 mM sodium phosphate buffer (pH 7.0) was used. For the GR analysis, 0.1 M Tris-HCl buffer (pH 7.8) containing 2 mM EDTA-Na and 2 mM dithiothreitol (DTT) was used. For APX, 50 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA and 2 mM DTT was used. The homogenate was filtered through Miracloth and then centrifuged at $27\,000 \times g$ for 30 min at 4°C. The resulting supernatants were used directly for the assays.

The SOD assay used the xanthine oxidase (XO)/ NBT system according to Ukeda *et al*. (1997). Into 2.4 mL of 50 mM sodium carbonate or sodium phosphate buffer, 0.1 mL each of 3 mM xanthine, 3 mM EDTA, 0.75 mM NBT, 15% (w/v) bovine serum albumin (BSA), and SOD solution or water were added. The reaction was initiated by the addition of XO. The absorbance change at 560 nm was monitored at 25°C. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease in SOD-inhibitable NBT reduction.

POD activity was assayed, using guaiacol as a substrate, by the method of Zhang *et al*. (2005) with slight modifications. The reaction mixture (3 mL) contained 0.5 mL of enzyme extract, 2.48 mL of 0.05 M phosphate buffer (pH 7.0), 0.1 mL of 20 mM H_2O_2, and 0.1 mL of 20 mM guaiacol. The increase in POD activity at 470 nm, due to guaiacol oxidation, was recorded after 2 min. One unit of enzyme activity was defined as the amount that caused an absorbance change of 0.01/min.

CAT activity was measured according to Wang *et al*. (2005) with slight modifications. The reaction mixture consisted of 2 mL sodium phosphate buffer (50 mM, pH 7.0), 0.5 mL H_2O_2 (40 mM), and 0.5 mL enzyme solution. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm. The specific activity was calculated using the extinction coefficient 40 mM^{-1} cm^{-1}.

GR activity was assayed according to Smith *et al*. (1988) by monitoring glutathione-dependent oxidation of NADPH at 340 nm. Glutathione disulfide (GSSG) was added to start the reaction, and the rate of oxidation was calculated using the extinction coefficient of NADPH (6.22 $mmol/L^{-1}$ cm^{-1}). GR activity was expressed as micromoles of NADPH oxidized per milligram of protein per minute.

APX activity was assayed, with some modification, according to the method of Amako *et al*. (1994) by the decrease in absorbance at 290 nm as ascorbate was oxidized by H_2O_2. The assay mixture contained 0.1 mL enzyme extract, 0.1 mL of 5 mM sodium ascorbate, 0.2 mL of 1.0 mM EDTA, and 1.2 mL of sodium phosphate buffer (50 mM, pH 7.0). Then, 0.4 mL of 60 mM H_2O_2 was added to start the reaction. Enzyme activity was expressed as micromoles of ascorbate oxidized per milligram of protein per minute.

Total protein content was measured according to the method of Bradford (1976), using BSA as the standard protein.

DNA extraction and agarose gel electrophoresis

DNA extraction was performed as described by Wagner *et al*. (1987) with modification. Five

grams of sample were ground into fine powder with liquid nitrogen with a Multi-Beads Shocker (Yasuikikai, Osaka, Japan) and then immediately transferred into 40 mL of isolation buffer (10% polyethylene glycol, 0.35 M sorbitol; 0.1 M Tris-HCL, pH 8.0; 0.5% spermidine; 0.5% spermine; 0.5% β-mercaptoethanol). After centrifugation at 27 000 × *g* (10 min at 4°C), the precipitate was extracted again with 25 mL of lysis buffer (0.35 M sorbitol; 0.1 M Tris-HCL, pH 8.0; 0.5% spermidine; 0.5% spermine; 0.5% β-mercaptoethanol; 1% PVPP), and then 10% sarcosine was added and the mixture incubated for 10 min at ambient temperature. After centrifugation, the precipitate was transferred to an extraction buffer (100 mM Tris–HCl, pH 9.5; 20 mM EDTA; 2% CTAB; 1.4 M NaCl; 0.5% β-mercaptoethanol), and incubated at 65°C for 10 min. DNA was then extracted with phenol:chloroform:isoamylalcohol (25:24:1, by volume) and precipitated with isopropanol. RNase was used to digest existing RNA, and the precipitate was extracted again, first with phenol/chloroform (1:1, by volume) and then with chloroform, and DNA was precipitated again with isopropanol. Finally, the pellets were washed with 70% ethanol and dissolved in TE buffer (10 mM Tris–HCl; 1 mM EDTA, pH 8.0). Extracted DNA, 10 μg from each sample, was subjected to agarose gel electrophoresis (100 mV) on a 1.2% agarose gel, and stained with ethidium bromide.

Real-time (quantitative PCR) expression analysis

Total RNA was extracted from banana peel by a method modified from Chang *et al*. (1993). First-strand cDNA was prepared from 1 μg extracted RNA using a Prime® RT Kit with gDNA Eraser (perfect Real Time) (TAKARA, Shiga, Japan) following the manufacturer's instructions and used as template for quantitative PCR (Q-PCR). Forward and reverse primers were designed based on the conserved nucleotide sequences from genes in the National Center for Biotechnology Information (NCBI) database. The accession numbers are given below. The primer set for the genes encoding heat-shock protein 70 (Hsp70; accession no. EU126852) was 5'CAAGATGGACAAGAGCAGTGTC 3' and 5'-ACAGCAGCACCATAAGCAACT-3'. Primers for the actin gene (ACT1; accession no. EF672732) were 5'-ATTGTGCTTGATTCTGGTGATG-3'and 5'-TTCAGCAGTGGT AGTGAAGGAA-3'. The amplification reactions were performed using Brilliant® SYBR®Green QPCR Master Mix (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions in a Real-Time QPCR System (Mx3005P, Stratagene). For amplification,

the reaction mixtures were initially held at 95°C for 3 min, followed by 40 cycles of 95°C for 20 s, 60°C for 20 s, and 72°C for 30 s; the melting curve was from 55°C to 95°C. Each reaction was performed in triplicate. The amplification efficiency was estimated from the melting curve, and amplification products were visualized on agarose gels (1.5%, w/v). The relative expression levels were normalized against the actin gene expression levels and compared with those of non-treated fruit samples as a control, with day 0 was assigned a nominal value of 1.

Statistical analysis

Experiments were performed according to complete randomized design (CRD). Data were analyzed by ANOVA. All data are presented as means ± SE. The statistical analysis program procedure was used, and mean separation was determined by the least significant difference (LSD) method ($P \leq 0.05$).

Results and Discussion

*Effects of UV-C treatment on reducing CI symptoms and oxidative stress*

Browning of the peel vascular tissues throughout the peel surface is the typical symptom of CI in banana, and it is usually apparent only in the peel (Pantastico *et al*., 1967). Bananas stored at 5°C in this study showed initial visible CI after 2 days of storage, and its severity increased with storage time (Figure 1). However, UV-C treatment significantly reduced CI severity; the initial visible CI symptoms were observed only after 4 days of storage in UV-C-treated fruit, and its severity was consistently lower than in the control until the end of the storage period.

Low temperature induces oxidative stress in the cell, and chilling temperatures alter the equilibrium between ROS generation and oxidative stress defense mechanisms. Antioxidant enzyme activity may also be related to chilling tolerance, as chilling-tolerant cultivars of several crops have higher antioxidant enzyme activity than susceptible cultivars (Imahori *et al*., 2008). The synthesis of lipid and water-soluble antioxidants, such as ascorbic acid, glutathione, α-tocopherol, flavonols, carotenoids, and other phenolic compounds, are part of a complex mechanism of chilling tolerance that involves both restricting the production of ROS and protection from the ROS produced (Walker and Mc Kersie, 1993). In this study, PAL activity of banana fruits stored at 25°C was higher than that of fruit stored at low temperatures, and UV-C treatment significantly increased PAL activity (Figure 2A). Changes in total free phenolics similar to those in PAL activity

were also observed in response to UV-C treatment (Figure 2B). Our results are consistent with those of many previous studies showing the accumulation of phenolic and flavonoid compounds in plants in response to UV irradiation (González-Aguilar *et al.*, 2007). Moreover, in combination with our results on CI severity, these results suggest that both PAL activity and accumulation of free phenolics may contribute to the defense of plant tissues against chilling injury by scavenging harmful ROS generated during chilling stress, and that both are activated by UV-C treatment.
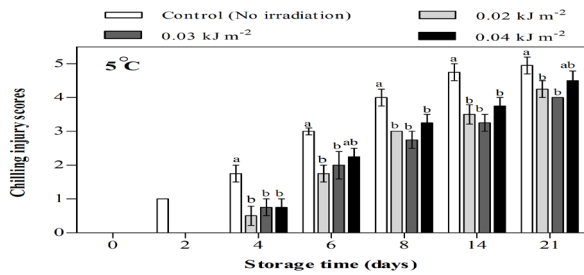

Figure 1. Changes in chilling injury (CI) scores of banana fruit treated with different UV-C dosages and then stored at 5 °C. Values are means ± SE (*n* = 10). Different letters above the bars indicate significant mean differences at each day of storage [least significant difference (LSD) test; *P* ≤ 0.05]
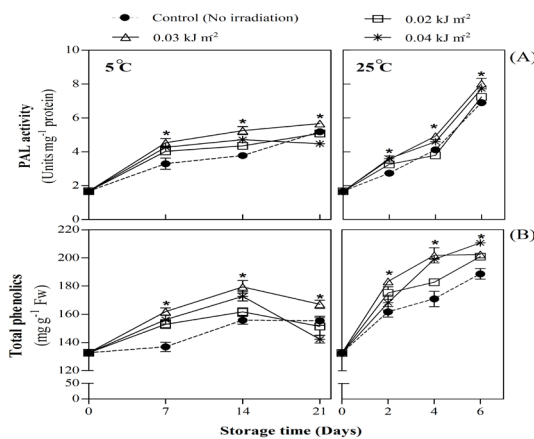
Figure 2. Changes in phenylalanine ammonia-lyase (PAL) activity (A) and total phenolic compounds (B) of banana fruit treated with different UV-C dosages and then stored at 5 and 25°C. Values are means ± SE (*n* = 3); asterisks (*) above the lines indicate significant differences between the means at each day of storage; LSD test, *P* ≤ 0.05

As ROS are highly reactive to membrane lipids, proteins, and DNA, they are believed to be major contributing factors to stress injuries and to cause rapid cellular damage (Hariyadi and Parkin, 1991). When plants are exposed to low temperature, in particular, electron transport chains tend to form $O_2^{\bullet-}$, which dismutates to form H_2O_2 (Mittler, 2002). In this study, fruits stored at 5°C showed lower $O_2^{\bullet-}$ formation than those stored at 25°C, but $O_2^{\bullet-}$ generation trends were similar (Figure 3A). The maximum increase in $O_2^{\bullet-}$ with storage 5°C was observed on storage day 7, and then $O_2^{\bullet-}$ gradually decreased until the end of storage. UV-C treatment significantly reduced $O_2^{\bullet-}$ production compared with nontreated fruit (Figure 3A).
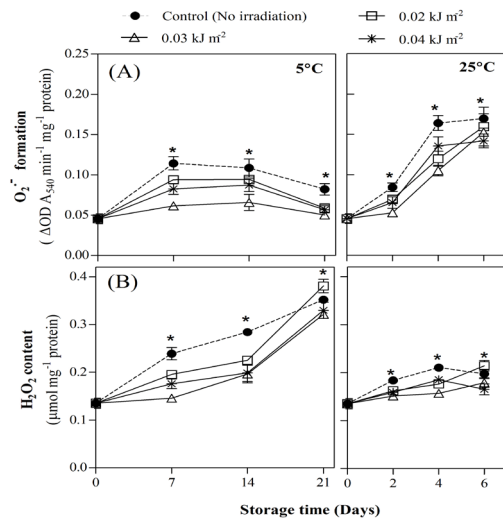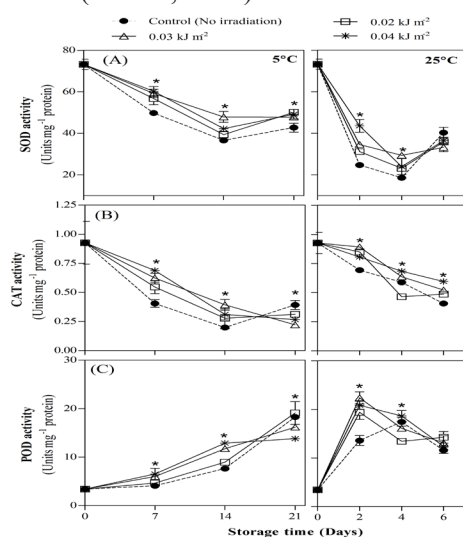

Figure 3. Changes in superoxide anion ($O_2^{\bullet-}$) formation (A) and hydrogen peroxide (H_2O_2) content (B) of banana fruit treated with different UV-C dosages and then stored at 5 and 25°C. Values are means ± SE (*n* = 3); asterisks (*) above the lines indicate significant differences between the means at each day of storage; LSD test, *P* ≤ 0.05

H_2O_2 is a stable molecular ROS species that is considered to be an environmental stress response signal; a transient increase in H_2O_2 may signal activation of protective mechanisms for acclimation to chilling (Foyer and Noctor, 2003). Our results showed that UV-C treatment reduced endogenous H_2O_2 production (Figure 3B) as well as alleviated CI symptoms during cold storage (Figure 1). Significantly lower levels of H_2O_2 were observed in fruits treated with UV-C after 7 and 14 days of storage. Interestingly, H_2O_2 levels increased in fruits stored at low temperatures, whereas in those stored at 25°C, the level was approximately constant. These results are consistent with H_2O_2 acting as a cold stress signal, as well as being a ROS intermediate involved in the CI mechanism, in banana fruit. We hypothesized that H_2O_2 production and H_2O_2 defense mechanisms might become equilibrated by UV-C treatment. This balance between the formation and detoxification of activated oxygen species is critical to cell survival during cold storage (Zhang *et al.*, 1995).

The level of ROS in pant tissue is controlled by an array of interrelated antioxidant enzymes such as SOD, CAT, and APX. $O_2^{\bullet-}$ is efficiently converted to H_2O_2 by the action of SOD, and H_2O_2 is destroyed mainly by APX and CAT (Mittler, 2002). Thus, the activities of these antioxidant enzymes in cells must be known to determine the steady-state levels of superoxide radicals and hydrogen peroxide. SOD, which belongs to a class of metal-containing proteins, catalyzes the dismutation reaction of superoxide anions to H_2O_2 and molecular oxygen. The results showed that, SOD activity in both control and UV-C-treated fruit decreased with storage time, with a slight increase at the end of storage, but fruit treated

with UV-C maintained remarkably higher SOD activity throughout storage (Figure 4A). CAT (Figure 4B) and GR (Figure 5A) activities in banana fruit exhibited a similar pattern of changes to SOD during the cold storage. In control fruit, the activities of both enzymes decreased continuously during storage, and markedly higher activities were observed in fruits stored at 25°C. UV-C treatment significantly delayed the decreases in CAT and GR activities, which were significantly higher in UV-C-treated fruit than in control fruit during the storage period (Figure 4B, Figure 5A). CAT protects cells against ROS by catalyzing the decomposition of H_2O_2 to form oxygen and water. In the presence of NADPH, GR contributes to the regeneration of ascorbate, which is required for APX activity (Saruyama and Tanida, 1995). Differences in CAT and GR activity at different storage temperatures reflect specific physiological processes related to the functioning of the antioxidant system in banana fruit. In contrast to SOD, CAT, and GR activities, POD and APX activities markedly increased in control fruit during cold storage, whereas they obviously decreased at 25°C (Figure 4C, Figure 5B). UV-C treatment also significantly enhanced the activities of POD and APX compared with those in control fruit. In addition, the patterns of change in APX activity were similar to those in H_2O_2 content (Figure 3B), which is removed by APX in the ascorbate–glutathione antioxidant cycle (Foyer *et al.*, 1997). APX is a major H_2O_2-detoxifying enzyme in plants. APX and CAT have different affinities for H_2O_2, suggesting that APX might be responsible for fine modulation of ROS for signaling, whereas CAT might be responsible for the removal of excess ROS under stress (Mittler, 2002).

Figure 4. Changes in superoxide dismutase (SOD) (A), catalase (CAT) (B) and peroxidase (POD) (C) activity of banana fruit treated with different UV-C dosages and then stored at 5 and 25°C. Values are means ± SE (*n* = 3); asterisks (*) above the lines indicates significant differences between the means at each day of storage; LSD test, *P* ≤ 0.05
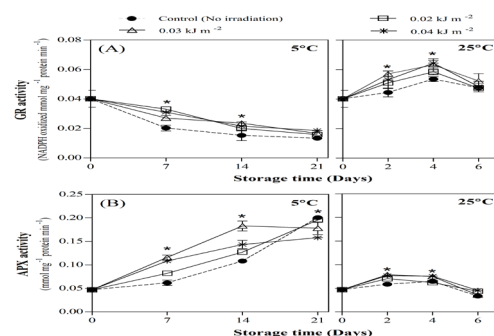
Figure 5. Changes in glutathione reductase (GR) (A) and ascorbate peroxidase (APX) (B) activities of banana fruit treated with different UV-C dosages and then stored at 5 and 25°C. Values are means ± SE (*n* = 3); asterisks (*) above the lines indicate significant differences between the means at each day of storage; LSD test, *P* ≤ 0.05

UV-C treatment reduced cellular oxidative stress damage

The activation and maintenance of the activities of the studied antioxidants by UV-C treatment protected the fruit against potentially dangerous superoxide radicals and H_2O_2. Moreover, UV-C treatment might efficiently eliminate these ROS, and consequently, protect cellular components. Low temperatures, as well as high light intensities, induce ROS production, which can then react with DNA, proteins, and lipids, cause breakdown of DNA strands, and initiate peroxidation of various compounds (Wise and Naylor, 1987).

MDA is a secondary end product of polyunsaturated fatty acid oxidation and thus is an indicator of membrane lipid peroxidation caused by ROS. The MDA content is therefore an indicator of the degree of plant oxidative stress (Hodges *et al.*, 1999). In this study, the MDA content of banana fruit increased continuously with storage time (Figure 6), and it was higher in fruit stored at 5°C than in that stored at 25°C. Changes in MDA content were also in accordance with the severity of chilling injury symptoms (Figure 1). The MDA content in UV-C-treated fruit was considerably significantly lower than that in control fruit both at 5 and 25°C. These data suggest that membrane lipid peroxidation in the peel of bananas stored at CI temperatures was effectively inhibited by UV-C treatment. Lipid peroxidation causes endogenous lesions by inducing formation of exocyclic DNA adducts. The lipid peroxidation product, MDA, reacts with the G residue in DNA to form pyridimidopurinone, an exocyclic adduct that can block the Watson-Crick DNA base pairing and thus can potentially damage DNA structure (Marnett, 1999). For example, mitochondria are a primary generator of endogenous ROS, and mitochondrial components, including mitochondrial DNA, are highly susceptible to oxidative damage (Qin *et al.*, 2008).

In this study, we also evaluated the potential of

UV-C treatment to protect the DNA of banana peel against CI. At 25°C, DNA damage increased as the fruit ripened. DNA damage was observed by 2 days of storage, and the extent of damage increased progressively with further ripening (Figure 7). In contrast, during storage at 5°C, severe DNA damage was observed that was not correlated with ripening. In bananas treated with UV-C (0.03 kJ m^{-2}), however, DNA damage was slight.
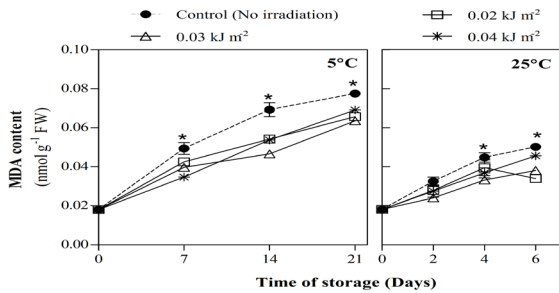
Figure 6. Changes in malondialdehyde (MDA) content of banana fruit peel treated with different UV-C dosages and then stored at 5 and 25°C. Values are means ± SE (*n* = 3); asterisks (*) above the lines indicate significant differences between the means at each day of storage; LSD, $P \leq 0.05$
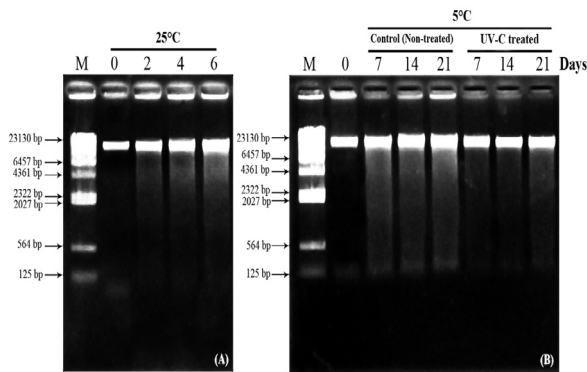

Figure 7. Agarose gel electrophoresis analysis showing DNA degradation: in banana fruit peel ripened at 25°C for 0, 2, 4, or 6 days (A); and in peel treated with UV-C at dosages of 0.03 kJ m-2 and stored at 5°C for 0, 7, 14, or 21 days (B). (Lane M, lambda DNA/*Hind*III marker)

Induction of chilling tolerance and HSP70 expression by UV-C treatment

The effects and mode of action of UV-C treatment in reducing cellular oxidative stress damage were supported by measurement of the expression of heat shock protein (HSP) genes. HSPs are a group of conserved proteins that are produced in prokaryotes and eukaryotes in response to many environmental factors, including oxidative stress as well as heat and cold (Boston *et al*., 1996). The HSP70 family proteins prevent the aggregation of non-native proteins and assist in refolding of proteins; furthermore, the expression of HSP70 genes is strongly upregulated under stress conditions (Ding *et al*., 2001; Sun *et al*., 2010). In this study, at the normal temperature, the level of HSP70 gene expression increased slightly by

2 days of storage at 25°C, during which ripening was observed (Figure 8A), and the level of expression decreased after full ripening. At 5°C, the expression of HSP70 increased continuously with storage time (Figure 8B), similar to the severity of CI symptoms, and HSP70 gene expression was higher in UV-C treated fruit than in control fruit (Figure 8B). In general, HSPs help maintain cellular homeostasis by functioning as molecular chaperons, which assist cells in maintaining polypeptides and proteins. Moreover, increases in HSPs are correlated with increased chilling tolerance (Ding *et al*., 2001; Sun *et al*., 2010). Thus, UV-C treatment may reduce cellular oxidative stress from CI by activation of HSP synthesis. Wang *et al*. (2004) suggested that there might be cross-talk between HSPs/chaperones and other stress response mechanisms in plants such as stress signals and antioxidative mechanisms, and that HSP synthesis is coordinated with those to prevent cellular damage and maintain cellular homeostasis. Consistent with that idea, in the present study, the enhancement and maintenance of higher levels of antioxidant capacity, particularly APX, as a consequence of CI might be related to HSP accumulation. Panchuk *et al*. (2002) also reported that in *Arabidopsis*, HSP transcription factors depend on the activity of antioxidants such as APX. However, information is lacking about the relationship between HSP levels and DNA damage and repair mechanisms; thus, further research is needed.
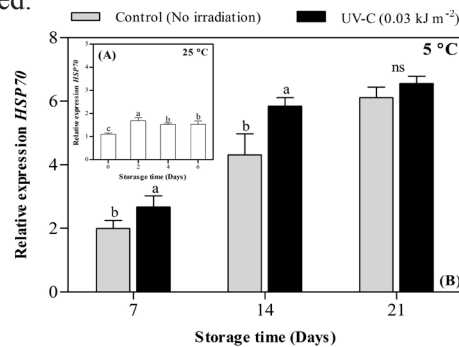
Figure 8. Changes in HSP70 mRNA expression in banana peel stored at 25°C for 6 days (A) or 5°C for 21 days (B). At 5°C, banana fruit was treated with UV-C (0.03 kJ m-2). Controls received no irradiation. Values are means + SE (*n* = 3). Different letters above the bars indicate significant differences in the means; LSD test, $P \leq 0.05$

In conclusion, the results of the present study support a possible role and mode of action of UV-C hormesis in inducing plant defense mechanisms against stress-induced damage. Taken together, our results provide evidence that the development of CI symptoms in banana fruit are associated with ROS accumulation those were inhibited by UV-C treatment. The activation of defense mechanisms such phenolic compounds and antioxidant enzymes (SOD, CAT, POD, APX, and GR) and increased HSP70 gene expression by UV-C treatment are likely to be at least

partly responsible for reductions in cellular oxidative stress, thus preventing membrane degradation and DNA damage. However, the mechanisms of cellular oxidative stress are complex, and other biochemical and physiological mechanisms may act in concert under chilling stress.

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