Scientia Horticulturae 225 (2017) 106–111

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

UV-C enhances resistance against gray mold decay caused by *Botrytis cinerea* in strawberry fruit

Peng Jin*, Huanyu Wang, Yu Zhang, Yuping Huang, Li Wang, Yonghua Zheng*

College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

ARTICLE INFO

Keywords:
Strawberry
UV-C
Botrytis cinerea
Decay
Induced resistance

ABSTRACT

The effect of ultraviolet C (UV-C) treatment on controlling gray mold caused by *Botrytis cinerea* in strawberry fruit and the possible mechanisms were investigated. The results showed that UV-C treatment significantly reduced lesion diameter and enhanced activities of chitinase (CHI), β-1, 3-glucanase, phenylalanine ammonia-lyase (PAL), peroxidase (POD) and polyphenoloxidase (PPO) in strawberry fruit. Total phenolic content were also increased by UV-C treatment. The activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were also higher in UV-C treated strawberry fruit than those in control. Expression of three defense related genes such as *CCR-1 allele, CAT, CHI2, PPO* and *PLA6* was greatly induced in UV-C treated strawberry fruit. These results suggested that UV-C treatment directly activated disease resistance against gray mold caused by *B. cinerea* in strawberry fruit.

1. Introduction

Strawberry fruit is highly perishable and susceptible to fungal and bacterial pathogen attack during ambient temperature storage. Gray mold decay caused by *Botrytis cinerea* is one of the main postharvest disease that limits the storage life of strawberry fruit (Civello et al., 1997). Traditionally, fungicide has been widely used to prevent postharvest decay of strawberry fruit. However, consumers concern over potentially harmful effects on the chamical residues, human health and environment pollution caused by synthetic fungicides. Thus, it is necessary to look for new strategies to control postharvest decay in strawberry fruit (Pombo et al., 2011).

Ultraviolet C (UV-C) treatment is a new and environment-friendly strategy to reduce naturally occurring infections during postharvest storage (Vicente et al., 2005; Pombo et al., 2011). UV-C treatment had been showing not only inhibiting microbial growth directly, but also inducing disease resistance of fruit and vegetables against pathogen attack. For example, Stevens et al. (1999) reported that UV-C could inhibit the dry rot caused by *Fusarium* in sweet potato during storage. Stevens et al. (1998) found that UV-C irradiation could enhance disease resistance in tomato throughout inducing a high level of lycopene content. UV-C treatment could inhibit the expression of genes related to cell wall degradation, photosynthesis and lipid metabolism, meanwhile, UV-C treatment promoted the expression of genes associated with signal transduction and defense response to improve disease resistance in tomato (Liu et al., 2011).

Induced disease resistance is a state of enhanced defensive capacity against pathogen attack after treatment with some physical, chemical of biological elicitors (Terry and Joyce, 2004). It has been considered that the cause of induced disease resistance of host against pathogens was usually along with stimulating respiration, increasing secondary metabolism and inducing expression of defense genes (Bostock, 2005). Recently, Wang et al. (2014) showed that methyl jasmonate treatment induced disease resistance against *Penicillium citrinumin* in Chinese bayberries. Heat treatment also inhibited blue mold in sweet cherry and gray mold in strawberry fruit throughout directly inducing disease resistance (Wang et al., 2015). However, there is not enough information about inducing disease resistance in strawberry fruit by UV-C treatment. The objective of this study was to evaluate the effect of UV-C treatment on disease resistance of strawberry fruit against *B. cinerea* and to evaluate the further mechanism study.

2. Materials and methods

2.1. Fruit material and treatment

Strawberry (*Fragaria ananassa* Duch. cv. Benihoppe) fruits were hand-harvested at commercial maturity stage (85–90% red color of fruit surface) from an orchard in Nanjing, Jiangsu Province, China. The strawberries were transported to the laboratory within 2 h and selected for uniform size, color and without damage. Sixty strawberry fruit were placed in trays under a bank of four germicidal UV lamps (ZW 30S19,

* Corresponding authors.
E-mail addresses: pjin@njau.edu.cn (P. Jin), zhengyh@njau.edu.cn (Y. Zheng).

http://dx.doi.org/10.1016/j.scienta.2017.06.062
Received 14 March 2017; Received in revised form 25 June 2017; Accepted 26 June 2017
Available online 11 July 2017

30W, Zhonghai Co., Ltd., Nanjing, China) and were irradiated at a distance of 30 cm to obtain doses of 2.0 KJ m^{-2}. The radiation intensity was measured by a UV-C digital radiometer (ZDZ-1, Xuelian Instrument Factory, Shanghai, China). Based on our previous study, 2.0 KJ m^{-2} of UV-C was the option dose for inhibiting decay and maintaining quality (Wang et al., 2015a,b). Thus, 2.0 KJ m^{-2} of UV-C was chosen in the present study to evaluate the further mechanism study. Another strawberry fruits without UV-C treatment were used for control. After UV-C treatment, all berry fruits were sterilized with 75% ethanol prior to wounding and inoculation.

2.2. Pathogen

The pathogen *B. cinerea* was isolated from the surface of infected strawberry fruit and cultured on potato dextrose agar (PDA) medium (containing the extract of 200 g boiled potatoes, 20 g dextrose and 20 g agar in 1000 mL of distilled water). Spore suspensions were prepared by removing the spores from the surface of 2-week-old PDA medium and suspending with 5 mL of sterile distilled water containing 0.05% (v/v) Tween 80. The number of spores was calculated with a hemocytometer counting chamber, and then the spore concentration was adjusted to 1×10^6 spores mL^{-1} with sterile distilled water.

2.3. Pathogen inoculation and fruit tissue sampling

Strawberry fruits were wounded (3 mm in depth and 3 mm in width) with a sterile nail at the equator of each fruit and then inoculated with 20 μL of a suspension of 1×10^6 spores mL^{-1} *B. cinerea* into each wound. Strawberries were transferred to incubator (MIR-253, Sanyo Inc, Japan) with refrigeration and humidifying systems set at 5 °C, 90–95% relative humidity for 12 days. Strawberry samples were taken every 3 day for lesion diameter, enzyme activity assay and total phenolic and H_2O_2 content analysis. There were three replicates of 150 fruit each for one replication per treatment.

2.4. Assay of enzyme activity

Chitinase (CHI, EC 3.2.1.14) and β-1, 3-glucanase (EC 3.2.1.58) activities were assayed according to the method of Abeles et al. (1971). One unit of CHI activity was defined as the amount of enzyme required to catalyze the production of 1 μg NAG per hour at 37 °C. One unit of β-1, 3-glucanase activity was defined as the amount of enzyme catalyzing the formation of 1 μmol glucose equivalents per hour.

Phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity was measured according to the method of Assis et al. (2001). Tissue sample was ground with 5 mL 0.2 M sodium borate buffer containing β-mercaptoethanol. One unit of PAL activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01at 290 nm per hour.

POD (EC 1.11.1.7) activity was measured according to the method of Kochba et al. (1977). The reaction mixture contained 50 mM sodium phosphate buffer (pH 8.7), 0.75% H_2O_2, 20 mM guaiacol and 0.2 mL crude enzyme extract. One unit of POD activity was defined as an increase of 0.01 in absorbance per minute at 460 nm.

PPO (EC 1.10.3.1) activity was measured according to the method of Murr and Morris (1974). One unit of PPO activity was defined as the amount of enzyme that caused the increase in absorbance of 0.01 at 410 nm in 1 min under the specified conditions.

SOD (EC 1.15.1.1) activity was measured according to the method of Rao et al. (1996). One unit of SOD activity was defined as the amount of enzyme that causes 50% inhibition of nitroblue tetrazolium.

CAT (EC 1.11.1.6) and APX (EC 1.11.1.11) activities were determined by the method of Jin et al. (2014). One unit of CAT activity was designated as the amount of enzyme that decomposes 1 μmol of H_2O_2 per minute at 30 °C. One unit of APX was defined as the amount of enzyme that oxidizes 1 μmol ascorbate per min.

Protein content was determined by the Bradford (1976) method, using bovine serum albumin as a standard. Specific activity of all the enzymes was expressed as units per milligram of protein.

2.5. Total phenolic and H_2O_2 content

Total phenolics content in strawberry fruit were measured with the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton (1977) using gallic acid as a standard. The results were expressed as milligram of gallic acid equivalent per gram of fresh weight.

For H_2O_2 assay, two gram of fresh tissue was homogenized with 5 mL acetone and then centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was collected immediately for H_2O_2 analysis according to the method of Patterson et al. (1984). H_2O_2 content was expressed as nanomoles per gram of fresh weight.

2.6. Analysis of defense-related gene expression by RT-PCR

In order to investigate the molecular mechanism of UV-C treatment induced disease resistance against *B. cinerea* in strawberry fruit, RT-PCR (Reverse transcription-polymerase chain reaction) was used to analyze the expression patterns of the defense-related genes (*CCR-1 allele, CAT, CHI2, PPO, PLA6*) in strawberry fruit.

Tissue samples from the UV-C or control fruit, with inoculation of *B. cinerea* were collected at the same intervals (0, 3, 6, 12 and 24 h after inoculation). Total RNA was extracted from tissue samples of strawberry fruit according to the method of Jin et al. (2016). The sequences of primers used for RT-PCR analysis were shown in Table 1. RT-PCR was performed using the PrimeScriptTM 16 1 st Strand cDNA Synthesis Kit (TaKaRa, Japan). Short and conserved segments of *CCR-1 allele* (GenBank ID: AY285922.1), *CAT* (GenBank ID: KC433883.1), *CHI2* (GenBank ID: FJ424410.1), *PPO* (GenBank ID: EU523113.1) and *PAL6* (GenBank ID: HM641823.1) were cloned by degenerate primers. Independent PCR with 30 cycles was performed using aliquots (1 μL) of cDNA samples, and a constitutively expressed gene 18S-rRNA (GenBank ID: X15590.1) was used as a quantitative control in the RT-PCR analysis. The RT-PCR program was initiated with a preliminary step of 5 min at 95 °C, followed by 30 cycles of 94 °C for 45 s, 55 °C for 30 s and 72 °C for 50 s. Relative mRNA levels of genes were analyzed based on densitometry values obtained using the Quality One software of Bio-Rad.

2.7. Statistical analysis

Experiments were performed using a completely randomized design. All statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The data were analyzed by one-way analysis of variance (ANOVA), and mean separations were performed using Duncan's multiple range tests. Differences at $P < 0.05$ were considered to be significant.

Table 1
Real-time PCR primer sequences for defense-related genes.

Gene	Forward primer sequence (5′ – 3′)	Reverse primer sequence (5′ – 3′)
CCR-1	GGTCCCGATGTCGTTGTC	ATGCTGGCGTTGATGGTT
CAT	GTCTTCTTCGTCC GTGAT	GTAGTTCCCAGCAGCAAT
CHI2	CCTTC ATCAGGGCAACCA	GGCTTCTGCTCCGTCATC
PPO	TCCTAAGTCGCCGCTCTA	GCACCATATTTG CGTTCTC
PAL6	GTGAAAGAAGCGAAGAAGG	GAAGCTCG GAGCAGTATG
18S-rRNA	AGCAAGCCTACGCTCTGG	GGTGC CCTTCCGTCAATT