Postharvest Biology and Technology 112 (2016) 241–246

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Effect of harpin on control of postharvest decay and resistant responses of tomato fruit

Zhu Zhu, Xueji Zhang*

Research Center for Bioengineering and Sensing Technology, University of Science & Technology Beijing, Beijing 100083, China

ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form 10 September 2015
Accepted 10 September 2015
Available online 19 September 2015

Keywords:
Defense-related gene
Fungal pathogens
Host response

ABSTRACT

Postharvest decay caused by fungal pathogens is responsible for significant economic losses in the tomato industry. Induction of disease resistance in harvested horticultural crops is an approach to controlling postharvest losses that is being actively investigated. In the current study, the effects of harpin (a bacterial hypersensitive response elicitor) on gray mold and black rot caused by *Botrytis cinerea* and *Alternaria alternata*, respectively, in tomato fruit were investigated. Harpin effectively controlled gray mold and black rot on inoculated tomato fruit, as well as natural infection. Harpin treatment at 90 mg L^{-1} induced the transcript expression of defense-related genes including *chitinase*, β-1,3-*glucanase* and *phenylalanine ammonia-lyase* based on RT-qPCR analysis, increased the activity of these enzymes, and the content of total phenolic compounds and lignin in tomato fruit. Moreover, harpin did not demonstrate any fungicide effect on *B. cinerea* or *A. alternata in vitro*. These findings suggest that the effect of harpin on postharvest diseases was attributed to the elicitation of resistant responses, and harpin treatment may represent a promising strategy for managing postharvest decay of tomato fruit.

1. Introduction

Tomato fruit suffers from postharvest decay resulting in significant economic losses. Gray mold and black rot, caused by *Botrytis cinerea* and *Alternaria alternata*, respectively, are among the most common postharvest diseases of tomato fruit (Cota et al., 2007; Fagundes et al., 2013; Zheng et al., 2014). Although utilization of synthetic chemical fungicides is still the main method to control postharvest diseases, increasing concerns about fungicide residues on development of resistant biotypes of pathogens, as well as public concern over food and environment safety, have created interest in new strategies for postharvest disease management (Fallik 2004; Droby et al., 2009; Janisiewicz and Conway, 2010).

Induction of disease resistance in postharvest horticultural crops is a promising strategy for decay management (Wilson et al., 1994; Terry and Joyce, 2004; Tian et al., 2007). Harpin is an acidic, heat-stable, glycinerich, 44-kDa protein, encoded by the hrpN gene of the bacterium *Erwinia amylovora* (Choi et al., 2013). Previous studies have shown that harpin triggers a variety of cellular responses, such as induction of defense-related gene expression (Qiao et al., 2010) and activation of reactive oxygen species (Sang et al., 2012), translocation of effector proteins into plant cytoplasm (Choi et al., 2013) and cell membrane depolarization (Dong et al., 1999). For instance, harpin could elicit hypersensitive response (HR) in tobacco, Arabidopsis and citrus leaves (Sgro et al., 2012; Li et al., 2013), and to induce disease resistance in harvested fruits and vegetables like apple (de Capdeville et al., 2003), melon (Bi et al., 2005) and pepper (Tezcan et al., 2013).

Harpin has been produced commercially as "Messenger" (Eden Bioscience, USA), which is currently being suggested as a plant growth enhancer for management of viral and fungal diseases, as well as control of selected insect populations (Wei et al., 1998; Fontanilla et al., 2005). The objective of the present study was to evaluate the effects of harpin on postharvest diseases and resistance response in tomato fruit. More specifically, this study investigated the effects of harpin on (i) infection from natural and artificial inoculum of *B. cinerea* and *A. alternata*; (ii) transcript expression of defense-related genes including *chitinase* (*CHI*), β-1,3-*glucanase* (*GNS*) and *phenylalanine ammonia-lyase* (*PAL*); (iii) enzyme activity of *CHI*, *GNS* and *PAL*, and total phenolic compounds in tomato fruit.

2. Materials and methods

2.1. Fruit

Tomato fruit (*Solanum lycopersicum* cv. Fenhong) were harvested at the mature green stage (average quality values: 5.6% of

* Corresponding author. Fax: +86 10 82376993.
E-mail address: zhangxueji@ustb.edu.cn (X. Zhang).

http://dx.doi.org/10.1016/j.postharvbio.2015.09.007
0925-5214/

total soluble solid content, 75 N of firmness and mass of 15 kg per 100 fruit). Fruit without wounds or rot were selected based on uniformity of size, disinfected with 2% (v/v) sodium hypochlorite for 3 min, rinsed with tap water, and air-dried (Liu et al., 2007).

2.2. Fungal pathogens

B. cinerea and *A. alternata* were isolated from infected tomato fruit and maintained on potato dextrose agar (PDA) at 4 °C. To verify their pathogenicity, the pathogens were inoculated into wounded tomato fruit and re-isolated onto PDA after infection was established. Spore suspensions of each of the pathogens were obtained from two-week-old PDA cultures at 25 °C. Spore concentration of the suspensions was determined using a hemocytometer and adjusted to 10^4 spores mL^{-1} with sterile distilled water prior to use.

2.3. Effect of harpin on in vitro growth of B. cinerea and A. alternata

The effect of harpin on mycelial growth was assayed according to Liu et al. (2007). Harpin (Messenger®, Eden Bioscience Co., Bothell, WA) was added to PDA after autoclaving for 15 min and cooling to 45–50 °C to reach the final concentrations of 0, 30, 60, 90 and 120 mg L^{-1}. The mycelial disks (5 mm in diameter) from two-week-old cultures of *B. cinerea* or *A. alternata* were placed in the center of Petri dishes (90 mm in diameter) with 20 mL of PDA containing harpin at the concentrations above, and then incubated at 25 °C. The mycelial growth was determined by measuring colony diameter at 3 d after inoculation. Each treatment contained three replicates and the experiment was repeated three times.

2.4. Effect of harpin on control of postharvest decay in tomato fruit

Tomato fruit were randomly grouped into four lots. Based on suggested commercial concentrations and previous studies (de Capdeville et al., 2003; Bi et al., 2005), three lots of fruit were immersed in harpin solutions at 30, 60, 90 and 120 mg L^{-1} for 10 min, respectively, while the fourth lot of fruit, immersed in water, served as a control. No phytotoxicity symptoms were observed on tomato fruit treated with the harpin solution above. Each treatment contained three replicates of 20 fruit and the experiment was repeated three times.

For assay of its effect on control of artificially inoculated gray mold and black rot in tomato fruit, two wounds (3 mm deep × 3 mm wide) were made with a sterile nail on the opposite sides at the equator of each fruit after 1 d of harpin immersion treatment. A 10 μL spore suspension of either *B. cinerea* or *A. alternata* (1×10^4 spores mL^{-1}) was then inoculated into each wound. Treated fruit were placed in a covered plastic food tray, and each tray was enclosed within a polyethylene bag and stored at 25 °C in a programmable environmental chamber for 4 d. Decay incidence and lesion diameter on each fruit was determined. Disease incidence represents the percentage of infected wounds, while lesion diameter was measured only on those wounds that were infected (Zhao et al., 2014). For assay of its effect on control of natural decay in tomato fruit, all harpin-treated fruit were stored at 25 °C for 15 d after which disease incidence was recorded (Zong et al., 2010).

2.5. RNA isolation and reverse transcription-quantitative real-time PCR (RT-qPCR) analysis of gene expression

Tomato fruit were treated with harpin at 90 mg L^{-1} for 10 min as described above, and the fruit without harpin treatment served as the control. Fruit samples were obtained from 20 fruit stored at 25 °C containing the pericarp and flesh each day for 5 d. Each treatment consisted of three replicates and the experiment was repeated three times. Total RNA from tomato samples at each time point was isolated using Concert™ Plant RNA Reagent (Invitrogen, USA) according to the manufacturer's instructions (Zhang et al., 2013). Extracted RNA was treated with DNase (Ambion, USA) and purified with RNeasy Mini Kit (Qiagen, Germany). RT-qPCR analysis was performed using 40 ng of total RNA, SuperScript III Platinum SYBR Green One-Step RT-qPCR Kit with ROX (Invitrogen), and 20 pmol of each primer per reaction. The ABI StepOne Plus (Applied Biosystems, USA) was set to cycle as follows: cDNA synthesis at 48 °C for 30 min; 95 °C denaturation for 5 min; 40 cycles of 95 °C for 15 s followed by 60 °C for 1 min; 40 °C for 1 min; dissociation step. The standard curve method was used to calculate transcript abundance relative to β-*actin* as a reference gene. The primers of the target genes of *CHI*, *GNS* and *PAL* and the five reference genes (β-*actin*, *GAPDH*, *EF1α*, *PP2Ac* and *RPL2*) in Table 1 were designed according to the previous studies (Wang et al., 2009; Van de Poel et al., 2012; Zhang et al., 2013). The expression level of each target gene was normalized against the average expression of the five references genes (Van de Poel et al., 2012; Landi et al., 2014). There were three replicates in each treatment, and the experiment was repeated three times.

2.6. Assay of enzyme activity

Tomato fruit were treated with harpin at 90 mg L^{-1} for 10 min as described above, and the fruit without harpin treatment served as the control. For the enzyme assay, fruit samples were obtained from 20 fruit stored at 25 °C containing the pericarp and flesh each day for 5 d. Each treatment consisted of three replicates and the experiment was repeated three times.

CHI and *GNS* enzymes were extracted according to Cao and Jiang (2006). Fruit tissue samples (10 g) were homogenized in 20 mL of ice-cold sodium acetate buffer (100 mM, pH 5.0) containing 5 mM β-mercaptoethanol and 1 mM ethylenediaminetetraacetic acid (EDTA) with a tissue grinder. The homogenate was centrifuged at 13,000 × *g* for 20 min at 4 °C, and the resulting supernatant was collected for the enzyme assay. *CHI* activity was measured with chitin azure (Sigma–Aldrich, USA) as the substrate, according to Liu et al. (2012). *GNS* activity was assayed with laminarin as the substrate, following the method described by Ippolito et al. (2000). Reaction production was measured spectrophotometrically at 550 nm for *CHI* or 500 nm for *GNS*. The specific activity of *CHI* was expressed as U kg^{-1}, where one unit was defined as the amount of the enzyme producing one

Table 1
Primers used in RT-qPCR analysis for defense-related gene expression.

Gene name	NCBI accession No.	Primer sequence
CHI	Z15140	F: CCCATGAAACTACTGGAGGATG
		R: TGGTGTACAGTAATCGCCAGG
GNS	M80608	F: AGGAACGATGTTAGATGGTTTACT
		R: ATCCATCGCAGCATAAACAG
PAL	M90692	F: CTTTGATGCAGAAGCTGAGACA
		R: TCGTCCTCGAAAGCTACAATCT
β-*actin*	U60480	F: ACATTGTGCTCAGTGGTGGTACT
		R: CCACCTTAATCTTCATGCTGCT
GAPDH	BT012693	F: GCTAAAGGTCAAGGATGAGAAGA
		R: CAACAACGAAGTCAGCACCA
EF1α	X14449	F: TTGGTCATGTTGACTCTGGAAA
		R: CCTTCTCGAACCTCTCAATAACAC
PP2Ac	AY325817	F: GATACACTTGATAACATCCGAGCA
		R: GAGGTGATATTCCCCAACCAC
RPL2	X64562	F: CCGATTCCGTACTCTCGATTT
		R: GCTCCTTCTGATGCTTGTACCT