Scientia Horticulturae 222 (2017) 187–192

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Changes in quality and defense resistance of kiwifruit in response to nitric oxide treatment during storage at room temperature

Xiaolin Zheng*, Bin Hu, Lijun Song, Jie Pan, Mengmeng Liu

College of Food Science and Biotechnology, Key Laboratory of Fruits and Vegetables Postharvest and Processing Technology Research of Zhejiang Province, Zhejiang Gongshang University, Hangzhou 310018, China

ARTICLE INFO

Keywords:
Disease resistance
Kiwifruit
Nitric oxide
Quality
Storage

ABSTRACT

The effects of nitric oxide (NO) treatment on quality and disease resistance of kiwifruit (*Actinidia deliciosa*) *cv.* 'Bruno' were investigated after the fruits were immersed in 0.2 mM sodium nitroprusside or water (as control) for 10 min and then stored at room temperature. NO treatment reduced diseases incidence; delayed the increase in soluble solid content and the loss of vitamin C (Vc); increased the activities phenylalanine ammonialyase (PAL), peroxidase (POD), and β-1,3-glucanase, with accompanying up-regulation of *PAL*, *POD* and *chitinase* (*CHT*) expression; and elevated the level of total phenolics, flavonoids, hydroxyproline-rich glycoprotein, and lignin during storage. Thus, NO treatment was beneficial to the maintenance of fruit quality and improvement of disease resistance of kiwifruit during storage.

1. Introduction

Nitric oxide (NO) is a gaseous-free radical and plays a number of diverse signal functions including regulating maturation and trigging defense responses to abiotic and biotic stresses in higher plants (Belligni and Lamattina, 2001). Many research works have reported that pre-storage application of NO delays the ripening process, improves chilling resistance, and inhibits browning in different fruits (Barman et al., 2014; Duan et al., 2007; Manjunatha et al., 2010; Singh et al., 2013; Wang et al., 2015; Zaharah and Singh, 2011; Zhu et al., 2009). Recently, influences of NO application in disease resistance of various fruits have been received much attention (Gu et al., 2014; Hu et al., 2014; Lai et al., 2011). For instance, NO treatment effectively increases the resistance against gray mold decay caused by *Botrytis cinerea* in tomato fruit (Lai et al., 2011) and against post-harvest anthracnose caused by *Colletotrichum gloeosporioides* in mangoes (Hu et al., 2014) and citrus fruit (Zhou et al., 2015).

Kiwifruit of *Actinidia chinensis* or *Actinidia deliciosa* is highly susceptible to infection of pathogenic fungi during post-harvest. *B. cinerea*, *Botryosphaeria dothidea*, and *Diaporthe actinidiae* are the major fungal pathogens causing stem-end rot, gray mold decay and soft rot decay in kiwifruit (Elmer and Michailides, 2007; Lee et al., 2001), and *Penicillium expansum* also causes blue mold decay in kiwifruit during storage (Hur et al., 2005), which result in the decreases in fruit quality, shelf life and consumer acceptability. Chemical treatments such as pre-harvest spraying of oxalic acid (Zhu et al., 2016), and pre-storage application of salicylic acid (Zhang et al., 2003), 1-methylcyclopropene (1-MCP) (Koukounaras and Sfakiotakis, 2007), ozone (Minas et al., 2010) or oxalic acid (Zhu et al., 2015) increase/induce disease resistance in different cultivars of kiwifruit. For example, pre-storage application of 1-MCP improves post-harvest quality of 'Hayward' kiwifruit due to delaying the ripening process and decay development caused by *B. cinerea* (Koukounaras and Sfakiotakis, 2007). Interestingly, a previous work has reported that NO treatment with 1 μM aqueous solution alleviates oxidative damage to kiwifruit associated with increases in the activity of antioxidant enzymes and the content of vitamins C (Vc) and E during storage (Zhu et al., 2008). However, little information is available about effects of NO treatment on quality and disease resistance of kiwifruit, which was the primary objective in our study.

2. Materials and method

2.1. Material and treatment

Kiwifruit (*A. deliciosa*) *cv.* 'Bruno' fruits were harvested at a commercial orchard in Wenzhou city, China. In a preliminary test, the effect of different sodium nitroprusside (SNP, a donor of NO) (Sigma) concentrations (from 0.0 to 0.5 mM) on decay of kiwifruit was evaluated, and a concentration of 0.2 mM showed desired result, so that this concentration was applied in the present experiment. Each of 180 uniform fruits without injuries and decay was immersed in water (as a

* Corresponding author. Tel.: +86 57128008958; fax: +86 57188053832.
E-mail address: zheng9393@163.com (X. Zheng).

http://dx.doi.org/10.1016/j.scienta.2017.05.010
Received 16 January 2017; Received in revised form 26 April 2017; Accepted 2 May 2017

Table 1
Sequences of specific primers used for qRT–PCR analysis.

Gene	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)	Product (bp)
AdPAL	GCCATGGCTGCTTATTGCTCTGA	GGTTGTGTTGCTCGGCACTTTG	82
AdPOD	GAGTGCCCTGGAGTTGTTTCTTGC	CTGCTCTTCCTACCATCCCTTCTTC	122
AdCHT	CAGTGGAAGCAGCAGCCGAGTT	GGTGCTCTGGAACCAATCCTTTAC	165
Actin	GCCCTATCAACTTTCGATGGTAGGA	CCTTGGATGTGGTAGCCGTTTCTCA	113

control) or 0.2 mM SNP aqueous solution for 10 min at 25 °C, respectively, and dried in air for about 2 h, and then each 20 fruits of the control or NO treatment was placed in a cleanly plastic box wrapped by an unsealed 0.07 mm polyethylene bag and stored in chambers (MIR-554, Sanyo, Oizumi, Japan) at 20 °C. Sixty fruits (three boxes) of the control or NO treatment was for observation of natural disease incidence during storage for 13 days. Moreover, flesh samples were collected from the middle part of 12 fruits without decay (from the other six boxes) of the control or NO treatment on the first day after the treatment and at 3 d intervals thereafter, and were rapidly frozen in liquid nitrogen and stored at −80 °C until use. Analysis in triplicate of flesh samples of the control or NO treatment was undertaken for measurements of quality parameters including soluble solid content (SSC), titratable acid (TA), and Vc, defense-related enzyme's activities, and resistance-related substance's contents.

2.2. Determinations of disease incidence, SSC, TA and Vc content

Disease incidence for fruit was observed in triplicate of 20 fruits each at 3-day intervals during storage. The individual fruit that showed visible disease symptoms on the surface was considered as decayed fruit, and the disease incidence was defined as the total percentage of decayed fruits. SSC in juice was measured with a refractometer (WYA-2S, Shanghai, China). An aliquot of 10 mL juice was titrated with 0.1 mM NaOH to pH 8.2 and TA was expressed as percentage malic acid equivalents. Two gram of sample was homogenized in 10 mL of 20 g L^{-1} oxalic acid, and Vc content was measured according to the method described by Bessey and king (1933), and was expressed as mg 100 g^{-1}(FW).

2.3. Determination of the activities of defense-related enzymes

Three grams of the frozen sample was ground with 5.0 mL sodium borate buffer (100 mM, pH 8.8) containing 5 mM β-mercaptoethanol (DTT), 4% (w/v) polyvinylpyrrolidone (PVP) and 2 mM EDTA and and then thoroughly centrifuged (10 000 × *g*, 4 °C, 30 min). The supernatants were used for assaying phenylalanine ammonialyase (PAL). PAL activity was assayed according to the method of Liu et al. (2005). One unit of PAL activity was defined as the amount of enzyme that resulted in the increase of 0.01 in the absorbance per hour at 290 nm.

Two grams of the frozen sample was homogenized with 5.0 mL sodium acetate buffer (100 mM, pH 5.5) containing 1 mM polyethylene glycol (PEG), 4% (w/v) PVP and 1% Triton X-100 and then centrifuged (10 000 × *g*, 4 °C, 30 min). The supernatant was used to assay peroxidase (POD). POD activity was determined following the method of Srivastava and Dwivedi (2000) with minor modifications. One unit of POD activity was defined as the amount of enzyme causing an increase of 0.01 in the absorbance per min at 470 nm.

Two grams of the frozen sample was homogenized with 5.0 mL sodium acetate buffer (100 mM, pH 5.2) containing 1 mM EDTA and 5 mM DTT and then centrifuged (10 000 × *g*, 4 °C, 30 min). The supernatant was used to assay chitinase (CHT). CHT activity was assayed as described by Boller (1983). One unit of CHT activity was expressed as the production of 1 μmol N-acetyl glucosamine per second per gram fresh weight.

Two grams of the frozen sample was homogenized with 5.0 mL sodium acetate buffer (100 mM, pH 5.2) containing 1 mM EDTA, 5 mM DTT and 1 g L^{-1} ascorbic acid and then centrifuged (10 000 × *g*, 4 °C, 30 min). The supernatant was used to assay β-1, 3-glucanase (GLU). GLU activity was measured as described by Mauch et al. (1984), and one unit of GLU was defined as the production of 1 μmol glucose equivalents per second per gram fresh weight.

2.4. Determination of the disease resistance-related substances

Two grams of flesh samples was ground in 20 mL 1% (v/v) HCl methyl alcohol at 4 °C and centrifuged (12,000 × *g*, 4 °C, 10 min) when extraction for 1 h without light, and the levels of total phenolics or flavonoids in the supernatant were determined at 280 or 325 nm, following the method described by Pirie and Mullins (1976).

The contents of hydroxyproline-rich glycoprotein (HRGP) and lignin were determined following the method of Hu et al. (1999) and were defined as A_{560} g^{-1}FW and A_{280} g^{-1} FW, respectively.

2.5. Q-PCR analysis for expression of genes encoding defense-related enzymes

Two grams of flesh samples were homogenized in liquid N_2, and total RNA was extracted from the frozen flesh according to the method reported by Zhu et al. (2013). Using a Takara PrimeScript® RT reagent kit with g DNA eraser, the first strand cDNA was synthesized by reverse transcription according to manufacturer's instruction. Transcript levels of APL, POD, and CHI were monitored via qPCR using the SYBR Green Master mix kit (Toyobo, Osaka, Japan) on a Chromo4 real time PCR Detection System (Bio-Rad, Hercules, USA). Real-Time PCR primers and conditions were shown in Table 1.

The PCR program was carried out as follows: 1 min at 95 °C; 40 cycles of 15 s at 95 °C, and 25 s at 63 °C and followed by an automatic melting curve analysis. Three independent biological replicates were determined for each sample. The relative expression level for each gene was calculated using the comparative Ct method (2 − ΔCt method) with a kiwifruit *actin* as a reference.

2.6. Statistical analysis

Data were expressed as mean values ± SD and were subjected to one-way analysis of variance using SPSS 13.0 software (SPSS Inc., USA) for comparing between treatments. All differences between means of the control and treatment were conducted using LSD tests at the 5% level, and the differences at $P \leq 0.05$ were considered as significant.

3. Results and discussion

3.1. Effects of NO treatment on disease incidence and fruit quality of kiwifruit

The visibly fungal decay in the control and treated fruit were first observed on days 7 and 10 after treatment, respectively; thereafter the disease incidence in fruits increased gradually during storage. However, the disease incidence of kiwifruit treated with NO was significantly lower than that of the control ($p < 0.05$) (Fig. 1A). SSC in the control and NO-treated kiwifruit increased gradually in the first 10 days after