Postharvest Biology and Technology 118 (2016) 128–133

Contents lists available at ScienceDirect

Postharvest Biology and Technology

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

Abscisic acid mediates wound-healing in harvested tomato fruit

Xiaoya Tao, Linchun Mao*, Jiayin Li, Jingxin Chen, Wenjing Lu, Si Huang

Department of Food Science and Nutrition, Zhejiang Key Laboratory of Agro-Food Processing, Fuli Institute of Food Science, Zhejiang University, Hangzhou 310058, People's Republic of China

ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 30 March 2016
Accepted 1 April 2016
Available online 19 April 2016

Keywords:
Abscisic acid
Tomato
Wound-healing
Suberin

ABSTRACT

The development of wound periderm is vital to prevent water vapor loss and pathogen invasion in wounded fruit. The effect of abscisic acid (ABA) on wound-healing in harvested tomato fruit was evaluated in this study. Wounded tomato fruit were treated with ABA or fluridone (FLD, an inhibitor of ABA biosynthesis), respectively, and allowed to wound-heal at 20 °C in darkness for 4 days. Weight loss of tomato fruit reflected the intuitionistic effects of different treatments during wound-healing. Autofluorescence and histochemical staining demonstrated the development of suberized wound periderm in harvested tomato fruit at 3–4 day after wounding. Alkanoic acids, *n*-alkanes, unsaturated fatty acids and pentacosane represented the major components in the suberin. ABA-treated fruit showed enhanced autofluorescence, histochemical intensity and suberin deposition along with increased activities of phenylalanine ammonia-lyase (PAL) and peroxidase (POD). FLD treatment, however, alleviated the features of suberization and enzyme activities during wound-healing. The results suggest that ABA is involved in the stimulation of wound-induced suberization in harvested tomato fruit.

1. Introduction

Fresh fruit often suffer mechanical wounds during harvest, transportation and storage. Physical wounding is an important abiotic stress that can significantly compromise fruit quality and shelf life and also elicit a series of physiological and pathological responses (Pēna-Cortés et al., 1989; Toivonen and Brummell, 2008; Tosetti et al., 2014). Therefore, rapid wound-healing is critical for harvested fruit. However, little is known about the regulation of healing processes at the wound periderm in fruit and knowledge about the regulators expediting the processes is very limited.

Suberization may be the most important process in potato tuber wound-healing (Lulai, 2007). The first stage of suberization, regarded as primary suberization, includes a closing layer formation whereby suberin biopolymers accumulate in the cell walls at the wound surface. The second stage, regarded as secondary suberization, involves wound periderm development whereby the newly formed cells below the closing layer become suberized (Graça, 2015; Vishwanath et al., 2015).

Suberin, a natural protective polymer, deposits mainly at plant-environment interfaces such as peridermal cell walls thereby protecting the plants or fruit from many stresses such as wounding and microbial attack (Graça, 2015; Vishwanath et al., 2015). Previous studies have shown that suberin is a cell wall-associated biopolymer composed of two primary domains (1) a suberin poly phenolic (SPP) forming the cell wall-localized suberin poly (phenolic) domain (SPPD) and (2) a suberin poly aliphatic (SPA) forming the suberin poly (aliphatic) domain (SPAD), localized between the cell wall and plasma membrane (Bernards and Lewis, 1998; Bernards, 2002). SPP appears upon wounding while SPA occurs after a 3-day lag period in wound-healing potato discs (Kolattukudy, 1980). SPP accumulation can be displayed by autofluorescence while SPA structure can be reflected by staining with Sudan dyes although SPA is colored weakly (Lulai and Morgan, 1992). SPPD contains primarily ferulic acid, coniferyl alcohol and vanillin (Schreiber et al., 1999; Graça and Pereira, 2000a). SPAD consists of long-chain α, ω-diacids, ω-hydroxyacids and a small amount of 1-alkanols and 1-alkanoic acids. Glycerol also appears in the periderm suberin of cotton stems and potato tubers and can cross-link SPAD and SPPD to form a three-dimensional structure in suberized cell walls (Moire et al., 1999; Graça, 2010).

Phenylalanine ammonia-lyase (PAL) is vital for the synthesis of suberin phenolic compounds, catalyzing the conversion of phenylalanine to cinnamic acid for further biosynthesis in phenylpropanoid metabolism (Rivero et al., 2001; Bernards, 2002; Kang and Saltveit, 2003). Peroxidase (POD), catalyzing the last step in lignin and suberin synthesis, comprises an enzyme

* Corresponding author.
E-mail address: linchun@zju.edu.cn (L. Mao).

http://dx.doi.org/10.1016/j.postharvbio.2016.04.002
0925-5214/

family and has important functions in defense-related cell wall fortification through phenol polymerization, lignification and suberification (Kolattukudy et al., 1992; Quiroga et al., 2000).

Abscisic acid (ABA) is considered to be a stress hormone and accumulates rapidly in response to stresses (Zhang et al., 2006). Early reports indicated that ABA might be involved in the regulation of wound-healing in potato tubers (Soliday et al., 1978; Cottle and Kolattukudy, 1982; Lulai et al., 2008; Kumar et al., 2010). The role of ABA on the formation of a suberized stem scar in tomato fruit has been investigated by Leide et al. (2012). Information about the involvement of ABA on wound-healing in harvested fruit, however, is very limited.

In this study, the wound-healing process in harvested tomato fruit was investigated with autofluorescence, histochemical staining and suberin analysis. Exogenous ABA and fluridone (FLD) were applied to evaluate the role of ABA in biosynthesis and deposition of suberin.

2. Materials and methods

2.1. Plant material and treatments

Cherry tomato fruit (*Lycopersicon esculentum var. cerasiforme* Xin Taiyang) were harvested at immature green stage from a greenhouse (20–25 °C, 70%–85% RH) located in Hangzhou, China. Fruit at about 25 day after anthesis were harvested on the basis of size and shape without physical injuries or infections. Harvested fruit were surface-disinfected with 0.5% (v/v) sodium hypochlorite solution for 5 min, washed with sterile deionized water and surface-dried at room temperature.

2.2. Wounding, ABA and FLD treatments

Mechanical wounds of approximate dimensions of 10 mm diameter and 1.5 mm deep were prepared with a sterilized scalpel around the fruit equator (Dean and Kolattukudy, 1976). The wounded fruit were randomly divided into three groups and treated with 1.0 mM ABA (≥90%, Aladdin Industrial Inc., China), 0.1 mM FLD (99.8%, Fluka Analytical, Germany) and deionized water (control), respectively. Each group was immediately placed into a vacuum dryer (SHZ-D III, Mingyuan Instrument Co., Ltd., China) containing related solution to vacuumize (0.07 MPa, 5 min) at room temperature. The vacuuming conditions and concentrations of ABA and FLD were based on results from preliminary experiments. After treatments, the fruit were air-dried and placed in a super-clean chamber (HWS, Ningbo Southeast Instrument Co., Ltd., China) at 20 °C with 90% RH in darkness for wound-healing.

2.3. Weight loss determination

Three batches of 30 tomato fruit for each group were weighed daily to evaluate weight loss. The results were calculated as the fresh weight change of tomato fruit at each test time divided by the initial weight and expressed as percentages.

2.4. Autofluorescence microscopy and histochemical staining

Cubic sections (15 mm × 3 mm × 3 mm) isolated with a scalpel from wound tissues were dehydrated in 20% (m/v) sucrose solution for 20 min followed by 30% (m/v) sucrose solution for 15 min twice. The sections were then transferred to embedding medium (Surgipath® (Leica) FSC 22®, Leica Biosystems, USA) and cooled to −15 °C. Frozen longitudinal sections of 5 μm thickness were obtained using a Cryostat Microtome (CryoStar™ NX50, Thermo Scientific, USA). Sections were analyzed with a microscope (Olympus BX61, Olympus Corporation, Japan) using fluorescence excitation filter at 470–495 nm and emission filter at 510–550 nm. The autofluorescence micrographs of cell wall-associated polyphenolics were obtained under 4× magnification (Olympus cellsens standard, Olympus Corporation, Japan).

After autofluorescence, the sections were immersed in Sudan IV saturated 70% ethanol solution (v/v, ethanol/water) as solvent for 20 min, and then cleaned with 50% ethanol for 2 min to wash away excess pigment. The sections were examined under 10× magnification (Olympus cellsens standard, Olympus Corporation, Japan). Five tomato fruit for each group were used daily for the measurement of autofluorescence and staining.

2.5. Analysis of phenylalanine ammonia-lyase (PAL) and peroxidase (POD) activity

All steps of enzyme extraction were performed at 4 °C. About 1 g wound tissue were homogenized in 4 mL of cold 0.1 M sodium borate buffer (pH 8.8) containing 1 mM EDTA and 3% (w/v) polyvinylpyrrolidone for PAL assay and 0.05 M cold sodium phosphate buffer (pH 7.8) containing 1 mM EDTA and 2% (w/v) polyvinylpyrrolidone for POD assay. The homogenates were centrifuged at 9000*g* for 15 min at 4 °C (Universal 320 R, Hettich Lab Technology, Germany). The supernatants were used separately for analysis of PAL and POD activity. Protein concentrations in the supernatants were determined according to Bradford (1976) with bovine serum albumin (BSA) as the standard.

PAL activity was assayed according to the method of Yingsanga et al. (2008) with slight modifications. The reaction mixture in a total volume of 4 mL contained 2 mL of 0.1 M sodium borate buffer (pH 8.8), 1 mL of 20 mM l-phenylalanine and 1 mL supernatant. The mixture was incubated in a water bath at 37 °C for 1 h. One unit of PAL was defined as the amount of enzyme that caused an increase of 1 in absorbance per second at 290 nm. PAL activity is expressed as unit per mass of protein ($U\,kg^{-1}$).

POD activity was determined with guaiacol as described by Zhang and Kirkham (1996). The reaction mixture in a total volume of 3 mL consisted of 2.83 mL of 10 mM sodium phosphate buffer (pH 7.0), 50 μL of 20 mM guaiacol and 0.1 mL supernatant. The reaction was initiated with the addition of 20 μL of 40 mM H_2O_2. POD activity was measured by following the change of 1000 in absorbance at 470 nm in one second due to guaiacol oxidation. Activity is expressed as unit per mass of protein ($U\,kg^{-1}$).

2.6. Isolation and purification of suberized cell wall

The suberized wound periderm of tomato fruit was removed using sterile forceps at the third and fourth day after wounding. The enzymatic isolation of suberized cell wall was adapted from Schönherr and Riederer (1986). The suberized tissue was separated in an enzymatic solution containing 1% (w/v) pectinase (3×10^7 U/kg, Aladdin Industrial Inc., China) and 1% (w/v) cellulase (10^7 U/kg, Aladdin Industrial Inc., China) in 20 mM citrate buffer (pH 3.0). After 1 h at 25 °C, the suberized cell-wall materials were obtained after the digested residue was rinsed three times with 10 mM borax buffer (pH 9.18) followed by rinsing with sterile deionized water. The residue was dried at 70 °C for 30 min in the thermostat (DHG-9075A, Linpin Instrument Co., Ltd., China).

2.7. Depolymerization and isolation of suberin

The suberized cell-wall materials were transesterified according to Zeier and Schreiber (1998) by incubating in 14% BF_3/methanol solution (ANPEL Scientific Instrument Co., Ltd., Shanghai, China) at 70 °C for 12 h to release methyl esters of suberin monomers. After the reaction mixture was ice-cooled, the liquid was removed and the solid residue was extracted three