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Characteristics of chilling injury-induced lignification in kiwifruit with different sensitivities to low temperatures

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ABSTRACT

The extent of chilling injury (CI) of kiwifruit varies greatly among cultivars. The characteristics of CI-induced lignification and the effects of 1-methylcyclopropene (1-MCP) on CI of kiwifruit has been studied on *Actinidia chinensis var. chinensis* 'Hongyang' and *A. chinensis var. deliciosa* 'Xuxiang'. 1-MCP delayed and reduced the CI proportion of the subcutaneous flesh. CI-induced lignification was mainly concentrated in the flesh of 'Hongyang' fruit, but in the core of 'Xuxiang'. The lignified cells were formed by the deposition of lignin particles on the inside of the cell wall. The accumulation of lignin in the flesh of both cultivars was decreased after treating with 1-MCP, but the more serious lignification was observed in core tissues, especially those of 'Xuxiang'. Activities of phenylalanine ammonia-lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), and peroxidase (POD) involved in lignin synthesis were analyzed. 1-MCP increased PAL and POD activities in both flesh and core tissues of the two cultivars, whereas CAD activity was enhanced in the core and decreased in the flesh. The results indicated that 1-MCP is effective in controlling the occurrence of CI, but the associated induced fruit core lignification in particular cultivars can be serious.

1. Introduction

Low-temperature storage of kiwifruit can result in chilling injury (CI), which is characterized by symptoms such as brown and black peel, epidermis pitting, a water-soaked and grainy tissue appearance, and lignification in the subcutaneous tissues (Burdon et al., 2014b; Gerasopoulos et al., 2010; Ma et al., 2014; Yang et al., 2012). However, the visible symptoms, appearance period and severity of CI in kiwifruit depend heavily on the cultivar, stage of maturity and storage duration (Burdon et al., 2007; Burdon et al., 2014a; Koutsoflini et al., 2013; Maguire et al., 2005). CI in horticultural products has been attributed to alterations in cell membrane structure and oxidative stress (Laura et al., 2009). *Actinidia chinensis* var. *chinensis* 'Hongyang' and *A. chinensis* var. *deliciosa* 'Xuxiang' are two major kiwifruit cultivars planted in China (Huang et al., 2013). The *chinensis* cultivars ('Hongyang' and 'Hort16A') are more sensitive to low temperature than *deliciosa* cultivars ('Xuxiang' and 'Hayward'), with earlier and with greater severity of CI symptoms (Burdon et al., 2014b; Yang et al., 2012; Yang et al., 2013a, 2013b; Yong et al., 2014). Despite the evident differences in CI characteristics between the two cultivar types, systematic comparisons of these characteristics are limited, and little attention has been paid to CI-induced lignification.

Lignification is considered as a response to abiotic stress and natural senescence (Jin et al., 2009; Kamdee et al., 2014; Vilanova et al., 2014), which were observed in many chilling-sensitive horticultural products, such as loquat (Cai et al., 2006a), mangosteen (Choehom et al., 2003), bamboo shoots (Song et al., 2011), and pears (Lu et al., 2015; Ning et al., 1992). It is the result of lignin synthesis and polymerization, and varies among different plant species. The lignin biosynthesis and polymerization are accompanied with a series of secondary metabolic reactions (Bonawitz and Chapple, 2010). Most studies on postharvest lignification have focused on the key enzymes: phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), and peroxidase (POD), and expression of associated genes (Shan et al., 2008; Whetten and Sederoff, 1995). In loquat fruit, the increase of firmness and lignin is associated with increased activities of PAL, CAD and POD (Cai et al., 2006b). 1-MCP is a key inhibitor of ethylene action and has been widely used to extend the storage life and improve postharvest quality

Abbreviations: CI, chilling injury; PAL, phenylalanine ammonia-lyase; CAD, cinnamyl alcohol dehydrogenase; POD, peroxidase; 1-MCP, 1-methylcyclopropene; XX, 'Xuxiang' kiwifruit; HY, 'Hongyang' kiwifruit

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(Watkins, 2006). Studies of the effects of 1-MCP on lignification in bamboo shoots (Luo et al., 2007), loquat (Liu and Jiang, 2006; Yang et al., 2008), green asparagus (Liu and Jiang, 2006), and melon (Jin et al., 2016) showed that 1-MCP alleviated lignification by repressing PAL, CAD and POD activity during cold storage. Previous studies indicated that responses to 1-MCP may vary among kiwifruit cultivars, and 'Xuxiang' is the most sensitive cultivar, followed by 'Huayou', 'Hongyang' and 'Hayward' (Xia et al., 2010, 2011; Park et al., 2015). 1-MCP reduced CI in some kiwifruit and enhanced the resistance to lignification (Mworia et al., 2012; Sooyeon et al., 2016). But little information is availabe on the effects of 1-MCP on CI and lignification in different cultivars of kiwifruit. In this study, we focused on the influence of 1-MCP on CI-induced lignification in *chinensis* cv. 'Hongyang' and *deliciosa* cv. 'Xuxiang' kiwifruit. The distinguishing features of CI and liginification in the two cultivars were identified by studying CI symptoms, and the microstructure and ultrastructure of flesh and core tissues. Our objective was to clarify the relationship between CI and lignification and provide insight into the different lignification characteristics between the flesh and core tissues.

2. Materials and methods

2.1. Plant materials

'Hongyang' (HY) (*A. chinensis*) and 'Xuxiang' (XX) (*A. deliciosa*) fruit were harvested at commercial maturity from an orchard located in Zhouzhi, Shaanxi Province, China, on 20th September and 13th October 2016, respectively. The harvesting standard for HY is uniform size (80 ± 10 g each fruit) and a soluble solid content of 8 ± 0.5%. The XX fruit were treated on the day of harvest and were selected according to uniform size (100 ± 10 g for each fruit) and a soluble solid content of 7 ± 0.5%. All fruit were free of any mechanical damage or disease and had no defects on their surfaces.

2.2. 1-MCP treatment

The fruit of each cultivar were divided into two lots: the control (no 1-MCP) and the 1-MCP treatment. Each lot contained 900 fruit, and was divided randomly into three groups as three biological replicates. For the 1-MCP treatment, fruit of HY and XX were treated with 0.5 μL L^{-1} and 0.25 μL L^{-1} of 1-MCP, respectively. The concentrations of 1-MCP were determined based on the results of our previous research on 1-MCP in kiwifruit (Xia et al., 2010). The fruit were exposed to 1-MCP in a sealed container at 20 °C for 24 h. Control fruit of the both cultivars were treated exactly the same but without the 1-MCP treatment. 1-MCP (active ingredient 0.14%) was obtained from SmartFresh™, Philadelphia, PA, USA. All treatment fruit were stored at 0 ± 0.5 °C (95% RH) for 100 d. For each replicate of each treatment, 15 fruit were taken to test the physiological and biochemical index at 10-d intervals. Samples of the flesh and core tissues were cut into small pieces, and were frozen in liquid nitrogen and stored at −80 °C. Three replicates of 45 fruit per treatment were removed to evaluate the CI index and incidence at 10 d intervals.

2.3. Evaluation of CI in flesh

CI assessment was based on the description of Yang et al. (2013a) and Ma et al. (2014). The fruit were transferred from cold storage to 20 °C for 5 d at ten-day intervals. The CI symptoms of the fruit surface and subcutaneous flesh (browning, grainy texture, water-soaking and pitting) were analysed to define the CI index where 0 = normal (no CI), 1 = trace (0 < visible disorder covering ≤ 1/4), 2 = slight (1/4 < visible disorder covering ≤ 1/3), 3 = moderate (1/3 < visible disorder covering ≤ 1/2), and 4 = severe (visible disorder covering > 1/2). The CI index was calculated as Σ [(CI level) × (number of fruit at the CI level)]/(4 × total number of fruit). The CI incidence (%) was calculated as (the number of CI fruit/the total number of fruit recorded) × 100%.

2.4. Measurements of firmness and ethylene production

Fruit firmness was determined using a firmness detector (FT327, Effegi, Alfonsine, Italy) fitted with a 7.9 mm diameter probe. The fruit were peeled, and two points with a 90° intersection angle were selected on the equator of each fruit. The detector was operated with a penetration speed of 1 mm s^{-1} and an insertion depth of 5 mm; the measurements were recorded in Newtons (N).

Ethylene production was measured by a gas chromatograph (GC-14A, Shimadzu, Kyoto, Japan) with a flame ionization detector. Fifteen fruit were selected randomly and sealed in a container equipped with a rubber septum for 1 h at 0 °C. The operating method was as described by Yang et al. (2013b). Production was expressed as ng C_2H_4 kg^{-1} h^{-1}.

2.5. Histochemical identification, microstructure and ultrastructure of the lignified tissues

Lignification in the flesh and core was evaluated by staining fruit slices with Weisner reagent (phloroglucinol/hydrochloric acid) as described by Blanco-Portales et al. (2002). Each fruit was cut into two 2 mm slices along the longitudinal direction. Which were steeped in a solution of 1% phloroglucinol and 70% ethanol for 2 h. The slices were placed on a glass plate, and a few drops of concentrated HCL were added. After 5 min, the lignified tissues were stained red.

The microstructure and ultrastructure of the lignified tissues and cells were evaluated by observing paraffin, semi-thin and ultra-thin sections. The sections were produced according to the methods of Li et al. (2016) and Khan et al. (2011). First, 1 mm^3 cubes of flesh and core tissue were fixed in solution (70% ethanol, 40% formaldehyde, and glacial acetic acid) for 48 h. Next, the tissue was gradually dehydrated with ethanol, and then the ethanol in tissue was replaced by 100% xylene solution. The tissue was then embedded in wax. The 8 μm paraffin sections were cut for staining with 1% safranin and fast green (FCF) (0.25 mg of fast green in 0.1 L of 60% ethanol) followed by rinsing with 100% ethanol. The sections were observed and photographed with a microscope (Olympus U-TV 0.63XC, Tokyo, Japan) after drying. For the semi-thin and ultra-thin sections, 1 mm^3 cubes of tissue were immersed in foetal bovine serum (FBS) for 30 s, dried at room temperature, and then placed in fixation solution (3.00% glutaraldehyde and 0.2 M phosphate pH 7.2). The processes of washing, fixing and embedding were performed following (Jin et al., 2013). The samples were embedded in pure Spurr resin and cut into semi-thin and ultra-thin sections with an LKB-2188 microtome. The semi-thin sections were stained with toluidine blue and observed and photographed under a microscope. The ultra-thin sections were double stained with uranyl acetate and lead citrate and then examined and photographed under a transmission electron microscope (JEM-1230, Japan).

2.6. Measurement of lignin content and PAL, CAD, and POD activities

The lignin content and PAL and CAD activities were determined according to the methods of Cai et al. (2006b) and Shan et al. (2008). Lignin was extracted using buffer solution (100 mM K_2HPO_4/KH_2PO_4, 0.5% Triton X-100, 50 g L^{-1} polyvinylpyrrolidone (PVP), pH 7.8). Two grams of tissue samples were ground, dissolved in 4 mL of K_2HPO_4/KH_2PO_4 buffer solution, and centrifuged (12,000 × g, 20 min, 4 °C). The deposition was washed three times with ethanol 95% (v/v) and 1:2 (v/v) ethanol: n-hexane solution. The residue was settled and dried at 65 °C for 12 h. Twenty milligrams of dried precipitate was suspended in a screw-cap tube in a 10 mL solution containing 2 mL of 2 M HCl and 0.5 mL of thioglcolic acid. The tube was immersed in boiling water for 8 h, cooled on ice and centrifuged (12,000 × g, 20 min, 4 °C) for 20 min. The residue was placed in 2 mL of 1 M NaOH and agitated for