Available online at www.sciencedirect.com
ScienceDirect
Postharvest Biology and Technology 49 (2008) 46–53
Postharvest Biology and Technology
www.elsevier.com/locate/postharvbio

Expression of expansin genes during postharvest lignification and softening of 'Luoyangqing' and 'Baisha' loquat fruit under different storage conditions

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Received 5 November 2007; accepted 2 January 2008

Abstract

Four expansin cDNA fragments, *EjEXPA1*, *EjEXPA2*, *EjEXPA3* and *EjEXPA4*, were isolated and characterized from loquat (*Eriobotrya japonica* Lindl.) fruit. *EjEXPA1* mRNA accumulated consistently with the increase in fruit firmness in 0 °C storage of 'Luoyangqing' (LYQ) fruit, where chilling injury with increased fruit firmness due to lignification was observed. *EjEXPA1* mRNA levels were lower in fruit that underwent low temperature conditioning (LTC, 6 d at 5 °C then 4 d at 0 °C), and in 1-methylcyclopropene (1-MCP) treated fruit, in both cases where chilling injury was alleviated. Fruit of the 'Baisha' (BS) cultivar soften after harvest rather than increase in firmness, and high expression levels of *EjEXPA1* and *EjEXPA4* accompanied the softening of BS fruit stored at 20 °C; such mRNA accumulation was much lower when fruit were stored at 0 °C, where softening was significantly inhibited by the low temperature. Very low expression of *EjEXPA2* and *EjEXPA3* was observed during storage of both LYQ and BS fruit under the different storage conditions. Our results showed that of the four genes characterized, *EjEXPA1* might be associated with chilling-induced lignification while both *EjEXPA1* and *EjEXPA4* were closely related to softening of loquat fruit during the postharvest period.

Keywords: Chilling injury; Expansin; Lignification; Loquat fruit; LTC; 1-MCP; Softening

1. Introduction

Fruit textural change is one of the most important processes taking place during fruit ripening and senescence, directly affecting postharvest life and commercial value of the fruit. While fruit softening caused by cell wall degradation is a common phenomenon occurring in many fruit during postharvest storage and ripening, an increase in fruit firmness caused by cell wall secondary lignification is more unusual, being reported in several fruit such as loquats (Cai et al., 2006a,b,c), mangosteens following impact injury (Bunsiri et al., 2003), and coconuts (van Dam et al., 2004). This poses a question on the interplay between lignification and cell wall enzymes conventionally associated with fruit softening.

Numerous studies have been conducted on the roles of cell wall hydrolases and modifying enzymes during postharvest softening of fruit, including polygalacturonase, xyloglucan endotransglycosylase, cellulase, pectin esterase, and β-galactosidase (Brummell and Harpster, 2001). However, such enzymes might not be the sole determinants in the disassembly of the cell wall since genetic modification of these enzyme activities has often not greatly retarded fruit softening in many cases (Smith et al., 1988; Giovannoni et al., 1989; Tieman and Handa, 1994).

More recently, expansins, a class of nonenzymatic cell wall proteins, have been found to play an important role in cell wall loosening and extension (Cosgrove, 2000). Close relationships between expansins and postharvest fruit softening have

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doi:10.1016/j.postharvbio.2008.01.005

been shown in a range of fruit including tomato (Rose et al., 1997; Brummell et al., 1999a,b; Brummell et al., 2002), peach (Hayama et al., 2000, 2003), strawberry (Harrison et al., 2001), pear (Hiwasa et al., 2003), banana (Wang et al., 2006), and kiwifruit (Yang et al., 2007).

Loquat (*Eriobotrya japonica* Lindl.) is a nonclimacteric fruit that can be separated into two categories according to the flesh color: white-fleshed and red- or orange-fleshed cultivars (Zhou et al., 2007). We have previously reported flesh lignification as one of the characteristics of ripening 'Luoyangqing' (LYQ) fruit, which is a red-fleshed loquat (Cai et al., 2006a,b,c; Shan et al., 2008). Increase in fruit firmness in relation to accumulation of lignin and change in activities of lignification enzymes such as phenylalanine ammonia lyase (PAL), cinnamyl alcohol dehydrogenase (CAD), guaiacol-POD (G-POD) and syringaldazine-POD (S-POD) were significantly correlated in LYQ fruit during postharvest storage and ripening, resulting in greater flesh firmness, toughness of the texture and loss of juiciness (Cai et al., 2006a; Shan et al., 2008). Lignification also occurred as a low temperature stress response in loquat fruit stored at 0 °C. (Cai et al., 2006c; Shan et al., 2008). Postharvest treatments, such as low temperature conditioning (LTC) and 1-methylcyclopropene (1-MCP), alleviated such lignification in LYQ loquats stored at 0 °C and inhibited the increase in fruit firmness and lignin accumulation (Cai et al., 2006b,c). 'Baisha' (BS) is another loquat cultivar representative of the white-fleshed loquat, which in contrast to LYQ fruit, softens continuously during postharvest ripening (Shan et al., 2008). The contrasting differences in textural changes between LYQ and BS fruit, i.e. lignification and softening, within the same fruit species provide valuable perennial fruit material for studies on postharvest textural changes.

In addition to their role in cell wall loosening, expansins have also been reported to be involved in plant xylogenesis and cell wall secondary differentiation (Gray-Mitsumune et al., 2004; Pesquet et al., 2005). The present study was designed to investigate the hypothesis that expansin genes might be involved in both postharvest lignification and softening processes of loquat fruit under different storage conditions, using the contrasting LYQ and BS cultivars as fruit material.

2. Materials and methods

2.1.1. Fruit material

Fruit of two cultivars of loquat, *Eriobotrya japonica* Lindl., cvs. Luoyangqing and Baisha, were harvested from an orchard in Luqiao, Zhejiang, China. Harvest time was based on the fruit being at commercial maturity and the fruit were transported to the laboratory on the day of harvest, and then screened for uniform size and maturity and absence of disease and mechanical damage.

2.2. Treatments and sampling

For both LYQ and BS cultivars, fruit were divided into lots of 270 fruit, and each lot was divided into three replicates of 90 fruit. Each set of three replicates of LYQ and BS fruit was given one of two treatments: (1) fruit were stored at 20 °C for 10 d; (2) fruit were stored at 0 °C for 10 d. Another two sets of LYQ fruit were given two treatments: (1) fruit were stored at 5 °C for 6 d, and then transferred to 0 °C for another 4 d as a low temperature conditioning (LTC) treatment; (2) fruit were treated with 5 $\mu L\,L^{-1}$ 1-MCP for 12 h before 0 °C storage for 10 d. 1-MCP treatment was carried out as described by Cai et al. (2006b). A 1000 $\mu L\,L^{-1}$ 1-MCP stock gas was made by dissolving 1.6 g EthylBloc™ powder (a.i. 0.14% 1-MCP) with water (at 35–40 °C) in a sealed 1000 mL glass flask. The appropriate volumes of 1-MCP stock gas determined to be necessary to attain the desired 1-MCP concentration (0.5, 5.0, 50 $\mu L\,L^{-1}$) in each container (6 L) was injected into the sealed container containing 80 (at 20 °C) or 100 (at 0 °C) fruit using a syringe. Injection holes were immediately sealed with adhesive tape, and the container with fruit was then left to stand for 12 h at 20 °C. All storage was at 92–98% relative humidity.

During the experiments, 15 fruit per treatment, 5 from each of 3 replicates, were sampled every other day during the postharvest periods. One more sampling was done 1 d after the LTC or 1-MCP treatment for the LYQ fruit stored at low temperature. The three sample replicates were kept separate and the fruit were cut into small pieces and frozen in liquid nitrogen immediately. Samples were kept at −80 °C for molecular assays.

2.3. Fruit firmness

Fruit firmness was determined on 9 individual fruit per treatment, 3 fruit from each of the 3 replicates. Measurements were made using a TA-XT2i (Stable Micro Systems, England) texture analyzer with a probe 5 mm in diameter, a penetration depth of 4 mm and rate of penetration 1 mm s^{-1}. Measurements were made on two sides of each fruit after removal of a small piece of peel, and the data expressed in newtons (N).

2.4. Gene cloning and amino acid sequence analysis

Total RNA was extracted from loquat fruit tissue following our previously published protocol (Zhang et al., 2006). A degenerate sense primer EXPSP, 5′-ACAATGGGNGGDGCDTGTGG-3′ (D = A/G/T, N = A/C/G/T), and an antisense primer EXPAP, 5′-TGCCARTTYTGNCCCCARTT-3′ (R = A/G, Y = T/C) were designed according to the conserved regions of plant expansin sequences from *Prunus armeniaca* (AF038815), *Lycopersicon esculentum* (AF096776), *Pyrus communis* (AB093029), *Prunus persica* (AB029083), *Fragaria x ananassa* (AF159563). The 3′-end of the fragments was amplified with a 3′-RACE kit (Takara, Dalian, China) following the instructions of the supplier. The primers used in the 3′-RACE were: EjEXPA1-3SP, 5′-CGGCTGGCAAGCAATGTCCA-3′; EjEXPA2-3SP, 5′-TTACAGAAGGGTGCCTTGCTT-3′; EjEXPA3-3SP, 5′-CAAGACAGGGTGGCAAACCT-3′; EjEXPA4-3SP, 5′-TGTGTCGATCAAGGGCTCCAAC-3′. Sequence alignment was analyzed by ClustalX 1.81 (Thompson et al., 1997) and phylogenetic analysis was carried out with DNAMAN 5.1 (Lynnon corporation, USA).