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Research note

Expression of genes encoding xyloglucan endotransglycosylase/hydrolase in 'Saijo' persimmon fruit during softening after deastringency treatment

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ABSTRACT

Persimmon (*Diospyros kaki* Thunb.) fruit undergoes intensive cell wall modification during postharvest fruit softening. Xyloglucan metabolism is important in cell wall disassembly. We cloned cDNAs for two xyloglucan endotransglycosylase/hydrolase genes (*DkXTH*1 and *DkXTH*2) from 'Saijo' persimmon fruit treated with dry ice to remove astringency. In order to determine the ethylene dependence of *XTH* gene expression, fruit were exposed to 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, prior to removal of astringency. Ethylene production increased in mature control and 1-MCP-pretreated fruit after dry-ice treatment, and flesh firmness decreased to the same extent during dry-ice treatment in the control and 1-MCP-pretreated fruit. After dry-ice treatment, control fruit softened completely, but fruit firmness was maintained in 1-MCP-pretreated fruit. Accumulation of *DkXTH*1 mRNA was induced simultaneously with commencement of ethylene production in mature control fruit. Pretreatment with 1-MCP delayed accumulation of *DkXTH*1 mRNA. *DkXTH*2 expression also coincided with fruit softening but was intensified by 1-MCP treatment during the deastringency treatment. These results indicate that fruit softening was related to both *DkXTH*1 and *DkXTH*2 expression in 'Saijo' persimmons.

1. Introduction

Persimmon (*Diospyros kaki* Thunb.) fruit have a short shelf-life due to fruit softening, which is accelerated after the removal of astringency, and this greatly influences consumer acceptability of the fruit. Ethylene plays an important role in fruit softening in persimmon (Itamura et al., 1991; Nakano et al., 2001) and 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, prolongs persimmon shelf-life in combination with different deastringency methods (Harima et al., 2003; Xu et al., 2004).

Plant primary cell walls consist of cellulose microfibrils and matrix substances including pectic polysaccharides and hemicelluloses. Xyloglucans are the predominant hemicellulosic polysaccharide and the degradation of both xyloglucans and polyuronides is cooperatively involved in fruit softening processes (Wakabayashi, 2000). Cutillas-Iturralde et al. (1994) reported that hemicellulosic polymers, in particular xyloglucans, are likely to participate in the maintenance of cell wall integrity and their depolymerization is closely related to fruit softening in persimmon fruit. These findings indicate that an understanding of xyloglucan metabolism during fruit softening is important in persimmons. Genes encoding members of the xyloglucan endotransglycosylase/hydrolase (*XTH*; previously known as xyloglucan endotransglycosylase, XET) enzyme family have been characterized in a number of fruit, including tomato (Arrowsmith and de Silva, 1995; Saladieĭ et al., 2006), kiwifruit (Schröder et al., 1998), grape (Nunan et al., 2001; Ishimaru and Kobayashi, 2002), pear (Hiwasa et al., 2003), banana (Lu et al., 2004), and apple (Goulao et al., 2008), but not yet in persimmon. In this study, we isolated cDNAs encoding *XTH*s from persimmon fruit pulp and characterized their expression during fruit softening following treatment with 1-MCP, in order to investigate the relationship between rapid fruit softening and *XTH* expression.

2. Materials and methods

Mature persimmon (*D. kaki* cv. Saijo) fruit of uniform weight and shape and without visual defects were harvested in a local commercial orchard, Shimane Prefecture, in late October. Fruit were randomly divided into two groups after harvest. One was immediately treated with 0.5 $\mu L L^{-1}$ 1-MCP at 20 °C, 60% RH for 16 h in a 117 L plastic container with a plastic lid sealed by a water moat (Zheng et al., 2006a). The other control group was stored under the same conditions without 1-MCP treatment. Both con-

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trol and 1-MCP-pretreated fruit received dry-ice treatment for removal of astringency (Zheng et al., 2006b). Fruit were immediately sealed in 0.06-mm thick polyethylene bags together with dry-ice (1.5% of the total fruit weight) and held at 20 °C, 60% RH for 4 days.

Ethylene production and fruit firmness were measured daily. Fresh pulp samples (5 g; 5 mm × 5 mm cubes) were sealed in a 30 mL vial for 20 min at 20 °C to avoid contamination with wound-induced ethylene, after which 0.5 mL headspace gas was drawn from the vial with a glass syringe and injected into a gas chromatograph (Shimadzu GC-14, Kyoto, Japan) fitted with an activated alumina column and a flame ionization detector. After the ethylene production assay, fruit firmness was measured at four pared points along the equatorial region of the same fruit with a fruit hardness tester (Fujiwara KM-1, Tokyo, Japan) fitted with a core tip plunger. The mean of four replicate tests was calculated for each of three fruit. Finally, the pulp tissues from each fruit were separately frozen in liquid nitrogen and stored at −80 °C until extraction of total RNA.

Total RNA was isolated using the hot borate method (Wan and Wilkins, 1994). The first-strand cDNA was synthesized with a first-strand cDNA synthesis kit (Amersham Pharmacia Biotech, England). The partial *XTH* gene was amplified using 100 ng cDNA from control mature pulp, for which fruit softening had been induced by deastringency treatment, and the degenerate primers, 5′- GA(C/T)TT(C/T)GA(A/G)TT(C/T)(C/T)T(A/G/T)GG(C/T)AA-3′ and 5′- ATC(A/G/T)G(C/T)(A/G)CA(A/G)TA(A/G)TT(A/G)TA-3′ based on sequences from tomato (Arrowsmith and de Silva, 1995) and kiwifruit (Schröder et al., 1998). The PCR reaction procedure comprised initial denaturation at 94 °C for 5 min, 35 cycles with denaturing at 94 °C for 1 min, annealing at 37 °C for 1 min and elongation at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. The PCR products (about 500 base pairs (bp) in length) were ligated into the pGEM-T vector (Promega, USA). After screening, target fragments were sequenced using the BigDye™ Terminator kit and an ABI PRISM 377-18 sequencing system (Applied Biosystems, USA). DNA sequences, excluding the primer sequences, were translated and multiple sequences of the *XTH* genes were aligned using GENETYX version 9.0.4 (GENETYX Corp., Tokyo, Japan). The cloned *XTH* sequences were compared against the DDBJ database using BLASTp. Northern hybridization and signal detection were carried out as described by Nakatsuka et al. (2003). Cross-hybridization between two *XTH* genes was not observed under these stringent conditions.

3. Results and discussion

Fruit softening is accelerated by ethylene in persimmon (Itamura et al., 1991; Nakano et al., 2001). Ethylene production is promoted in mature persimmon fruit by external factors, such as water and CO_2 stress (Nakano et al., 2001, 2002), and deastringency treatment with ethanol (Kubo et al., 2003; Ortiz et al., 2005) and dry ice (Xu et al., 2004). In this study, ethylene production and fruit softening were investigated after harvest in 'Saijo' persimmon fruit. Mature fruit were treated with dry ice for 4 days to remove the astringency. Little ethylene was detected in both the control and 1-MCP-pretreated fruit during deastringency treatment. However, ethylene production by control fruit increased immediately after astringency removal and peaked 5 days after harvest (Fig. 1A) The control and 1-MCP-pretreated fruit softened to identical extents during the 4 days of dry-ice treatment (Fig. 1B). The control fruit, thereafter, softened rapidly concomitant with the sudden increase in ethylene production and had entirely softened 10 days after harvest (Fig. 1B). In contrast, ethylene production

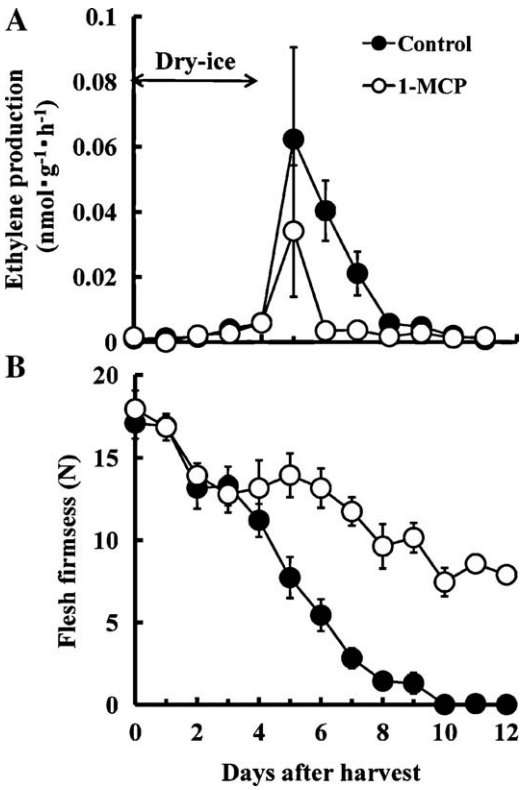

Fig. 1. Effects of 1-methylcyclopropene (1-MCP) treatment on ethylene production (A) and flesh firmness (B) in pulp of mature 'Saijo' persimmon fruit. Each point represents the mean value for five replicate fruit and vertical bars represent the SE.

by the 1-MCP-pretreated fruit peaked on day 5 after harvest and was inhibited by about 50% compared with the maximum level of control fruit (Fig. 1A). This result indicated that dry-ice-induced ethylene production is regulated by a positive feedback system and that 'Saijo' persimmon softening is dependent on ethylene. Almost no softening had occurred on days 5 and 6 in the 1-MCP-pretreated fruit (Fig. 1B). However, fruit softened during the first 4 days during deastringency treatment in both the control and 1-MCP-pretreated fruit (Fig. 1). A similar result was reported for 'Hiratanenashi' fruit treated with ethanol to remove astringency and it was suggested that some fruit softening can still occur in an ethylene-independent manner in control fruit without any treatment and 1-MCP with ethanol treatment (Kubo et al., 2003). Our results support the contention that fruit softening is controlled by ethylene-dependent and -independent systems in mature persimmon fruit.

We cloned two *XTH* isogenes and investigated their expression. Two different *XTH* homologs were isolated from softening persimmon fruit, which were designated *DkXTH*1 (465 bp; DDBJ accession no. AB474948) and *DkXTH*2 (492 bp; DDBJ accession no. AB474949). The deduced amino acid sequences of the *DkXTH*1 and *DkXTH*2 fragments shared only 55% identity. A BLASTp search showed that *DkXTH*1 and *DkXTH*2 had 67% and 60% homology with deduced amino acid sequences for the *Cucumis melo CmXTH*3 gene (GenBank accession no. ABI94063), which is involved in fruit softening (Nishiyama et al., 2007).

Disassembly of hemicelluloses (Itamura et al., 1989, 1995) and XET activity (Cutillas-Iturralde et al., 1994) are correlated with fruit softening in persimmon. These results indicate that xyloglucan metabolism, such as xyloglucan depolymerization, wall restructuring or incorporation of newly synthesized xyloglucan polymers, is important for fruit softening during ripening. In persimmon fruit, *DkXTH*1 mRNA abundance was not detectable during