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Research note

Enzymatic activities and gene expression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase in persimmon fruit

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Abstract

The role of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase in ethylene biosynthesis in persimmon fruit (*Diospyros kaki* Thunb. cv. Hiratanenashi), both intact and wounded, was characterized. In young intact fruit, ethylene production was detected 2 days after harvest, and peaked at 4 days. Little ACC content was detected at 1 day, rapidly increasing 4 days after harvest, and peaking at 7 days. *DK-ACS2* was strongly expressed during almost all periods after it commenced at 3 days, followed by a rapid increase in ACC synthase activity at 4 days and a peak at 5 days. *DK-ACO1* mRNA accumulation was initiated at harvest time, dramatically increased at 2 days; as a result, high ACC oxidase activity was detected at the beginning of harvest, and peaked at 3 days. *DK-ACO1* mRNA accumulation continued during the subsequent days, whereas ACC oxidase activity decreased to a low level. Wounding treatment induced ethylene biosynthesis and ACC accumulation. The strongest *DK-ACS2* expression was induced 1 day after wounding, followed by the highest ACC synthase activity, which paralleled ACC accumulation. Abundant *DK-ACO1* mRNA accumulation and high ACC oxidase activity were observed at the initiation of wounding and remained at high levels during the days that followed.

Keywords: 1-Aminocyclopropane-1-carboxylic acid (ACC); ACC synthase; ACC oxidase; Ethylene production; Persimmon

1. Introduction

Persimmon fruit have a short shelf-life due to fruit softening, which is accelerated after the removal of astringency, and this significantly influences the acceptability of the fruit. Ethylene plays an important role in fruit softening (Itamura et al., 1991) and therefore, understanding of the mechanism of ethylene biosynthesis in persimmon fruit is important to inhibit fruit softening and prolong the shelf-life.

Usually, persimmon is classified as a climacteric fruit (Abeles, 1992). However, unlike typical

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climacteric fruit, persimmons have some unique characteristics: the younger the stage at which the fruit is detached, the greater the amount of ethylene produced, and ethylene biosynthesis is induced only when the fruit is detached from the parent tree (Takata, 1983; Itamura, 1986). As demonstrated in other plant species (Kende, 1993), the regulating enzymes ACC synthase and ACC oxidase are also encoded by multigene families in persimmon. Recently, three ACC synthase genes (*DK-ACS1*, *DK-ACS2* and *DK-ACS3*) and two ACC oxidase genes (*DK-ACO1* and *DK-ACO2*) were cloned from young persimmon fruit and characterized (Nakano et al., 2002). Initiation and progress of ethylene biosynthesis in young detached persimmon fruit was found to be temporally and spatially coordinated with the transcription of ACC synthase and ACC oxidase genes (Nakano et al., 2003).

Several reports have shown that mechanical wounding (e.g., cutting, bruising) also induces ethylene production via induction of ACC synthase and ACC oxidase gene expression in some plants (Kende, 1993), although this is not clear in persimmon fruit. Furthermore, analysis of ACC synthase and ACC oxidase have never been reported in persimmon fruit, because of the high content of soluble tannins which greatly hinders the extraction of ACC synthase and ACC oxidase from persimmon fruit tissue. In this paper, these enzymes were extracted, and their activities, as well as gene expression, were investigated.

2. Materials and methods

Young and mature persimmon (*Diospyros kaki* Thunb. cv. Hiratanenashi) fruit were harvested from the experimental fields of Shimane University on July 23 and Experimental Station of Shimane Prefecture on September 27, and then held at 20 °C. For wounding, the mature flesh tissue was cut into 5 mm cubes and incubated at 20 °C.

Measurements of ethylene production, ACC content, and the activities of ACC synthase and ACC oxidase were repeated three times. Three fruit per replication were used for young intact fruit. Ethylene production was assayed daily. The fruit were sealed in a 900 ml airtight container for 5 h at 20 °C, and 0.5 ml gas headspace was withdrawn from the container 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. For mature wounded persimmon fruit, 10 fruit were used in each replication. The equatorial parts of 10 fruit were cut into small cubes and mixed together; about 5 g of wounded tissues were sealed in a 32 ml vial with a silicon rubber cap and incubated at 20 °C for 2 h to measure ethylene production with the same method as described above. After ethylene production was determined, the pulp tissues were frozen in liquid nitrogen and stored at −80 °C for the extraction of ACC, ACC synthase, ACC oxidase, and total RNA.

ACC was assayed according to the method described by Itamura et al. (1990). ACC synthase and ACC oxidase extraction and their activities were measured according to the method of Zheng et al. (2005).

Total RNA was isolated using the hot borate method (Wan and Wilkins, 1994). Northern hybridization and signal detection were carried out as described by Nakano et al. (2002). Twenty micrograms of RNA from each sample was used.

3. Results and discussion

Ethylene production in whole fruit was detectable 2 days after harvest and peaked at 4 days, rapidly decreasing to the basal level at 7 days (Fig. 1A). ACC formation usually precedes or accompanies ethylene production in fruit tissue (Hyodo et al., 1983). In young intact persimmon fruit, low levels of ACC accumulated 1 day before ethylene production initiation, and rapidly increased at 4 days, accompanying the peak of ethylene production (Fig. 1A). ACC content remained elevated at the late shelf-life stage when ethylene production decreased.

ACC synthase activity rapidly increased at 4 days in parallel with the highest ethylene production, peaking at 5 days followed by a rapid increase in ACC content, which then decreased to the basal level (Fig. 1B). Strong *DK-ACS2* expression was initiated at 3 days and peaked at 4 days. This was associated with the increase in ACC synthase activity, and remained at high levels during all subsequent days. The basal activity of ACC synthase and abundant mRNA of *DK-ACS2* at the late shelf-life stage, indicates that ACC synthase might be subjected to post-transcriptional regulation in per-