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Relationship between fruit cracking and expression of the expansin gene *MdEXPA3* in 'Fuji' apples (*Malus domestica* Borkh.)

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Abstract

In 'Fuji' apples, fruit cracking causes great economic loss. To understand its mechanisms, we analyzed the relationship between fruit cracking and the expression of an apple expansin gene (*MdEXPA3*) in pericarp and mesocarp during fruit growth. Fruit cracking is divided into two types; internal ring cracking (IRC) and stem-end splitting (SES). The former is an early symptom sometimes followed by the lattar. In this study, IRC mostly was observed during the phase of rapid fruit growth. *MdEXPA3* transcripts appeared in the mesocarp at 30 days after full bloom (DAFB), reached a maximum at 95 DAFB and then decreased, thus paralleling the fruit growth rate. In contrast, the transcript level in the pericarp was below the detection limit until 50 DAFB, then increased until 109 DAFB to remain high until the end of observation. As IRC began to occur just before the increase of *MdEXPA3* transcript levels in the pericarp, the differential expression in pericarp and mesocarp may be related to the initiation of IRC. Bagging reduced the incidence rate of both IRC and SES to one eighth without affecting fruit enlargement, and induced *MdEXPA3* expression at earlier stage in the pericarp but not in mesocarp. These results suggested that induced accumulation of *MdEXPA3* mRNA in pericarp reduced the susceptibility of fruit cracking. Thus, early symptoms of fruit cracking coincide with situations in which *MdEXPA3* expression in the mesocarp exceeds that in the pericarp. In such situations, pericarp cells may be unable to follow the expansion of mesocarp cells due to insufficient levels of growth promoting expansins. If so, IRC appears as a consequence of the imbalance of expansin-dependent tissue growth rates.

Keywords: 'Fuji' apples (*Malus domestica* Borkh.); Fruit cracking; Fruit growth; Pericarp; Expansin gene expression

1. Introduction

'Fuji' is one of the superior apple cultivars, which was a cross between 'Ralls janet' and 'Delicious' and was introduced in 1962 in Japan (Sadamori et al., 1963). Its sweet taste and long-term storability have made its production expand across the world and the quantities produced exceeded those of any other cultivar worldwide in 2001 (O'Rourke et al., 2003). However, fruit cracking, a pre-harvest physiological disorder, occurs in its stem cavity (Sadamori et al., 1963) which often causes great economic loss to apple growers in Japan. To control this disorder, understanding its cause and mechanism is indispensable.

Fruit cracking was also reported in 'Gala' apples (Opara, 1996; Opara et al., 2000; Andrews et al., 1999) and was divided into two types based on the symptoms observed. One is 'Internal Ring-Cracking' (IRC) which occurs at the fruit-stem joint and the other is 'Stem-End Splitting' (SES) originating in the stem cavity (Opara, 1996). As IRC was observed in all fruits in which SES occurred (Opara, 1996), IRC is considered an early symptom of fruit cracking followed by SES.

Research into the effects of orchard management practices showed that frequent irrigation increased the proportion of fruits with SES or IRC compared to fruits without irrigation (Opara et al., 2000). Therefore, cell enlargement promoted by water uptake may have induced IRC or SES. The imbalance of cell enlargement within a fruit, aggravated by fruit growth promotion, may cause minute cracks between cells, leading to IRC. Since IRC occurs just underneath the pericarp tissue, an imbalance between the cell enlargement in pericarp and mesocarp appears to trigger it. In apples, the possible dependence of fruit cracking on the extensibility of pericarp tissue has been discussed for a long time (Verner, 1938; Costa et al., 1983; Weiser, 1990; Yamamoto et al., 1996), but as the

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measurement of tissue extensibility is difficult, there is no unambiguous evidence available to evaluate the role of pericarp mechanic properties.

Expansins are cell wall proteins capable of inducing wall loosening during cell expansion under tensile stress (McQueen-Mason et al., 1992; McQueen-Manson and Cosgrove, 1995; Cosgrove, 2000). Expansins extracted from cucumber hypocotyls were shown to stimulate enlargement of living cells (Cosgrove, 1999). Expansins do not exhibit any hydrolytic activity and are believed to disrupt bonds at the interface between cellulose and hemicellulose (McQueen-Manson and Cosgrove, 1994; Whitney et al., 2000). Expansin isoforms have been found in a variety of plant species and tissues, and some of them have been shown to be associated with cell growth (McQueen-Manson et al., 1995; Cosgrove, 1999). In tomato and peach, expression of several expansin genes appears correlated with fruit development (Brummell et al., 1999; Hayama et al., 2001). In apples, six expansin genes were isolated and their expression patterns were characterized during fruit growth (Wakasa et al., 2003). Among them, *MdEXPA3*, which had been previously described as *MdEXP2*, is mainly expressed during fruit enlargement (Wakasa et al., 2003). Since expansins have the ability to enhance cell wall extensibility and to induce cell expansion, we hypothesize that expansins have a role in fruit cracking. Recently, an expansin gene (*LcExp2*) has been identified that is expressed at different levels in pericarp tissue of a cracking susceptible and a cracking-resistant litchi cultivar (Wang et al., 2006). Therefore, an analysis of the expression patterns of fruit growth-related expansin genes might help to elucidate the mechanisms of fruit cracking in apples.

In this study, we analyzed the expression patterns of *MdEXPA3* in pericarp and mesocarp tissues during fruit growth, in order to clarify the relationship between the mRNA accumulation and fruit cracking in 'Fuji' apples. The effects of fruit bagging which reduces fruit cracking on the mRNA accumulation patterns were also investigated.

2. Materials and methods

2.1. Incidence of IRC and SES

'Fuji' apple trees planted in the experimental orchard of the Apple Experiment Station in Aomori, Japan, were studied. In 2004, 36 fruits (three fruits per tree) were randomly picked at about 10-day intervals from 70 to 170 DAFB (days after full bloom) and the presence of SES and IRC was examined by cutting them longitudinally.

2.2. Fruit growth

Sixty fruits (30 fruits per tree) were randomly selected and tagged individually. Equatorial diameters and lengths of these fruits were measured at about 10-day intervals from 30 to 170 DAFB in 2004. From these measurements, fruit volume was estimated as volume = $4/3 \times \pi \times \{(\text{diameter} + \text{length})/4\}^3$.

2.3. Fruit bagging

Bagging treatment was conducted in 2005. About 130 fruits were covered with commercial double paper bags (lightproof type, Osanai seitaisho) at 35 DAFB and the bags were removed at 125 DAFB according to the standard commercial practice in Japan. Both bagged and non-bagged fruits were picked at the commercial harvesting time of 166 DAFB and examined for IRC and SES.

2.4. Fruit materials for northern blot analysis

Fruit samples for the comparative analysis of mesocarp and pericarp tissues were picked at about 20-day intervals from 30 to 150 DAFB in 2004. Fruits for the comparison between bagged and non-bagged fruits were picked at about 20-day intervals from 35 to 171 DAFB in 2005. Mesocarp and pericarp tissues (<2 mm thick) were separately diced with a kitchen knife, immediately frozen in liquid nitrogen, and then stored at −80 °C for RNA extraction.

2.5. RNA extraction and Northern blot analysis

Total RNA was extracted from the frozen tissue (10 g) of mesocarp or pericarp using the hot borate method (Wan and Wilkins, 1994). Total RNA (30 μg per lane) was subjected to electrophoresis on 1.2% agarose gel containing 5% (v/v) formaldehyde, and was blotted onto a nylon membrane (Hybond N^+, Amersham Biosciences). *MdEXPA3* was labeled with the PCR DIG Probe Synthesis Kit (Roche Diagnostics) and used as a probe. Hybridization was performed in hybridization buffer (DIG Easy Hyb Granules, Roche Diagnostics) at 50 °C overnight. The membrane was washed twice for 15 min with 0.1 × SSC and 0.1% SDS at 68 °C, and then an X-ray film (Fuji Film) was exposed to it.

3. Results

3.1. Incidence of fruit cracking and fruit growth

The first symptoms of IRC were observed at 92 DAFB; the incidence rate increased rapidly until 120 DAFB (33.3%) and then remained at this level until the commercial harvesting time (Fig. 1A). SES was first observed at 141 DAFB but its incidence rate did not increase afterwards (11.1%; Fig. 1A). All of the fruits with SES also showed IRC. Thus, SES occurred after IRC, confirming previous results from 'Gala' and 'Fuji' apples (Opara, 1996). The growth curve of apple fruits followed a sigmoidal path (Fig. 1B) and IRC occurred in the phase of rapid fruit growth from 80 to 120 DAFB.

3.2. Expression patterns of MdEXPA3 in mesocarp and pericarp tissue during fruit growth

To compare the expression patterns of *MdEXPA3* between mesocarp and pericarp during fruit growth, *MdEXPA3* mRNA accumulation was determined by northern blot analysis. In