Expansin and XET Genes Are Differentially Expressed During Aril Breakdown in Harvested Longan Fruit

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ABSTRACT. Fresh fruit of longan (*Dimocarpus longan* Lour.) are susceptible to pericarp browning and aril breakdown. Aril breakdown in longan fruit is regarded as one of the most important factors reducing quality and shortening storage life of the fruit. To better understand the molecular mechanism of aril breakdown, the expression patterns of three expansin (*EXP*) and three xyloglucan endotransglucosylase (*XET*) genes in relation to the aril breakdown of longan fruit stored at room temperature (25 °C) or low temperature (4 °C) were investigated. The results showed that aril breakdown index increased progressively during storage at 25 and at 4 °C. Northern blotting analysis revealed that the accumulations of three *EXP* and three *XET* genes exhibited differential characteristics with the occurrence of aril breakdown. During storage at 25 °C, the accumulations of *DI-XET3* increased after 1 day, suggesting that *DI-XET3* correlated well with the early aril breakdown, while *DI-EXP3* together with *DI-XET1* and *DI-XET2* was involved in later aril breakdown. However, expression of *DI-XET1* and *DI-XET2* could be mainly involved in aril breakdown of longan fruit stored at 4 °C. In addition, *DI-EXP2*, whose accumulation increased sharply when longan fruit were transferred from low temperature to room temperature within 12 hours, was related to the aril breakdown in this storage period. These data indicated that *DI-EXPs* and *DI-XETs* were closely related to aril breakdown in longan fruit.

Longan is a nonclimacteric subtropical fruit with high value (Huang, 1995; Paull and Chen, 1987). The edible portion of longan fruit is a fleshy and translucent white aril. However, the fruit deteriorate rapidly after harvest, due to pericarp browning and aril breakdown, resulting in reduced market value (Jiang et al., 2002). Aril breakdown involves loss of turgidity and translucency, and thus the fruit become bland in taste. The disorder starts near the pericarp and appears to be more prevalent at the distal end (Jiang et al., 2002). Some researchers also mentioned aril breakdown in longan fruit as “autolysis” (Jiang et al., 2002; Lin et al., 2007). Storage at low temperature can delay the occurrence of aril breakdown and extend storage life, but when longan fruit were transferred to room temperature after low-temperature storage, the aril breakdown occurred very rapidly (Jiang et al., 2002). Thus, delaying or reducing aril breakdown should be an important approach to extend storage life or maintain quality of longan fruit.

It has been considered that cell-wall components are important for fruit texture (Manganaris et al., 2005, 2006). Cellular-wall modification-related enzymes, including endo- β -1,4-glucanase (EGcase), polygalacturonases (PGcase), pectate

lyase, and pectin methyl esterase (PME), are involved in cellular-wall degradation (Rose and Bennett, 1999). Recently, expansin and xyloglucan endotransglucosylase/hydrolase (*XET/XTH*) have been suggested to be involved in fruit softening (Asha et al., 2007; Hayama et al., 2003; Hiwasa et al., 2003; Lu et al., 2004).

It has been proposed that a function of EXPs loosens the cellular walls by disrupting non-covalent linkages at the cellulose–hemicellulose interface, resulting in relaxing an important constraint to turgor-driven cell expansion (Cosgrove, 2000; McQueen-Mason and Cosgrove, 1994). EXPs are involved in fruit softening in tomato (*Solanum lycopersicum* L. cv. T5) (Rose et al., 1997), strawberry (*Fragaria ananassa* Duch. cv. Brighton) (Harrison et al., 2001), and peach [*Prunus persica* (L.) Batsch] (Hayama et al., 2003). In addition, EXP proteins have been detected in a range of ripe fruit (Rose et al., 2000).

XET/XTH, one of the best-studied enzymes in fruit ripening and softening, is able to disassemble the cellulose–xyloglucan matrix that has been associated with the fruit softening process. *XTH* genes have been determined to be related to fruit softening in banana (*Musa acuminata* Colla cv. Grand Nain) (Lu et al., 2004), tomato (cv. 83-G-38) (MacLachlan and Brady, 1994), persimmon (*Diospyros kaki* L.) (Cutillas-Iturralde et al., 1994), kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson var. *deliciosa* cv. Hayward] (Redgwell and Fry, 1993), and pear (*Pyrus communis* L. cv. La France) (Fonseca et al., 2005).

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It has been reported that the aril breakdown of longan fruit resulted from the degradation of cellular-wall components and the decline in aril firmness accompanied with the aril breakdown (Lin et al., 2007). However, the molecular mechanism of longan aril breakdown associated with expressions of *DL-EXP* and *DL-XET* has not been investigated.

The aim of the present work was to investigate the expression patterns of *EXP* and *XET* genes in relation to aril breakdown in harvested longan fruit stored at various temperatures.

Materials and Methods

PLANT MATERIAL. Longan fruit at physiological maturity stage were harvested from the Dongguan Agricultural Research Center plantation near Guangzhou, China. Fruit were transported to laboratory within 2 h and then selected for lack of visual defects and for uniformity of weight, shape and maturity.

TREATMENTS. The selected longan fruit were divided randomly into four groups. All of the selected fruit were placed into unsealed plastic bags (0.04 mm thick), and each bag contained 50 individual fruit; 12 bags were used as one group. Fruit of Group 1 were stored at 25 °C (room temperature) for 6 d until aril breakdown occurred and were sampled every day. Fruit of Group 2 were stored at 4 °C (low temperature) for 45 d and sampled at 5-d intervals. Fruit of Group 3 and Group 4 were stored at 4 °C for 20 and 40 d, respectively, and then removed to room temperature and sampled every 6 h until aril breakdown. Each time, 60 individual fruit were withdrawn, sampled randomly from 6 bags. Aril tissues (the whole aril of fruit) of each sample were frozen in liquid nitrogen and stored at -80 °C until use. All assessments were conducted with three replicates.

ARIL BREAKDOWN INDEX EVALUATION. Aril breakdown index was assessed by determining the ratio of the area of aril breakdown to the entire aril area using 50 individual fruit per treatment and calculated by the following scale: 0 (no breakdown), 1 (<1/4), 2 (1/4 to 1/2), 3 (1/2 to 3/4), 4 (3/4 to 1). The aril breakdown index was calculated as $\sum(\text{breakdown scale} \times \text{proportion of fruit within each scale})$ (Lin et al., 2007). For the fruit of Group 1 (storage at 25 °C), the index of aril breakdown was assessed beginning at 2 d of storage with assessment every day thereafter until 6 d, when aril breakdown occurred completely; for fruit of Group 2 (storage at 4 °C for 45 d), the index of aril breakdown was assessed beginning at 20 d of storage with 5-d assessment intervals thereafter, while for fruit of Group 3 and Group 4 (storage at 4 °C for 20 and 40 d, respectively, and then removed to 25 °C), the index of aril breakdown was assessed beginning at 6 h of storage at 25 °C until 84 and 48 h when aril breakdown occurred completely, respectively.

RNA EXTRACTION AND NORTHERN HYBRIDIZATION. Frozen tissues taken from the whole aril part of fruit (10 g) were ground to a fine powder in a mortar using a pestle in the presence of liquid nitrogen. Total RNA was extracted using the hot borate method of Wan and Wilkins (1994). Total RNA (10 µg) was separated on a 1.2% agarose-formaldehyde gel and capillary-blotted onto a PVDF membrane (Biodyne B, 0.45 µm; Pall Co., Sarasota, FL). The RNA was fixed to the membrane by baking for 2 h at 80 °C and then cross-linked to the membranes using an ultraviolet cross-linker (Amersham Biosciences, Piscataway, NJ). The membranes were prehybridized for more

than 3 h in SDS buffer [50% deionized formamide (v/v), 5× SSC, 7% SDS, 2% blocking reagent (Roche, Mannheim, Germany), 50 mmol·L⁻¹ sodium phosphate (pH 7.0), and 0.1% *N*-lauroylsarcosine (w/v)], and hybridization was then performed overnight in the same buffer containing the gene-specific DIG-labeled probes at 45 °C. Probes were prepared with a DIG probe synthesis kit (Roche) according to the manufacturer's instructions. All probes were synthesized from the 3'-untranslated regions of the genes. Following hybridization, membranes were washed twice for 10 min with 2× SSC containing 0.1% SDS at 25 °C, followed by washing twice for 30 min in 0.1× SSC containing 0.1% SDS at 62 °C. The signals were detected with chemiluminescence using CDP-Star (Roche) as described by the manufacturer. The specific primers used for synthesis of three *DL-XETs* DIG-labeled probes were according to Feng et al. (2008), and the specific primers used for synthesis of three *DL-EXPs* DIG-labeled probes were designed on the bases of sequences registered in GenBank (accession numbers EU416313, EU416314, and EU416315, respectively; listed in Table 1).

Results

ARIL BREAKDOWN AND DIFFERENTIAL EXPRESSION OF *EXP* AND *XET* GENES IN LONGAN FRUIT STORED AT ROOM TEMPERATURE. Longan fruit began to show aril breakdown symptoms after 2 d of storage at 25 °C. The aril breakdown index increased markedly after 3 d of storage and increased progressively with increasing storage (Fig. 1). The breakdown index after 6 d of storage was about 16-fold higher than that after 2 d of storage.

The accumulations of three *DL-EXP* and three *DL-XET* genes in longan fruit stored at 25 °C were detected using the specific DIG-labeled probes. As shown in Fig. 2, the three *DL-EXP* mRNAs and three *DL-XET* mRNAs accumulated in different patterns in aril tissues of longan fruit during storage. *DL-EXP1* mRNA decreased initially and increased slightly at Day 5, *DL-EXP2* mRNA remained almost constant during the entire storage, and accumulation of *DL-EXP3* mRNA increased gradually, especially at Day 5 and Day 6 (Fig. 2). In addition, mRNAs of both *DL-XET1* and *DL-XET2* first decreased and then accumulated obviously at Day 5 and Day 6, while accumulation of *DL-XET3* mRNA increased at Day 1, peaked at about Day 2, and finally decreased at Day 4 (Fig. 2). The increase in the accumulation of *DL-XET3* mRNA coincided well with the aril breakdown index. These results suggested that *DL-XET3* may be involved in the aril breakdown in longan fruit at the early storage stage and that *DL-EXP3* together with *DL-XET1* and *DL-XET2* were involved in aril breakdown at the later storage stage at room temperature.

Table 1. Sequences of specific primers used for the syntheses of three DIG-labeled *DL-EXP* probes.

Gene	Primer sequences for DIG probes (5'-3')
<i>DL-EXP1</i>	DIG-For: GGCGTGCTTTGAGCTAAAGTGCGTG DIG-Rev: TTCACGCTCACCTTCACTATATCCC
<i>DL-EXP2</i>	DIG-For: ATCTTGCTATGAGATTAAGTGTGTG DIG-Rev: TTGATGGAAACAGCATGGACATCAC
<i>DL-EXP3</i>	DIG-For: GGCAACCAATGTCAAGGAAGT DIG-Rev: CACACAAACAATCAATACCACC

Fig. 1. Changes in aril breakdown index of longan fruit stored at 25 °C. Each value represents the mean of three replicates, and vertical bars indicate the SE.

ARIL BREAKDOWN AND EXPRESSION CHARACTERISTICS OF *EXP* AND *XET* GENES IN LONGAN FRUIT STORED AT LOW TEMPERATURE. Storage at low temperature delayed the appearance of aril breakdown symptoms of longan fruit. As shown in Fig. 3, aril breakdown symptoms were observed after 25 d of storage at 4 °C, with the breakdown index increasing sharply after 35 d of storage. The breakdown index of the fruit stored for 45 d was about 27-fold higher than that after 25 d of storage at 4 °C.

Fig. 2. Differential expression patterns of three *DI-EXP* and three *DI-XET* genes in aril tissues of longan fruit stored at 25 °C. Total RNA (10 µg per lane) was used for RNA gel blot analysis and hybridized with DIG-labeled probes. The top section shows the Northern blot hybridization, and the bottom section shows the ethidium bromide-stained rRNA bands as a loading control for the gel.

Fig. 3. Changes in aril breakdown index of longan fruit stored at 4 °C. Each value represents the means of three replicates, and vertical bars indicate the SE.

Northern blotting analyses of three *DI-EXP* and three *DI-XET* genes in aril tissues of longan fruit stored at 4 °C are shown in Fig. 4. Expression of *DI-EXP3* was barely detected during the entire storage at 4 °C, while *DI-EXP1* and *DI-EXP2* transcripts continuously decreased with increasing storage, with higher transcript accumulation in the former than in the latter. In contrast, *DI-XET1* transcripts increased obviously after 2 d of storage at 4 °C, remained almost constant until 30 d when aril breakdown occurred, and finally decreased slightly (Fig. 4). *DI-XET2* increased after 2 d of storage but decreased slightly after 10 d, suggesting that *DI-XET1* and *DI-XET2* may be related to the aril breakdown in longan fruit stored at low temperature.

ARIL BREAKDOWN AND EXPRESSION CHARACTERISTICS OF *EXP* AND *XET* GENES IN LONGAN FRUIT AT ROOM TEMPERATURE AFTER 20 AND 40 DAYS OF STORAGE AT LOW TEMPERATURE. Changes in aril breakdown index of longan fruit during storage at 25 °C after 20 and 40 d of storage at 4 °C are shown in Figs. 5 and 6,

Fig. 4. Differential expression patterns of three *DI-EXP* and three *DI-XET* genes in aril tissues of longan fruit stored at 4 °C. Total RNA (10 µg per lane) was used for RNA gel blot analysis and hybridized with DIG-labeled probes. The top section shows the Northern blot hybridization, and the bottom section shows the ethidium bromide-stained rRNA bands as a loading control for the gel.

Fig. 5. Changes in aril breakdown index of longan fruit stored for 20 d at 4 °C and then transferred to 25 °C and stored for 84 h at this temperature. Each value represents the means of three replicates, and vertical bars indicate the SE.

Fig. 6. Changes in aril breakdown index of longan fruit stored for 40 d at 4 °C and then transferred to 25 °C and stored for 48 h at this temperature. Each value represents the means of three replicates, and vertical bars indicate the SE.

respectively. The aril breakdown index increased markedly when fruit were removed from 4 to 25 °C, with a higher level in aril breakdown of fruit stored for 40 d at 4 °C than that for 20 d. The aril breakdown index values were 2.72 and 3.92 in fruit stored for 20 d and 40 d at 4 °C and then stored at 25 °C for 84 and 48 h, respectively, whereas the breakdown index value of the fruit stored for 42 d at 4 °C was only 1.29 (Fig. 3).

Expression patterns of three *EXP* and three *XET* genes in longan fruit removed to 25 °C after 20 or 40 d of storage at 4 °C are shown in Figs. 7 and 8. *DI-EXP2*, *DI-EXP3*, and *DI-XET3* transcripts began to accumulate and then reached their peaks at 24, 84, and 36 h of storage at 25 °C, respectively, but *DI-EXP1* transcripts continuously decreased while *DI-XET1* and *DI-XET2* decreased and then increased to a high level after 84 h of storage at 25 °C (Fig. 7), after which these fruit exhibited serious aril breakdown symptoms (Fig. 5).

Fig. 7. Differential expression patterns of three *DI-EXP* and three *DI-XET* genes in aril tissues of longan fruit stored for 20 d at 4 °C and then transferred to 25 °C and stored for 84 h at this temperature. Total RNA (10 µg per lane) was used for RNA gel blot analysis and hybridized with DIG-labeled probes, and ethidium bromide-stained rRNA is shown as the loading control.

Fig. 8. Differential expression patterns of three *DI-EXP* and three *DI-XET* genes in aril tissues of longan fruit stored for 40 d at 4 °C and then transferred to 25 °C and stored for 48 h at this temperature. Total RNA (10 µg per lane) was used for RNA gel blot analysis and hybridized with DIG-labeled probes, while ethidium bromide-stained rRNA is shown as the loading control.

After these fruit were removed to 25 °C after 40 d of storage at 4 °C, the *DI-EXP2*, *DI-XET1*, and *DI-XET2* transcripts increased, reached their peaks after 24, 36, and 24 h of storage at 25 °C, respectively, and finally decreased, but the hybridized signal of *DI-EXP3* was hardly detected while *DI-EXP1* and *DI-XET3* transcripts decreased progressively during the entire 48 h of storage (Fig. 8).

Discussion

Senescence and deterioration of postharvest longan fruit are characterized by increased pericarp browning, aril breakdown, and reduced flavor and nutritive quality (Jiang et al., 2002). Moreover, the fruit have a very short shelf life at ambient temperature due to pericarp browning and aril breakdown during storage and transportation (Duan et al., 2007; Jiang and Li, 2001; Jiang et al., 2002). Storage at low temperature effectively delayed occurrence of aril breakdown (Jiang et al., 2002). Similar results were obtained in this study (Fig. 3). The aril breakdown index of longan fruit increased markedly after 3 d of storage at room temperature (Fig. 1) and increased after 25 d of low-temperature storage. Furthermore, the aril breakdown index increased rapidly when fruit were removed from low-temperature storage to room-temperature storage (Figs. 5 and 6).

It is generally accepted that fruit softening or deterioration results from a number of changes in cellular-wall components, but cellular-wall modifications have also played a major role in fruit softening (Giovannoni, 2001). Furthermore, fruit softening or deterioration is accompanied by depolymerization and solubilization of several classes of cellular-wall components, such as pectins and hemicelluloses, elevated expression levels of genes or proteins, or increased enzymatic activities associated with cell-wall degradation (Brummell and Harpster, 2001; Giovannoni, 2001; Hiwasa et al., 2003; Manganaris et al., 2006; Nishiyama et al., 2007). Cellular-wall modification-related enzymes, including PG, PME, EGcase, β -galactosidase (β -GAL), EXP, and XET/XTH, have been implicated in fruit softening or deterioration, which suggests that fruit softening or deterioration is a complex system involving several cellular-wall modified genes (Bennett, 2002; Giovannoni, 2001; Harrison et al., 2001; Hiwasa et al., 2003; Kitagawa et al., 1995; Smith and Gross, 2000). The present study provides further evidence for this complexity as indicated by the simultaneous expression of multiple genes of the same family during aril breakdown of longan fruit. The accumulations of three EXP and three XET genes exhibited differential expression patterns which were involved in aril breakdown of longan fruit stored at various temperatures. During storage at room temperature, *DI-XET3* correlated with the initiation of aril breakdown while *DI-EXP3* together with *DI-XET1* and *DI-XET2* were involved in the occurrence of the aril breakdown (Fig. 2). It is noteworthy that the increased expressions of both *DI-XET1* and *DI-XET2* at 4 °C could be mainly attributed to aril breakdown of longan fruit stored at low temperature (Fig. 4). In addition, accumulations of the *DI-EXP2*, *DI-EXP3*, *DI-XET1*, *DI-XET2*, and *DI-XET3* increased in longan fruit during storage at room temperature after 20 d or during storage after 40 d at low temperature. However, an increase in *DI-EXP2* expression corresponded only with initiation of aril breakdown while the increased accumulations of the other four genes were observed after occurrence of aril breakdown. It is suggested that

DI-EXP2 was responsible for the early stage of aril breakdown of longan fruit at room temperature that had been removed from low-temperature storage while *DI-EXP3*, *DI-XET1*, *DI-XET2*, and *DI-XET3* may be involved in the later stage of the aril breakdown (Figs. 7 and 8). Further, expressions of expansin and XET at protein levels correspond well with gene expression during ripening and softening in tomato (Rose et al., 1997), strawberry (Harrison et al., 2001), and peach (Obenland et al., 2003). Thus, these data indicate that *DI-EXPs* and *DI-XETs* are closely related to aril breakdown in longan fruit and that considerable variation exists between expression patterns of individual members of EXP and XET gene families.

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