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# Sucrose-metabolizing enzymes and their genes in the arils of two *Dimocarpus longan* cultivars

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## Abstract

This study aimed to investigate sucrose-metabolizing enzymes and their genes in fruits of two longan (*Dimocarpus longan* Lour) cultivars Cihezhong (CHZ) and Lidongben (LDB). Content of sucrose, glucose, and fructose were measured by high-performance liquid chromatography. The genes of sucrose-metabolizing enzymes were cloned by combining reverse transcription polymerase chain reaction and rapid amplification of cDNA ends, and enzyme activities were analyzed at various points in the fruiting cycle. The total soluble solid (TSS) content of longan arils rose and was positively correlated with sucrose content during maturation and then declined as the fruit senesced. Cihezhong showed a more rapid decrease in sucrose content than LDB. The activities of both sucrose phosphate synthase (SPS) and sucrose synthase (SS) were lower in CHZ, whereas the activities of soluble acid invertase (SAI) and neutral invertase (NI) were higher. The full-length cDNA of the genes of the sucrose-metabolizing enzymes were cloned successfully. The patterns of changes of sucrose synthase-2 (*DlSS-2*), sucrose synthase-3 (*DlSS-3*), and neutral invertase-3 (*DlNI-3*) gene expressions corresponded to those of SS and NI activities. The rate of sucrose decline in the longan fruits was related to sugar receding, sucrose metabolizing enzyme activities, and corresponding gene expressions.

*Additional key words*: gene expression, invertase, longan, sucrose phosphate synthase, sucrose synthase.

## Introduction

Longan (*Dimocarpus longan* Lour.), an important evergreen fruit crop of the *Sapindaceae* family, is mainly cultivated in Southeast Asia (Yonemoto *et al*. 2006). Longan arils accumulate sugars, which comprise between 16 and 25 % of fresh fruit mass (Menzel and Waite 2005), of which sucrose, glucose, and fructose are the major sugars. Content and composition of soluble sugars are key factors determining fruit taste (Itai and Tanahashi 2008). Sucrose is the major product of photosynthesis in higher plants, and it is used for long-distance transport and re-distribution (Ren and Zhang 2013). It is the most dominant metabolite involved in growth and development of fruits (Park *et al*. 2009).

Plants have evolved a sucrose/hexose interchange system for governing sugar metabolism and accumulation in fruit (Nguyen-Quoc and Foyer 2001), and invertase (EC 3.2.1.26), sucrose synthase (E.C.2.4.1.13, SS), and sucrose phosphate synthase (E.C. 2.4.1.14, SPS) are the key enzymes of the processes (Koch 2004). Invertase, an enzyme that catalyzes irreversible cleavage of sucrose into glucose and fructose, has three different isoforms: cell wall acid invertase (CWAI) which is bound to the

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*Abbreviations*: BSA - bovine serum albumin; CHZ - Cihezhong; CWAI - cell wall acid invertase; DAA - days after anthesis; HPLC - high-performance liquid chromatography; LDB - Lidongben; NI - neutral invertase; PVPP - polyvinylpolypyrrolidone; qPCR - quantitative PCR; RACE - rapid amplification of cDNA ends; SAI - soluble acid invertase; SPS - sucrose phosphate synthase; SS - sucrose synthase; TSS - total soluble solid.
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cell walls, soluble acid invertase (SAI) which is found in vacuoles, and neutral invertase (NI), which is found in the cytosol (Pan *et al*. 2006). Sucrose synthase generally catalyzes formation of uridine diphosphate (UDP)-glucose and fructose from sucrose, and the catalytic reaction is reversible (Coleman *et al*. 2009). The same enzyme, in source tissues, is known to play a role in sucrose synthesis by using UDP-glucose and fructose as substrates (Qazi *et al*. 2012). During maturation stages of organ development, the cleavage process predominates King *et al*. 1997, Fernie *et al*. 2002). Sucrose phosphate synthase is the key enzyme in sucrose formation in the source tissues, converting hexoses to sucrose. In tomato, SPS overexpression increases the sink strength of the fruit (Nguyen-Quoc *et al*. 1999).

Longan fruits mature quickly during the hot and rainy summer months (July - September), making the harvest time quite short. After the proper harvest period, the colour of the pericarp turns brown, the pulp becomes fibrous because of rising cellulose content, and sweetness declines. In recent years, the key enzymes in sucrose metabolism and their genes have been studied in different fruits such as grapes (Xie *et al*. 2009), peaches (Zhang *et al*. 2013), pineapples (Zhang *et al*. 2010), apples (Li *et al*. 2012), and lychees (Yang *et al*. 2013). Studies of sucrose-metabolizing enzymes and their genes in longan fruits are rare. Therefore, we evaluated changes in sugar content at varying stages during fruit maturation and aging in two longan cultivars. At the same time points, expression of several genes encoding the key enzymes involved in sugar metabolism and activities of the corresponding enzymes were analyzed.

## Materials and methods

Longan (*Dimocarpus longan* Lour.) fruits were provided by the experimental orchard of the Fruit Tree Research Institute at the Guangdong Academy of Agricultural Science, Guangzhou, China. Standard horticultural practices were applied for pest and disease control. Fruits of the cultivars Cihezhong (CHZ) and Lidongben (LDB) were used for this study. Harvested fruit samples were taken to the College of Horticulture, the South China Agricultural University, where arils were separated, frozen in liquid nitrogen, and stored at -80 °C. The sampling dates of CHZ were 93, 101, 109, 117, 125, and 133 days after anthesis (DAA), and the sampling dates of LDB were 115, 125, 134, 143, 151, and 160 DAA. The commercial dates of CHZ and LDB were 101 and 125 DAA, respectively.

The total soluble solid (TSS) content was determined with a *PR-32α* digital handheld refractometer (*ATAGO*, Tokyo, Japan) in juice of fresh tissue.

Sucrose, glucose, and fructose were extracted and their content was determined by high-performance liquid chromatography (HPLC) according to Yang *et al*. (2013) with slight modifications. Frozen longan aril tissue (1 g) was heated in a microwave oven for 45 s to deactivate enzymes and homogenized in a mortar with 5 $cm^3$ of distilled water, then centrifuged at room temperature (4 000 *g*, 10 min). The precipitate was washed with distilled water and re-centrifuged. We combined the supernatants and added distilled water to make a final volume of 10 $cm^3$. Two $cm^3$ of this extract was centrifuged at room temperature (12 000 *g*, 15 min). The supernatant was passed through a *Sep-Pak C18* cartridge (*Waters Corporation*, Milford, MA, USA). An *Agilent 1200* HPLC system (*Agilent Technologies*, Waldbronn, Germany) coupled with a *G1362A* refraction index detector cell and a transgenomic *CARB Sep Coregel 87C* column with a guard column cartridge (*CARB Sep Coregel 87C*) were used for measuring sugar content. The temperature of the column oven was set to 80 °C, and the injection volume was 0.01 $cm^3$. The column was eluted at a rate of 0.6 $cm^3$ $min^{-1}$, and sugar content in each eluate was determines according to standard solution calibrations.

All enzymes were extracted at 4 °C using a method previously described (Zhu *et al*. 1997) with some modifications: 1 g of the frozen longan aril tissue was homogenized in 1.5 $cm^3$ of a buffer composed of 100 mM Hepes-NaOH (pH 7.5) containing 5 mM $MgCl_2$, 2.5 mM dithiothreitol, 1 mM $Na_2EDTA$, 10 % (v/v) glycerol, 1 % (v/v) *Triton X-100*, 0.5 % (m/v) bovine serum albumin (BSA), and 5 % (m/v) polyvinylpolypyrrolidone (PVPP). Then, the homogenate was centrifuged (13 000 *g*, 4 °C, 20 min), and 1 $cm^3$ of the supernatant was desalted in a *Sephadex G25 PD-10* column and equilibrated with 25 mM Hepes-NaOH (pH 7.5) containing 30 % glycerol, 5 mM $MgCl_2$, and 1 mM $Na_2EDTA$ to a final volume of 2 $cm^3$. These crude extracts were used for determining SAI, NI, SS, and SPS activities.

For CWAI, 1 g of the frozen tissue was homogenized in 2 $cm^3$ of buffer A composed of 200 mM Hepes-KOH (pH 8.0) containing 5 mM $MgCl_2$, 2 mM benzamidine, 2 mM $Na_2EDTA$, 2.5 mM dithiothreitol, 0.1 % (m/v) BSA, 0.1 mM leupeptin, 1 % (v/v) *Triton X-100*, 10 % (v/v) glycerol, and 4 % (m/v) PVPP. Then, the homogenate was centrifuged (13 000 *g*, 4 °C, 20 min) and the precipitate washed three times with buffer B composed of 50 mM Hepes-KOH (pH 7.4), containing 5 mM $MgCl_2$, 2 mM benzamidine, 2 mM $Na_2EDTA$, 0.1 mM leupeptin, 10 % glycerol, and 0.1 % BSA. The precipitate was soaked at 4 °C overnight with buffer C composed of 200 mM Hepes-KOH (pH 8.0) containing 5 mM $MgCl_2$, 2 mM $Na_2EDTA$, 2.5 mM dithiothreitol, 2 mM benzamidine, 0.1 % BSA, 1 % *Triton X-100*, 10 % glycerol, 0.1 mM leupeptin, and 1 mM NaCl. The extract was centrifuged (13 000 *g*, 4 °C, 20 min) and then

desalted.

Neutral invertase was measured in a reaction mixture (1 $cm^3$) containing 100 mM Hepes-NaOH (pH 7.5), 1 % (m/v) sucrose, and 0.2 $cm^3$ of the desalted extract. This was incubated at 34 °C for 1 h, and the reaction was stopped with 1.5 $cm^3$ of 3,5-dinitrosalicylic acid (Zrenner *et al*. 1995). Absorbance was determined at 540 nm and compared to glucose standards.

For SAI and CWAI activities, a reaction mixture was identical to that for NI except that a 100 mM citrate buffer (pH 5.0) was used to replace the Hepes-NaOH (pH 7.5) (Zrenner *et al*. 1995). Absorbance was determined at 540 nm and compared to glucose standards.

Sucrose synthase activity was assayed according to Koch (2004). A reaction mixture (0.2 $cm^3$) contained 0.1 $cm^3$ of the desalted extract, an 80 mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer (pH 5.5), 5 mM UDP, 100 mM sucrose, and 5 mM NaF. This was incubated at 30 °C for 30 min, and the reaction was stopped with 1.5 $cm^3$ of 3,5-dinitrosalicylic acid. Absorbance was determined at 620 nm and compared to sucrose standards.

Sucrose phosphate synthase was measured according to Xie and Li *et al*. (2009). A reaction mixture (0.2 $cm^3$) was as follows: 0.100 $cm^3$ of the desalted extract, a 100 mM Hepes-NaOH buffer (pH 7.5), 15 mM $MgCl_2$, 5 mM fructose-6-phosphate, 1 mM $Na_2EDTA$, 10 mM UDP-glucose, and 15 mM glucose-6-phosphate. The mixture was incubated at 34 °C for 30 min and the reaction stopped with 0.2 $cm^3$ of 30 % (m/v) KOH. The tubes were boiled for 10 min and then allowed to cool. Then, 3.5 $cm^3$ of a mixture of anthrone (0.15 %, m/v) in 100 $cm^3$ of 80 % (m/v) $H_2SO_4$ was added to the tube, and the tube was incubated at 40 °C for 20 min. After cooling, absorbance was determined at 620 nm and compared to sucrose standards. All activities were determined using a spectrophotometer *UV-2600* (*Shimadzu*, Kyoto, Japan)

The total RNA was extracted from the frozen longan arils using an *RNAOUT* kit (*Huayueyang*, Beijing, China), and the genomic DNA was cleared using an RNase-free *DNaseI* set (*TaKaRa*, Tokyo, Japan). The first-strand cDNA was synthesized using a *SuperScript III* reverse transcriptase kit (*Invitrogen*, Carlsbad, CA, USA), in order to obtain partial sequences of genes related to sugar metabolism. Specific and degenerate primers were designed using *DNAMAN 6.0* by comparing their conserved amino acid regions with the sequences of sugar metabolism-related genes in other species, listed in *NCBI* (http://www.ncbi.nlm.nih.gov/), such as *Citrus sinensis, Populus trichocarpa,* and *Vitis vinifera* (Table 1 Suppl.). We used the following procedure for PCR: 94 °C for 5 min, 30 cycles at 94 °C for 30 s, 50 - 60 °C for 30 s, and 72 °C for 10 min. The PCR products were cloned into the pMD19-T vector (*TaKaRa*) and sequenced. For 3' and 5' ends of the sequence, we used a *3' rapid amplification of cDNA ends* (*RACE*) kit (*TaKaRa*) and a *Clontech* (Mountain View, CA, USA) *Smart* kit according to the sequenced segment design-specific primers. We then obtained the full-length sequences of *SAI*, *CWAI*, *NI-1*, *NI-2*, *NI-3*, *SS-1*, *SS-2*, *SS-3*, and *SPS*, which were named *DlSAI*, *DlCWAI*, *etc.*

Real-time quantitative PCR (qPCR) was performed using a *Lightcycler 480* real-time system (*Roche*, Basel, Switzerland) and a *SYBR Green I Master* mix (*Roche*). Every 0.02 $cm^3$ of a reaction mixture contained 0.006 $cm^3$ of sterilized distilled water, 0.01 $cm^3$ of a *SYBR Green I Master* mix, 0.001 $cm^3$ of each primer (to achieve a final concentration of 0.5 μM), and 0.002 $cm^3$ of a cDNA template. The real-time qPCR procedure was as follows: preincubation at 95 °C for 5 min followed by 40 cycles at 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 20 s. A melting curve was determined after each reaction. Each assay was replicated three times. Real-time qPCR primers of the sugar metabolism-related genes were designed using *BatchPrimer3* (You *et al*. 2008, Table 2 Suppl.). *Actin* and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) were used as reference genes to normalize expression levels of the target genes across different samples (Lin and Lai 2010, Zhong *et al*. 2011). The relative expression of the target genes were finally calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

All statistical analyses were carried out using *Excel 2013* and the *SPSS v. 19.0* statistical software package. *Origin 8.5* was used for diagraph analysis. Data are expressed as means ± standard deviations (SDs). $P < 0.05$ was considered to be statistically significant.

## Results

During maturation and aging the fruits on the tree, their TSS content tended to rise initially and then decline in proportion with the delay of harvest (Fig. 1). A change in content of TSS in the LDB longan aril was smaller (20.94 - 18.33 %) than in the CHZ longan aril (20.58 - 12.33 %).

In this study, the amount of sucrose was the highest among all of the sugars for both cultivars (Fig. 2). Firstly, it tended to increase and then to decrease as the fruit became overly ripe. Sucrose content in CHZ decreased more compared with LDB after maturity (Fig. 2). Additionally, TSS content was positively correlated with the total sugar and sucrose content ($r^2$ = 0.849** and 0.851**, respectively; Table 1). We also found that hexoses (the sum of glucose and fructose) content continuously increased up to the end of fruit aging for both cultivars.

Activities of NI, SAI, and SS in the CHZ longan aril

rose to a peak at 117 DAA and then declined (Fig. 3*A*,*B*,*D*). In LDB, activity of SS increased slowly and protein content increased constantly (Fig. 3*D*,*F*). Activities of SPS and SS were lower in CHZ compared with LDB (Fig. 3*D*,*E*), whereas SAI activity was higher in CHZ. Activity of SPS in the arils of the two cultivars showed opposite patterns of change (Fig. 3*E*). Activity of CWAI in both CHZ and LDB arils was relatively constant (Fig. 3*C*). All of those above mentioned results show that activities of the sycrose metabolizing enzymes in LDB and CHZ were different during fruit development.

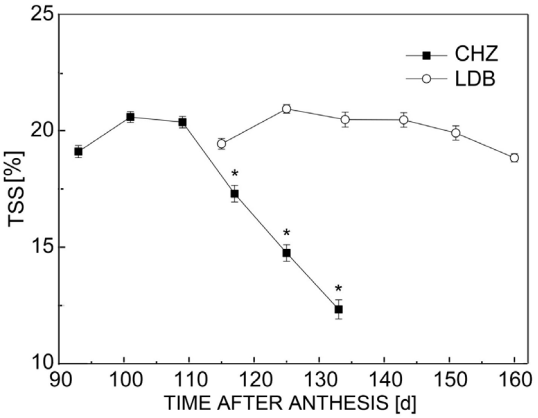


Fig. 1. Changes in the total soluble solid (TSS) content in the arils of two longan cultivars during fruit maturation and aging on trees. Means ± SEs, $n$ = 3, * - significant differences at $P < 0.01$ *versus* day 93. The maturation times of the 2 cultivars were 101 and 125 d for Cihezhong (CHZ) and Lidongben (LDB), respectively.

Real-time qPCR and RACE were performed to get the full-length cDNAs of *SAI*, *CWAI*, three members of *NI*, three members of *SS*, and *SPS* genes from the longan arils, which were named *DlSAI*, *DlCWAI*, *DlNI-1*, *DlNI-2*, *DlNI-3*, *DlSS-1*, *DlSS-2*, *DlSS-3,* and *DlSPS*, respectively (Table 2). The characteristics of the obtained sequences were compared with known sequences from other species using the *NCBI BLAST* server. The expression of the sugar metabolism genes showed different trends in the arils of the two cultivars. On the whole, the relative expressions of *DlN-3, DlSPS, DlSS-2, DlSS-3*, and *DlSAI* in CHZ were higher than in LDB. In CHZ, the relative expressions of *DlNI-3, DlSPS*, and *DlSS-2* increased until 117 DAA and then declined (Fig. 4*C*,*D*,*G*). *DlSS-3* also increased dramatically until 117 DAA, but *DlSS-1* decreased throughout the sampling period (Fig 4*F*,*H*). In LDB, *DlN-1, DlNI-3*, *DlCWAI,* and *DlSS-1*, the expressions decreased significantly during fruit maturation and aging (Fig. 4*A*,*C*,*E*,*F*). The expressions of *DlSS-2* and *DlSS-3* in LDB showed no evident changes (Fig. 4*G*,*H*). *DlNI-2* in both cultivars showed no evident changes (Fig 4*B*,*E*).

Table 1. Correlation coefficients between the total soluble solid (TSS) and sugar content (mean values of two longan cultivars). *, ** - significant at 0.05 and 0.01 levels, respectively.

| | TSS | Sucrose | Glucose | Fructose | Total sugars | Hexoses | Soluble protein |
|---|---|---|---|---|---|---|---|
| TSS | 1 | | | | | | |
| Sucrose | 0.851** | 1 | | | | | |
| Glucose | 0.639* | 0.206 | 1 | | | | |
| Fructose | -0.056 | -0.042 | 0.003 | 1 | | | |
| Total sugars | 0.849** | 0.950** | 0.291 | 0.254 | 1 | | |
| Hexose | 0.186 | 0.082 | 0.305 | 0.947** | 0.387 | 1 | |
| Soluble protein | 0.033 | -0.318 | 0.610* | 0.311 | -0.140 | 0.462 | 1 |

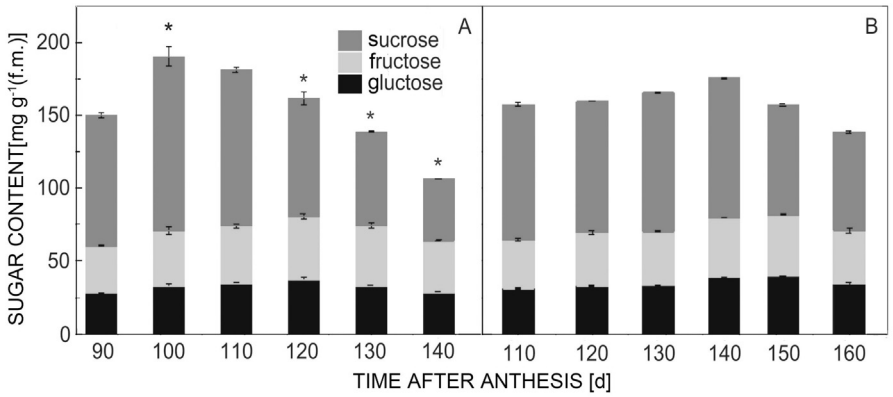


Fig. 2. Changes in sucrose, glucose, fructose, and the total sugar content in the arils of two longan cultivars Cihezhong (*A*) and Lidongben (*B*) during fruit maturation and aging on trees. Means ± SEs, $n$ = 3, * - significant differences at $P < 0.01$ *versus* day 90.

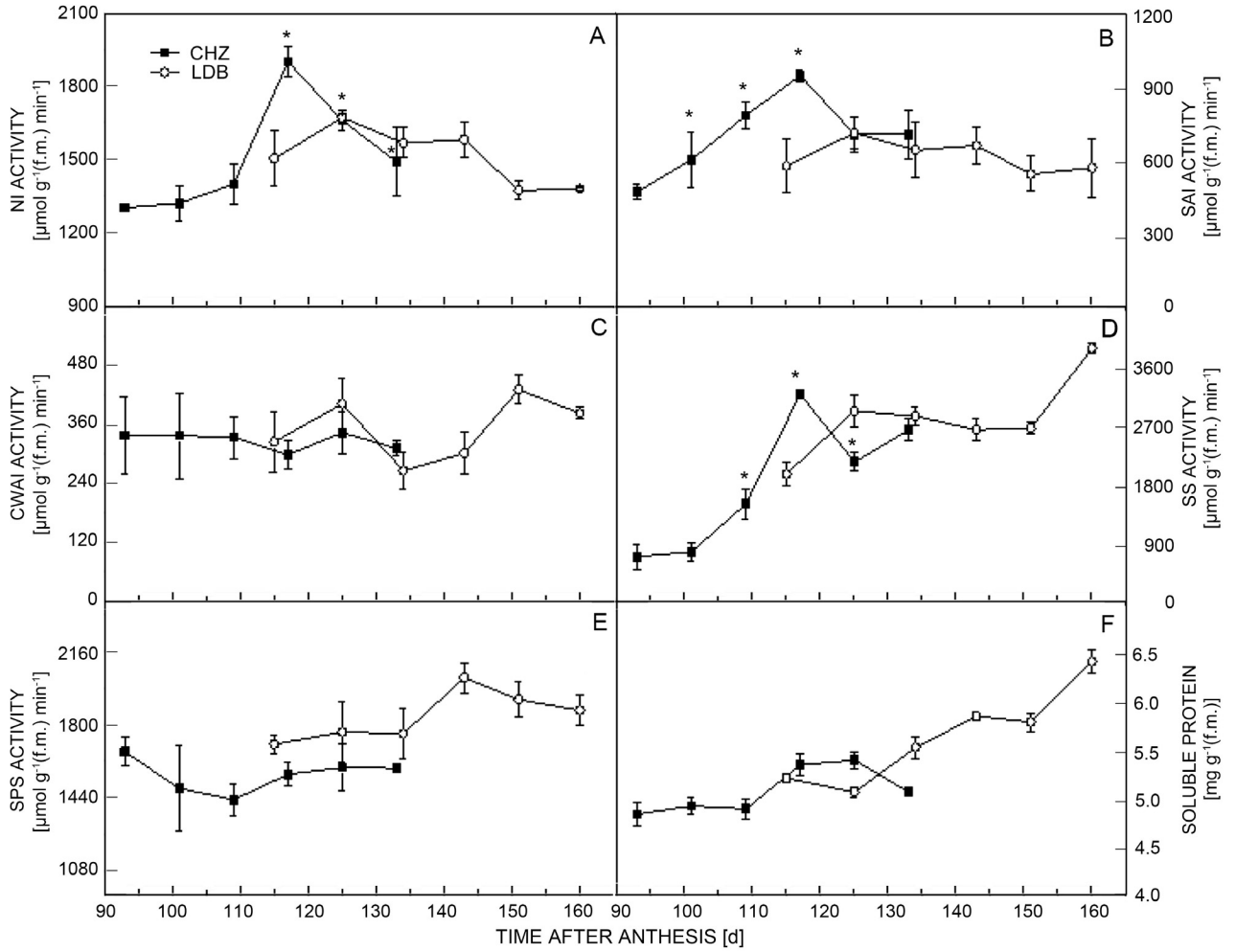


Fig. 3. Changes in activities of neutral invertase (NI, *A*), soluble acid invertase (SAI, *B*), cell wall acid invertase (CWAI, *C*), - sucrose synthase (SS, *D*), sucrose phosphate synthase (SPS, *E*), and in content of soluble protein (*F*) in the arils of two longan cultivars Cihezhong and Lidongben during fruit maturation and aging on trees. Means ± SEs, $n = 3$, * - significant differences at $P < 0.01$ *versus* day 93.

## Discussion

The accumulation of sugars is one of the main features of ripening process in longan fruits, and it is very important for longan growers (Hu *et al*. 2006). In practice, TSS is index for judging the maturity of longan fruits (Beckles 2012). In this study, TSS content of the longan arils rose during maturation and then declined as fruit senesced in later stages. Additionally, the change of sucrose content played an important role in TSS content. Sucrose content increased reaching a peak and then dropped gradually. Meanwhile, the total sugar content began to fall, and the colour of the pericarp began to darken. Successively, the pulp turned fibrous, and pulp cellulose content increased. Finally, sweetness declined. Collectively, these phenomena are known as sugar receding.

The different longan cultivars varied in their abilities to retain their high sugar content after sucrose reached its peak value. In this study, LDB had a slower sugar receding rate than CHZ. When TSS content reached its peak value, sucrose content was also at its maximum value in the CHZ fruit, which suggests that TSS might be a reliable and feasible index to guide harvesting in practice. Sucrose and TSS content then dropped rapidly, but glucose and fructose content still rose. These results suggest that the inversion of sucrose into glucose and fructose was faster than sucrose synthesis after maturation in CHZ (Fig 2). The LDB cultivar, however, showed a different pattern of change; content of TSS peaked at 128 DAA and then declined. During the sugar receding stage, sucrose content remained relatively stable, whereas glucose and fructose content continued increasing, which suggests that sucrose degradation and synthesis were at equilibrium (Fig. 2).

Previous studies have shown that differences in sucrose content change are closely related to activity of sucrose-metabolizing enzymes. Li *et al.* (2012) discovered that starch breakdown and up-regulation of sucrose synthesis through SPS play an important role in the increase of the total soluble sugars at maturity. A positive correlation between SPS activity and sucrose accumulation has been shown in sugar cane (Botha and Black 2000), tomatoes (Dali *et al*. 1992), and melons (Hubbard *et al*. 1989). Cihezhong showed a faster decrease in sucrose content than LDB. Soluble protein content and activities of SPS and SS were lower in CHZ, whereas activities of both SAI and NI were higher (Fig. 3), which is likely due to the fact that the sucrose synthesis ability of CHZ was lower than of LDB after the maturation period, while the degradation rate gradually increased with aging (Fig. 3). By 117 DAA, both glucose and fructose content and activities of NI, SS, and SAI reached a peak in CHZ, which is consistent with the patterns of change of the two kinds of monosaccharides. Lidongben maintained relatively high SPS and SS activities until the end of the experiment when the enzyme activities and content of the two hexoses declined rapidly, whereas activities of NI and SAI were low and continued to decrease gradually.

Analysis of a relationship between enzyme activities, their gene expression, and sugar metabolism helps clarify possible functions of these genes. The genes of sugar-metabolizing enzymes have been isolated from grapes (Xie *et al*. 2009), peaches (Zhang *et al*. 2013), pineapples (Zhang *et al*. 2010), apples (Li *et al*. 2012), and lychees (Yang *et al*. 2013). Li *et al*. (2012) suggested that both *MdSPS5* and *MdSPS6* may contribute significantly to elevating SPS activity for sucrose accumulation towards fruit maturity in apples. Itai and Tanahashi (2008) showed that an accumulation of hexose and a decrease in sucrose in Japanese pear during cold storage might be due to up-regulation of soluble acid invertase (*PpAIV1*) and down-regulation of sucrose phosphate synthase (*PpSPS1*) expression. Although the genes of sucrose-metabolizing enzymes have not been previously isolated from the longan aril, members of various gene families that encode key enzymes in sucrose metabolism have been isolated from *Arabidopsis* (Bieniawska *et al*. 2007) and different

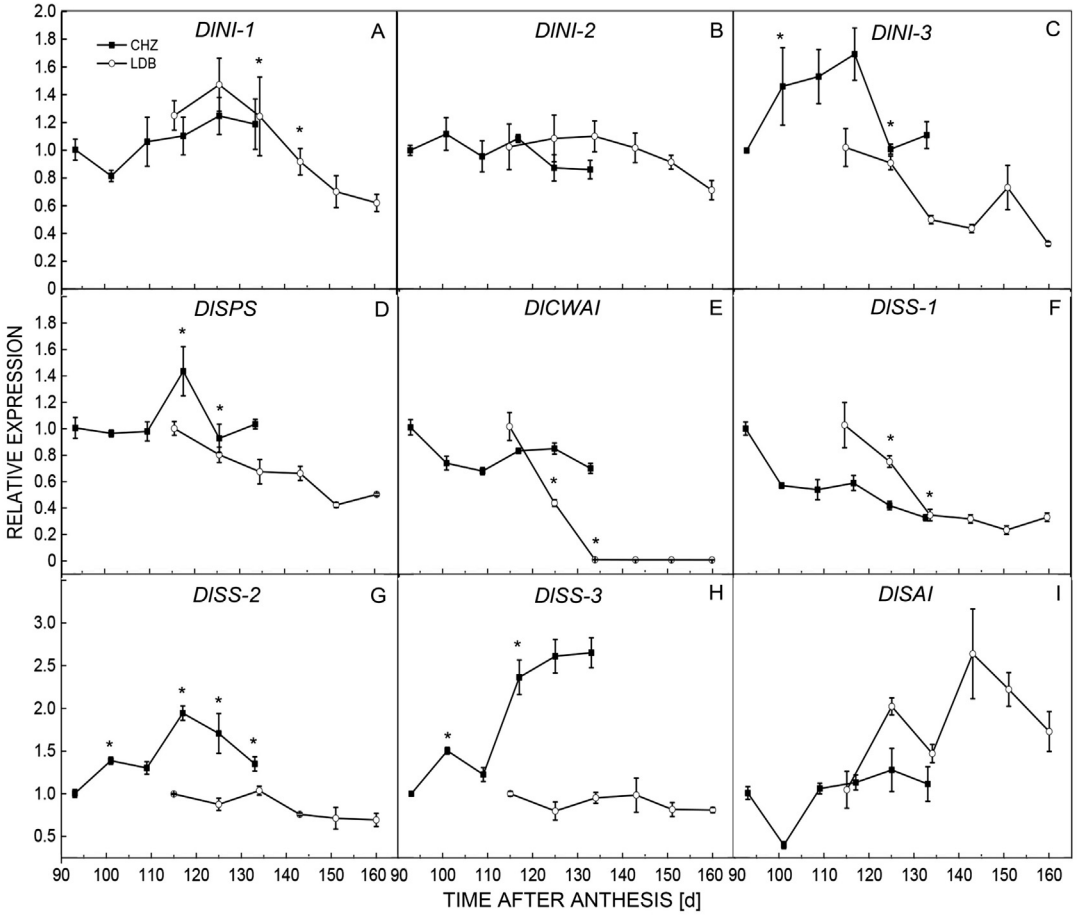


Fig. 4. Changes in expression of *Dimocarpus longan neutral invertase 1* (*DlNI-1*, *A*), *DlNI-2* (*B*), *DlNI-3* (*C*), *sucrose phosphate synthase* (*DlSPS*, *D*), *cell wall acid invertase* (*DlCWAI*, *E*), *sucrose synthase 1* (*DlSS-1*, *F*), *DlSS-2* (*G*), *DlSS-3* (*H*), and *DlSAI* (*I*) in the arils of two cultivars during fruit maturation and aging on trees. Means ± SEs, $n = 3$, * - significant differences at $P < 0.01$ *versus* day 93.

Table 2. Basic information and homologies based on the nucleotide sequences of genes for sucrose metabolism isolated from longan. ORF - open reading frame; UTR - untranslated region.

| Name of genes | Length [bp] | Genbank number | ORF length | 5' UTR [bp] | 3' UTR [bp] | Number of amino acid | *Top BLAST* match | Homology [%] |
|---|---|---|---|---|---|---|---|---|
| *DlCWAI* | 2064 | KP769771 | 1752 | 136 | 176 | 583 | XM_006465966.1 *Citrus sinensis* | 78 |
| *DlSAI* | 2273 | KP769772 | 1971 | 120 | 182 | 656 | AB074885.1 *Citrus unshiu* | 80 |
| *DlNI-1* | 2090 | KP769773 | 1770 | 96 | 224 | 589 | XM_006471320.1 *Citrus sinensis* | 88 |
| *DlNI-2* | 2558 | KP769774 | 2130 | 113 | 315 | 709 | XM_006472173.1 *Citrus sinensis* | 81 |
| *DlNI-3* | 2444 | KP769775 | 2037 | 259 | 148 | 678 | XM_006488730.1 *Citrus sinensis* | 84 |
| *DlSS-1* | 2738 | KP769776 | 2418 | 96 | 224 | 805 | KF694989.1 *Citrus uavissima* | 87 |
| *DlSS-2* | 2719 | KP769777 | 2436 | 100 | 183 | 811 | JQ773415.1 *Litchi chinensis* | 98 |
| *DlSS-3* | 2917 | KP769778 | 2436 | 239 | 242 | 811 | XM_006488970.1 *Citrus sinensis* | 85 |
| *DlSPS* | 3504 | KP769779 | 3174 | 69 | 271 | 1057 | JQ773416.1 *Litchi chinensis* | 97 |

fruit trees. For example, nine genes encoding invertase have been identified in apples, including three *CWINVs*, three *NINVs*, and three *AINVs* (Li *et al*. 2012). In this study, we cloned just one member of the *SPS*, *SAI*, and *CWAI* genes termed *DlSPS*, *DlSAI,* and *DlCWAI*, respectively, and three *NI* and *SS* gene members named *DlNI-1*, *DlNI-2*, and *DlNI-3*; and *DlSS-1*, *DlSS-2*, and *DlSS-3,* respectively. There were obvious differences in expressions of these genes between the two cultivars during the maturation and aging periods (Fig. 4). Expressions of *DlSS-2* and *DlSS-3* of CHZ showed marked increases, compared with those of LDB, corresponding to the change in SS activity. The latter suggests that SS contributed significantly to sucrose degradation in CHZ during the maturation and aging periods. Furthermore, the patterns of change in *DlNI-3* expression were similar to those in NI activity, which suggests that the *DlNI-3* gene might play an important role in sucrose degradation in longan flesh. These co-regulate activities of several key enzymes of sugar metabolism and lead to the sugar receding phenomenon in longan flesh. In summary, activities of NI and SAI in the longan fruits increased resulting in the increased accumulation of sucrose and regulating sink-source relation during this developmental stage. However, when sugar content reached a threshold value, activities of NI and SAI and expression of *DlCWAI* and *DlSPS* showed a downward trend.

In conclusion, TSS can be used as a reliable index to guide harvesting in practice. Decrease in sucrose may be an important factor to sugar receding in longan fruits. Cihezhong had a faster sucrose degradation rate than LDB. The high SAI activity and low SPS and SS activities in the CHZ pulp were the direct cause of the more rapid sucrose degradation rate in the cultivar. The observed differences were evidenced by the differences in expression of genes, especially *DlSS-2*, *DlSS-3*, and *DlNI*-3.

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