



Gene Expression and Activity of Enzymes Involved in Sugar Metabolism and Accumulation During “Huangguan” and “Yali” Pear Fruit Development

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Abstract

Since the carbohydrate content affects pear flavor during the process of growth, it is necessary to determine the sugar components that accumulate in the fruit. We analyzed the fruit carbohydrate content, and the gene expression and activity of acid invertase (AI), neutral invertase (NI), sucrose synthase (SS), and sucrose phosphate synthase (SPS) during the development of “Huangguan” and “Yali” pears. The results demonstrate that during development, the fruit sugar metabolism of the “Huangguan” pear follows a typical sorbitol–starch–soluble sugars middle model, whereas the “Yali” pear fruit follows a typical sorbitol–sucrose–starch–soluble sugars middle model. In the “Huangguan” pear, we found the AI and NI gene expressions, as well as AI ($P < 0.05$) and NI ($P < 0.01$) enzyme activities, to be positively correlated, whereas we found the NI gene expression and NI enzyme activity of “Yali” pear to be negatively correlated ($P < 0.01$). We observed the high levels of late-stage AI and early-stage SS during development to roughly correspond with the gene expression found in the late and early stages, respectively, suggesting their potential regulatory roles in “Huangguan” pear fruit development. Our results indicate that the primary function of SPS during the early developmental stage is to accumulate sucrose, whereas the primary function of AI is to promote hexose accumulation during the late developmental stage of mature “Yali” pear fruit.

Keywords “Huangguan” pear · “Yali” pear · Sugar metabolism · Enzyme activity · Gene expression

Introduction

The amount and types of sugars in fresh fruit directly influence its quality and flavor, so it is necessary to determine the sugar components that accumulate in fruit. During development, fruits accumulate carbohydrates, generally in the form of starch, sucrose, or hexose sugars. In the family *Rosaceae*, fruits synthesize sorbitol and glucose, and both sorbitol and sucrose are translocated and utilized in the fruit. The composition of stored sugar in several plant species has been associated with the key enzymes, which are responsible for sucrose metabolism, and the relationships between these compounds and fruit ripening have been extensively studied in various fruits, such as peach, jackfruit (*Artocarpus*

heterophyllus Lam.), Feizixiao and Heiye litchis (*Litchi chinensis* Sonn.), citrus fruit, strawberry and banana [1–6].

Sucrose metabolism enzymes, including invertase (EC 3.2.1.26), sucrose synthase (SS, EC 2.4.1.13) and sucrose phosphate synthase (SPS, EC 2.4.1.14), are key determinants of fruit sugar accumulation and composition. Soluble acid invertase (AI, EC 3.2.1.26) and soluble neutral invertase (NI, EC 3.2.1.26) are located in the cytoplasm and vacuoles. Soluble invertases control the amount of soluble sugars in plant tissues and determine the soluble carbohydrate composition in sink organs, as well as during drought stress, hypoxia, and gravitropism [7]. Manning and Maw [8] reported that an increase of AI activity during fruit development was directly correlated with the hexose content of the mature fruit, the loss of which seems to be a prerequisite for sucrose accumulation. By contrast, the physiological roles of NI remain elusive with only limited and fragmentary information available, even though a slight transcriptional upregulation has been reported to occur in developing organs. This is mainly due to low levels of enzyme activity and gene expression, which are generally steady-state [7, 9, 10]. The unloading

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and utilization of sucrose depend on its cleavage into hexoses, and this process is catalyzed by SS and/or invertase [11]. SS and SPS play more important roles than invertase in sucrose accumulation [12]. The genes of sucrose metabolism enzymes have been isolated from tomato (*Lycopersicon*) (Sturm, 1999), banana, citrus, and peach, the expression patterns of which have also been investigated in relation to sugar accumulation [1, 4, 6, 13].

“Huangguan” and “Yali” pears are routinely planted in China and are valued for their unique fragrance, subtle aroma, sweetness and crispness. The “Yali” pear (*Pyrus bretschneideri* Reld), which belongs to the super-white pear group and has been grown in China for more than 2000 years, matures in middle or late September. The “Huangguan” pear, which matures in early August, is a new white pear cultivar developed from an interspecies crossing of the “Xuehua” pear (*Pyrus bretschneideri* L. cv. Xuehua) and the “New Century” pear, a new variety of *Pyrus pyrifolia* Nakai (*Pyrus pyrifolia* Nakai cv. Shinseiki).

Our understanding of the mechanisms controlling sugar metabolism and accumulation in “Huangguan” and “Yali” pears is as-yet quite limited. Data on genes, as well as the relationships between genes, enzymes, and sugars are scant. In this study, we investigated the accumulation of carbohydrates (starch, sorbitol, sucrose, fructose and glucose), enzyme activities of AI, NI, SS and SPS, and gene expression in “Huangguan” and “Yali” pears during various fruit development stages to elucidate the relationships between sugar metabolism and gene expression. In addition, we identified the key enzymes responsible for imported sugar allocation.

Materials and Methods

Plant Materials

We collected “Huangguan” and “Yali” pear samples randomly from the “Shenyuwang” orchard in Gaocheng city, Hebei Province, China, 18 days after full bloom and at an interval of 15 days thereafter until harvest. For this experiment, we selected about 100 trees for each cultivar, one or two samples were taken from each tree. After weighing and measuring the samples, we immediately cut the collected fruit (flesh) into small pieces, immersed the pieces in liquid nitrogen and stored them at -80°C . We repeated all experiments of index determination three times.

Fruit Mass and Form Index

We weighed ten fruits randomly selected from each cultivar on an electronic balance and calculated the average mass.

We measured the transverse and longitudinal diameters of the fruits with a vernier caliper.

Determination of Carbohydrate Content (Sorbitol, Starch, Sucrose, Fructose and Glucose)

With minor adjustments to the chromatographic conditions, we used high-performance liquid chromatography (HPLC), as reported by Lopez-Gomez and Gomez-Lim [13], to determine the fructose, glucose, sucrose and sorbitol contents. We pulverized the flesh (4 g) in a mortar with liquid N_2 , diluted to 20 mL with ultrapure water, and filtered it three times through a $0.45\text{-}\mu\text{m}$ Millipore filter, then utilized an Agilent 1200 apparatus (Agilent 1200, USA) with a quaternary pump, a refractive index detector, and an injection valve fitted with a $20\text{-}\mu\text{L}$ loop. The mobile phase consisted of a solvent system of acetonitrile–water (7:3, v/v) at a flow rate of 1.0 mL/min at 25°C . We studied the linear range with solutions derived by sequentially diluting a standard stock solution. We determined the repeatability of the method by taking three injections from the same sample and calculating the relative standard deviation (RSD) of each analyzed content. We determined the starch content by the Official Standard Method AOAC 996.11 (1996), using the total starch assay kit supplied by Megazyme International Ireland Ltd. (Bray Business Park, Co. Wicklow, Republic of Ireland).

Assays of the AI, NI, SPS and SS Enzymes

Using a modified version of the method in Nielsen et al. [14], first, we added 1 g of each freeze-dried sample to a 5-mL extraction buffer containing 300 mmol/L HEPES–NaOH buffer (pH 7.5), 10 mmol/L MgCl_2 , 1 mmol/L ethylenediaminetetraacetic acid, 0.1% (w/v) bovine serum albumin, 2.5 mmol/L dithiothreitol, 0.5% (w/v) polyvinylpyrrolidone, and 10 mmol/L vitamin C. Next, we clarified the extract by centrifugation ($15000g$, 15 min, 4°C) for immediate use in the enzyme assays. For AI, we incubated $200\text{ }\mu\text{L}$ of the extract and $800\text{ }\mu\text{L}$ of the reaction mixture containing 0.1 mol/L acetate buffer (pH 5.5) and 10 g/L sucrose for 30 min at 37°C , and then boiled the mixture for 10 min. After cooling, we added 0.5 mL 3,5-dinitrosalicylic acid reagent containing 6.3 g 3,5-dinitrosalicylic acid and 185 g K/Na-tartrate (w/v) dissolved in 2 mol/L NaOH and boiled the mixture again for 10 min. We measured the color development at 510 nm. We assayed NI as above, using a different reaction mixture (10 g/L sucrose, 0.1 mol/L phosphate buffer (pH 7.5), 5 mmol/L MgCl_2 , and 1 mmol/L ethylenediaminetetraacetic acid). We used the anthrone sulfuric acid method to assay SS and SPS. For SS, we incubated $50\text{ }\mu\text{L}$ of extract and $50\text{ }\mu\text{L}$ of reaction mixture containing 4 mmol/L uridine diphosphate glucose, 0.06 mol/L fructose, 15 mmol/L MgCl_2 , and 0.1 mol/L phosphate buffer (pH 8.0)

for 30 min at 37 °C, and terminated the reactions by boiling the samples for 10 min. After cooling, we quantified the reaction mixture to 1 mL, added 5 mL cooled anthracenone reagent (0.14 g anthracenone with 100 mL 30% H₂SO₄), boiled the mixture again for 10 min, rapidly cooled the samples in ice water, and placed them in the dark for 20 min. We measured the color development at 630 nm. We assayed SPS as above, using a different reaction mixture containing 4 mmol/L uridine diphosphate glucose, 0.06 mol/L fructose-6P, 15 mmol/L MgCl₂, and 0.1 mol/L phosphate buffer (pH 8.0). As a blank control for all the enzymes used in the experiment, we boiled the isometric liquid of the enzyme for 10 min. We express the enzyme activity as the micromoles of glucose produced for AI and NI, and as the sucrose or sucrose-P produced for SS or SPS per min per milligram of sample. We performed all experiments in triplicates ($n = 3$) in the temperature range 0–4 °C.

Expression of AI, NI, SS and SPS

We extracted the total RNA from the samples using a Column Plant RNAOUT Kit (Invitrogen, Beijing, China), examined the quality of the RNA solution on an agarose gel, and quantified it using a Nanodrop 2000 spectrophotometer. We used approximately 0.3 µg of RNA to make standard cDNA with a cDNA Synthesis SuperMix Kit (TransGen Biotech Co., Ltd., Beijing, China). Table 1 shows the primers we used in the real-time polymerase chain reaction (PCR) analysis and their products. We designed the primers using Primer 5.0 software and determined their specificity by reverse transcription polymerase chain reaction (RT-PCR) and melting curve analysis.

We used the real-time quantitative polymerase chain reaction (qPCR) method to analyze the expressions of the AI, NI, SS and SPS genes. We used *Actin* (CN938023) as an internal constitutively expressed control (housekeeping gene). For each sample, we extracted and mixed the total RNA from the respective three replicates in tubes for reverse transcription. We performed the qRT-PCR experiments for three technical replicates, following the work of Mbégué-A-Mbégué et al. [15]. Using specific primers, we followed a typical running profile of 95 °C for 5 min, 40 cycles at 95 °C for 5 s, 52 °C (NI) or 58 °C (AI, SS, SPS) for 10 s and 72 °C for 10 s. To ensure the purity of the amplified products, we generated a melting curve for each sample at the end of each run. Using

the gene-specific primers, a single correctly sized product was amplified in each assay with high PCR efficiency (90–110%). We used the $2^{-\Delta\Delta CT}$ formula in [16] to determine the relative fold differences of the genes. The reported values represent the average of three biological replicates.

Statistical Analysis

We produced three replicates of each sample ($n = 3$) and expressed the results as mean \pm SD. We made statistical comparisons via one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. We considered differences to be significant at $P < 0.05$. We analyzed correlations using SPSS 20.0.

Results

Fruit Quality Parameters

As shown in Fig. 1a, fruit growth was not constant during the season. The mass of the “Huangguan” pear fruit started to increase rapidly 33 days after full bloom (DAFB), whereas the mass of the “Yali” pear fruit increased continuously after 48 DAFB. Moreover, the masses of the “Huangguan” pear fruit were greater than those of the “Yali” pear fruit over the entire period.

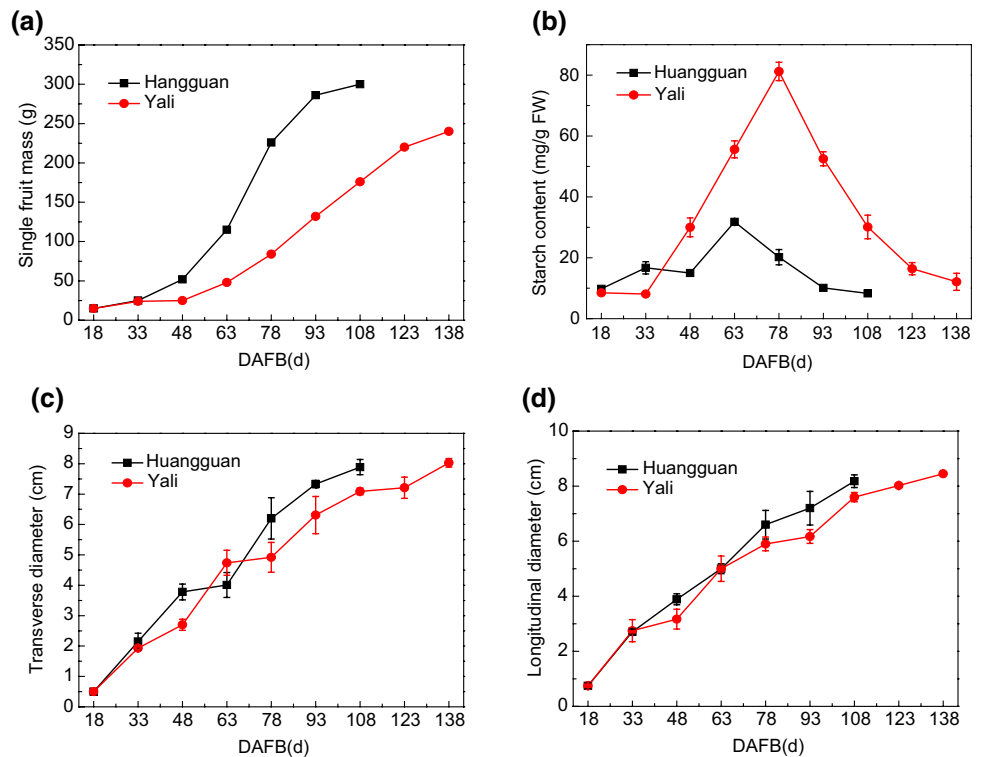
We found the growth of transverse diameter (TD) can be characterized by three phases. The first phase was associated with an exponential increase in fruit size, followed by a central lag-phase characterized by a reduction in the growth rate, and a final third-phase of rapid regrowth. In contrast, the longitudinal diameter (LD) increased gradually without significant fluctuation (Fig. 1c, d).

Figure 1b shows the observed starch fluctuations of the “Huangguan” and “Yali” pears during development. We can see that the starch content of the “Yali” pear fruit was very low during the young fruit stage and thereafter increased rapidly, reaching a peak at 78 DAFB. The starch content of the “Huangguan” pear accumulated slowly, reaching a peak at 63 DAFB. During the later development period in both pear varieties, the starch began to decompose and its content dropped rapidly.

Table 1 Primers for real-time PCR analysis and products

Gene	Forward primer (5'–3')	Reverse primer (5'–3')	Product (bp)
AI	TTGACTCCGCCATCTGGG	AGTCCACGCACTCCCACATAC	218
NI	TTGGCAATCTACAACCTGC	AATCATCCCATTGGCTTC	156
SS	TTTTCTCGTCCCACCC	CAGAGTAGGGTGTCTCGGGTT	228
SPS	ATCTGGTGCCTTGAATGTCC	CAAGCGCCATTGTGAGTCTA	235

Fig. 1 Changes of fruit mass (a), starch (b), transverse diameter (c) and longitudinal diameter (d) of “Huangguan” and “Yali” pear fruits in the developing stage. Data represent the average of the total masses of ten fruits randomly selected from each sample



Soluble Sugar Content Changes

As shown in Fig. 2a, the sorbitol content dropped dramatically from its maximum at 18 DAFB until 33 DAFB, followed by a gradual decrease. We observed a parallel gradual increase in the glucose and fructose contents throughout virtually all the “Huangguan” and “Yali” pear fruit development stages studied (Fig. 2), whereas sucrose, which was present at negligible level prior to 63 DAFB, exhibited a slight accumulation along with an increase in hexose (Fig. 2a). Prior to 48 DAFB, sorbitol was the predominant sugar present in the early stages of fruit growth. The variation of sucrose content was similar to that of sorbitol content (Fig. 2b). The total sugar content of “Huangguan” pear dropped rapidly during the early development stage, which

can be largely attributed to sorbitol cleavage and consumption. After that, the total sugar content of “Huangguan” pear rose rapidly with substantial accumulations of sucrose, glucose, and fructose (Fig. 2a), while the total sugar content of “Yali” pear reached a peak at 48 DAFB (Fig. 2b).

AI, NI, SS and SPS Enzyme Activities

The AI and NI activities of “Huangguan” pear plummeted to a minimum at 63 DAFB, after which the AI activity rose dramatically and maintained a gradual increase until harvest, whereas a peak of NI activity emerged at 78 DAFB with a slight decrease observed until harvest (Fig. 3a). The AI and NI activities of the “Yali” pear exhibited comparatively low levels in fruitlets, and AI rose markedly after 63 DAFB,

Fig. 2 Changes of soluble sugar contents of “Huangguan” (a) and “Yali” (b) pears in the developing stage. Total sugars represent the sum of sorbitol, sucrose, fructose, and glucose contents. Data represent three replicates and the vertical bars indicate the standard errors of three replicates. When no bar is shown, the error was smaller than the symbol

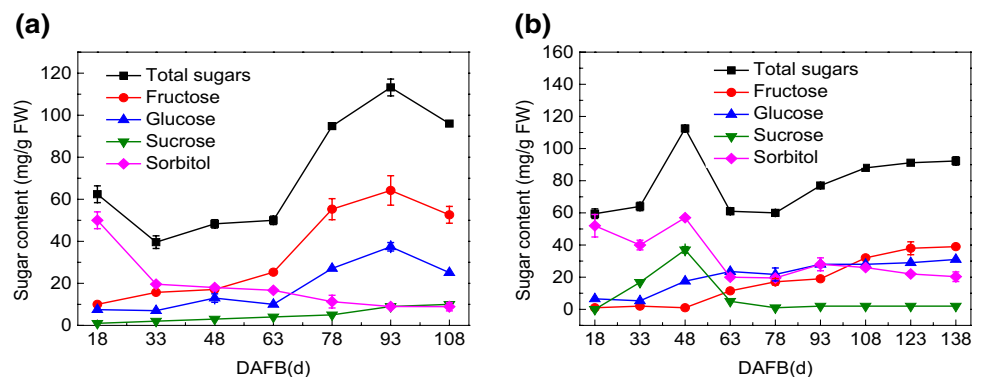
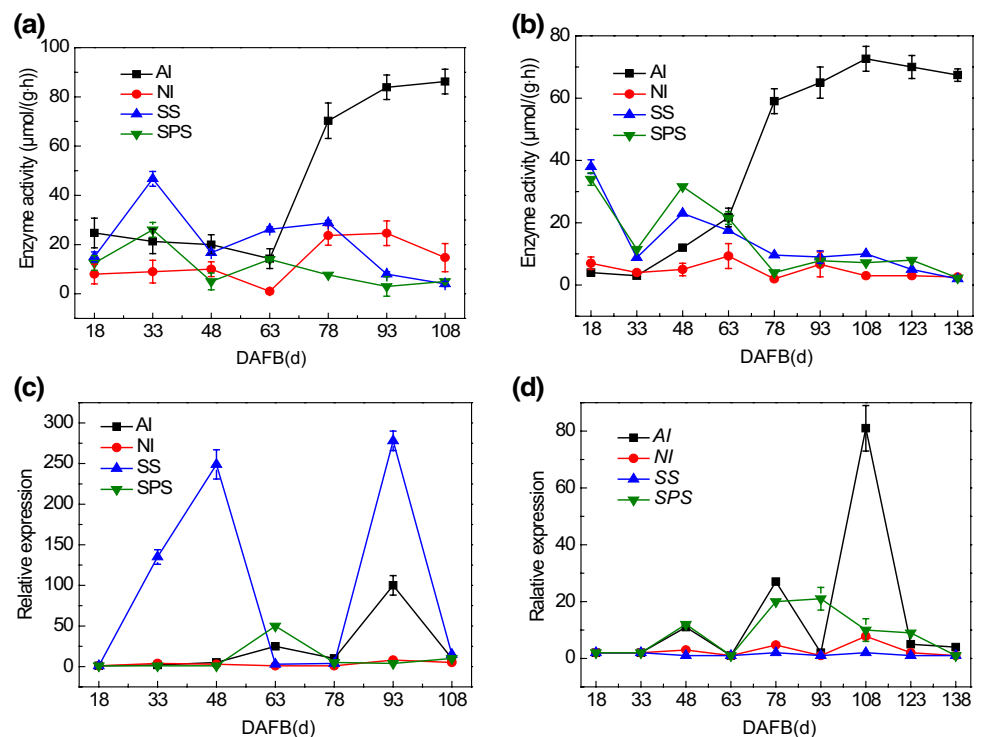


Fig. 3 Changes in AI, NI, SS, SPS activities and gene expressions of AI, NI, SS, SPS of “Huangguan” (a, c) and “Yali” (b, d) pears in the developing stage. Data represent three replicates and the vertical bars represent the standard error of the three replicates. When no bar is shown, the error was smaller than the symbol



whereas NI did not increase considerably with fruit maturity (Fig. 3b). The AI activity was nearly 6 times of NI in ripe “Huangguan” pear fruit and 30 times of NI in ripe “Yali” pear fruit (Fig. 3a, b). AI activity was predominant throughout development (Fig. 3a, b).

The SPS and SS activities in the “Huangguan” pear fruit experienced significant fluctuations during growth and exhibited a similar pattern, which was verified by the emergence of peaks in the early and late phases of SPS and SS development. After a sharp decline, their activity rose after 48 DAFB (Fig. 3a). Maxima of SS and SPS activities were observed in the “Yali” pear fruit at the initial stage of fruit development. The SPS and SS activities in the “Yali” pear fruit decreased dramatically at 33 DAFB and showed peak values at 48 DAFB, then decreased until the fruit matured (Fig. 3b).

Expressions of the AI, NI, SS and SPS Genes

Figure 3c shows the changes of the relative expressions of AI, NI, SS and SPS genes during “Huangguan” pear development. The relative expression of the AI gene was virtually undetectable during the early stage of development but reached a dramatically high level at 93 DAFB, 11 times higher than that of the NI gene. In contrast, the NI gene expression remained at basal levels throughout development, with only a slight increase observed at 93 DAFB. The SS gene expression profile climbed to two peaks of accumulation, with maxima at 48 and 93 DAFB, but exhibited

considerably low expression levels from 63 to 78 DAFB, presenting a pattern that lagged behind the changes observed in SS activity. The expression of the SPS gene was low, similar to that of the NI gene described above, and exhibited a slight increase only at 63 DAFB. The expression levels of all of the AI, NI, SS and SPS genes were low at harvest.

Figure 3d shows changes in the relative expressions of AI, NI, SS and SPS genes during the development of “Yali” pear. The relative expression level of the AI gene was low at the initial stage, thereafter rising markedly at 78 DAFB and 108 DAFB, concomitant with the significant level of AI enzyme activity. The NI and SS gene expression level were low and nearly undetectable throughout fruit growth. We detected high levels of SPS expression only at 48 DAFB during the initial stage of development. After a clear drop at 63 DAFB, SPS expression increased markedly from 78 to 93 DAFB, and then decreased until maturity.

Correlation Analysis of Sugar Content, Enzyme Activity and Corresponding Gene Expression

Table 2 shows the correlation analysis of sugar metabolism during development of “Huangguan” pear fruit. We detected a significant positive correlation among sucrose, fructose, and glucose contents during fruit growth ($P < 0.01$) (Table 2). Both the AI and NI activities were significantly positively correlated with the sucrose, fructose, and glucose contents ($P < 0.05$ and $P < 0.01$), and SS showed a negative correlation with sucrose content in “Huangguan” pears. The

Table 2 Correlation between sugar contents, related enzyme activities and related gene expressions in “Huangguan” pear

	LD	TD	Starch	Total sugars	Sorbitol	Glucose	Fructose	Sucrose	Al _a	Nl _a	SS _a	SPS _a	Al _c	Nl _c	SS _c	SPS _c	
Fruit mass	0.958**	0.969**	-0.257	0.897**	-0.417	0.947**	0.972**	0.926**	0.934**	0.986**	-0.968**	-0.943**	0.974**	0.855**	0.054	0.047	
LD		0.991**	-0.070	0.748*	0.846**	0.887**	0.916**	0.887**	0.828**	0.981**	-0.764*	-0.908**	0.776*	0.978**	0.090	0.188	
TD			-0.175	0.803**	-0.705*	0.932**	0.941**	0.877**	0.878**	0.774*	-0.702*	-0.754*	0.715*	0.821**	0.172	0.059	
Starch				-0.784*	-0.225	-0.316	-0.226	-0.704*	-0.720*	-0.778*	0.965**	0.342	-0.160	-0.786*	-0.243	0.916**	
Total sugars					-0.071	0.937**	0.928**	0.862**	0.950**	0.832**	-0.942**	-0.700*	0.934**	0.803**	0.090	-0.271	
Sorbitol						-0.391	-0.394	-0.324	-0.282	-0.250	-0.161	0.078	-0.145	-0.392	-0.412	-0.178	
Glucose						0.981**	0.865**	0.865**	0.935**	0.857**	-0.746*	-0.924**	0.954**	0.838**	0.260	-0.186	
Fructose							0.856**	0.856**	0.940**	0.805*	-0.766*	-0.926**	0.915**	0.812**	0.110	-0.075	
Sucrose									0.898**	0.906**	-0.916**	-0.732*	0.947**	0.961**	0.207	-0.037	
Al _a										0.840**	-0.747*	-0.704*	0.702*	0.835**	0.082	-0.286	
Nl _a											-0.301	-0.949**	0.786*	0.726*	0.354	-0.929**	
SS _a													0.853**	-0.402	-0.898**	-0.118	
SPS _a														-0.397	-0.725*	-0.244	
Al _c															0.781*	0.748*	
Nl _c																0.417	
SS _c																	-0.376

The symbols * and ** denote significance levels of $P < 0.05$ and $P < 0.01$, respectively

Al_a, Nl_a, SS_a, SPS_a indicate the related enzyme activities; Al_c, Nl_c, SS_c, SPS_c indicate the related gene expressions

expressions of the AI and NI genes were positively correlated to their enzyme activity (Table 2).

We found fruit mass, longitudinal diameter, and transverse diameter to be significantly positively correlated with fructose, glucose, and AI enzyme activity ($P < 0.05$), but negatively correlated with sorbitol content, SS and SPS ($P < 0.01$) (Table 3). The fructose and glucose contents were positively correlated with AI enzyme activity ($P < 0.01$) and negatively correlated with SS and SPS ($P < 0.01$), but sucrose content was positively correlated with SPS and negatively correlated with AI ($P < 0.01$). Theoretically, the above results make sense. AI gene expression and enzyme activity were positively correlated ($P < 0.05$). NI gene expression and enzyme activity were negatively correlated ($P < 0.01$). The expressions of the SS and SPS genes had no effect on their enzyme activities.

Discussion

Sugar Metabolism and Accumulation Patterns During Fruit Development

Increasing levels of fructose, glucose, and sucrose at advanced stages of fruit maturity have been reported in numerous studies. The accumulation forms of starch, sucrose, and hexose have also been identified in ripe fruits [17]. The sugar composition and content and the key enzymes related to sucrose metabolism differ in Japanese pear cultivars [18], and similar results have been observed in Chinese pear cultivars [19].

In “Huangguan” ripe pear fruit, our results indicate that hexose accounted for 89.52% of the total sugar content and fructose accounted for nearly 50%, illustrating that “Huangguan” pear fruit is a high-hexose-accumulation cultivar (Fig. 2a). The equimolar increase of glucose and fructose observed throughout “Huangguan” pear development has also been reported for bananas, *Lycium*, and kiwifruit [9, 17, 20]. At the starch accumulation stage, we observed the longitudinal diameter and fruit mass continue to increase. Interestingly, soluble sugars began to accumulate at 63 DAFB, which indicated that the high levels of starch stimulated the onset of soluble sugar accumulation. After 63 DAFB, the hexose content greatly increased and the transverse diameter entered a second period of growth escalation. These two periods of transverse diameter growth were followed by sorbitol degradation and a considerable accumulation of hexose, indicating that hexose was effective in promoting fruit set. However, high levels of fructose seemed to be more essential than glucose. Identical results were observed by Nitsch [21].

Interestingly, in the “Yali” pear, starch increased gradually as a second reserve material, reaching a maximum at

78 DAFB, and decreased thereafter. Similar growth patterns were reported regarding the “La France” pear (*Pyrus communis* L.) and sweet pepper (*Capsicum annuum*) [15, 22]. We found the longitudinal diameter of the fruit reach a second peak concurrent with the starch peak, suggesting that the accumulation of starch might be associated with longitudinal growth of the fruit. In contrast, a high accumulation of soluble sugars corresponded to rapid transverse growth (low fruit shape index), suggesting that the accumulation of soluble sugars might be related to transverse fruit growth.

Regulation Effects of Enzymes and Gene Expression Patterns on Sugar Metabolism

We found a highly significant positive correlation between AI activity and these three sugars used in our study. Manning and Maw [8] reported an increase in AI activity during fruit development, which can be directly correlated with the hexose content of the mature fruit. The loss of it seems to be a prerequisite for sucrose accumulation. The marked explosion of AI activity (after 63 DAFB) we observed in this study paralleled dramatic increases in glucose and fructose in equimolar quantities. We noted in particular that the expression of AI gene during the late stage of development in ripe “Huangguan” pear fruit was with respect to sugar accumulation. In “Yali” pear fruit, AI exhibited relatively higher activity during fruit expansion and maturation and was predominant during the fruit growth stage, leading to high hexose content in the mature fruit. High invertase activity and a high concentration of hexoses in mature fruit have also been found in apples and strawberries [5, 23]. The main function of AI is considered to be in the breakdown of sucrose and the accumulation of reducing sugars. We observed the highest activity of AI at fruit ripening when the lowest sucrose content was detected. Our results suggest that sucrose was the source of the hexoses required for metabolism during the development of “Yali” pear fruit and high AI activity prevented the accumulation of sucrose. During the early stages of fruit development, AI was apparently low enough to allow sucrose to accumulate. The activity of AI was negatively correlated with sucrose content (Table 3). This observation is similar to the results reported for the tomato, whereby invertase was reported to be a relatively effective regulator of total sugar content and sugar composition [24]. Thus, we suggest that AI plays a more predominant role than the other sucrose-cleaving enzymes in controlling hexose accumulation and fruit maturation.

All the cultivars showed low NI activity during fruit development (Fig. 3a, b). We observed the highest activity early in the fruit development and these NI activity levels did not change drastically during ripening. The “Huangguan” cultivar exhibited a higher NI activity than the “Yali” fruit. In addition, we found no significant correlation between

Table 3 Correlation between sugar contents, related enzyme activities and related gene expressions in “Yali” pear

	LD	TD	Starch	Total sugars	Sorbitol	Glucose	Fructose	Sucrose	Al _a	Nl _a	SS _a	SPS _a	Al _c	Nl _c	SS _c	SPS _c
Fruit mass	0.929**	0.943**	-0.082*	0.739*	-0.976**	0.812*-	0.985**	-0.749*	0.905**	-0.763*	-0.953**	-0.956**	0.285	0.187	0.351	0.105
LD		0.991**	0.220	0.737*	-0.795*	0.931**	0.934**	-0.344	0.927**	-0.712*	-0.845**	-0.894**	0.348	0.273	0.343	0.264
TD			0.183	0.795**	-0.807*	0.936**	0.944**	-0.709*	0.928**	-0.35	-0.831**	-0.897**	0.323	0.203	0.343	0.224
Starch				-0.264	-0.768*	0.717*	-0.018	-0.163	0.292	0.081	-0.165	-0.252	0.151	0.253	0.222	0.966**
Total sugars					0.179	0.381*	0.349	0.959**	0.310	-0.185	-0.244	-0.033	0.176	0.214	-0.051	0.158
Sorbitol						-0.784*	-0.955**	0.991**	-0.955**	0.237	0.237	0.795*	-0.215	-0.110	-0.308	-0.141
Glucose							0.828**	-0.360	0.886**	-0.114	-0.870**	-0.731*	0.282	0.182	0.277	0.352
Fructose								-0.926**	0.904**	-0.542	-0.935**	-0.957**	0.324	0.223	0.367	0.061
Sucrose									-0.908**	0.097	0.734*	0.989**	-0.121	0.022	-0.708*	0.045
Al _a										-0.756*	-0.724*	-0.897**	0.741*	0.784*	0.963**	0.759*
Nl _a											0.770*	0.994**	-0.366	-0.948**	-0.890**	-0.240
SS _a												0.919**	-0.161	-0.123	-0.218	-0.209
SPS _a													-0.321	-0.299	-0.902**	-0.333
Al _c														0.887**	0.843**	0.286
Nl _c															0.993**	0.751*
SS _c																0.776*

The symbols * and ** denote significance levels of $P < 0.05$ and $P < 0.01$, respectively

Al_a, Nl_a, SS_a, SPS_a indicate the related enzyme activities; Al_c, Nl_c, SS_c, SPS_c indicate the related gene expressions

sugar content and NI activity in the “Yali” pear (Table 3). These NI activity patterns were also observed by Miron and Schaffer [25]. The low levels of NI activity and NI gene expression observed throughout the development of “Yali” pear suggest that NI is not crucial to the growth and sugar metabolism of “Yali” pear.

SPS is the key enzyme involved in the conversion of starch to sucrose. In transgenic *Arabidopsis thaliana*, decreased SPS expression was reported to inhibit SS [26]. An increase in SPS activity has been reported during fruit ripening in starch-accumulated fruits, such as banana [6]. Our results show that the starch content of “Huangguan” pear accumulated slowly, reaching a peak at 63 DAFB. Thus, we can conclude that SPS in the “Huangguan” pear is involved in the conversion of starch to sucrose. However, the inconsistent enzyme activity and gene expression patterns of SPS suggest that these activities are regulated at the post-transcriptional level. We observed SPS and SS in “Yali” pear fruit to be conspicuous only in young fruit, resulting in the accumulation of sucrose. The expression of SPS gene fluctuated during the entire phase, which was consistent with high SPS enzyme activity and sucrose content, and was high after 78 DAFB. Similarly, Kajiura et al. [27]. reported that SPS activity was higher in young fruit, decreased and remained at a relatively low level during fruit maturation. SPS has an important function in sugar accumulation in several fruits in which sucrose is used as a form of transport [28]. In our study, we detected positive correlations between SPS and sucrose ($P < 0.01$), as well as AI and hexoses ($P < 0.01$) in “Yali” pear fruit.

SS converts sucrose to fructose and UDP-glucose, producing only half the hexoses yielded by invertase. Thus, the function of SS in determining the hexose/sucrose ratio is not as efficient as that of invertase [29]. As seen in Fig. 3a, SS activity was high in immature fruit and then declined rapidly with fruit development. The decrease in SS activity was associated with a greater reduction of sugar content. Yelle et al. [30]. concluded that the SS activity was high in *L. esculentum* during early fruit growth. Sun et al. [31]. reported that, in growing tomato fruit, SS is a biochemical determinant of sink strength and that the primary role of SS is to cleave sucrose in the cytoplasm of growing sink cells and then to feed hexoses into an intermediary metabolism via the sucrose synthase pathway. In most instances, SS is assumed to be responsible for the degradation of sucrose. However, in cucurbit and peach fruit, it has been reported that SS acts in the direction of sucrose synthesis [18]. In this study, we also observed high SS gene expression and low levels of sugar accumulation during the early stage of development in “Huangguan” pears. The SS gene expression pattern was similar to the pattern of SS enzyme activity, showing short-term hysteresis. We observed higher sucrose levels in the “Yali” cultivar, as well as higher SS

activity in early stage of fruit development (Fig. 3b). These results indicate a positive correlation between SS levels and sucrose accumulation (Table 3). As a crucial aspect of the early stage of fruit development, a high level of SS activity enables the rapid metabolism of imported sugars, thus satisfying the energy requirements of cell division and growth. We observed the AI and NI activity patterns to be identical to those of SS and SPS, respectively, indicating a potential synergistic effect, but additional research is necessary to support this conclusion. Although a full understanding of sugar metabolism and regulatory processes in “Yali” pear fruit remains an ambitious goal, integrative studies on gene expression and enzyme activity might substantially contribute to our knowledge of this process.

In this study, a speculative model showing the various mechanisms involved in “Huangguan” and “Yali” pears was generated (Fig. 4).

Conclusions

According to our results, AI plays a crucial role in sugar accumulation and composition in ripe fruit, while SS appears to be involved in the growth and the regulation of carbohydrate metabolism of “Huangguan” pear fruit. We found the high levels of late stage AI and early stage SS during development, which are roughly corresponding with the gene expressions found in the late and early stages, respectively, suggesting their potential regulatory roles in the development of “Huangguan” pear fruit. The SPS and AI enzymes played roles in the early and late stages,

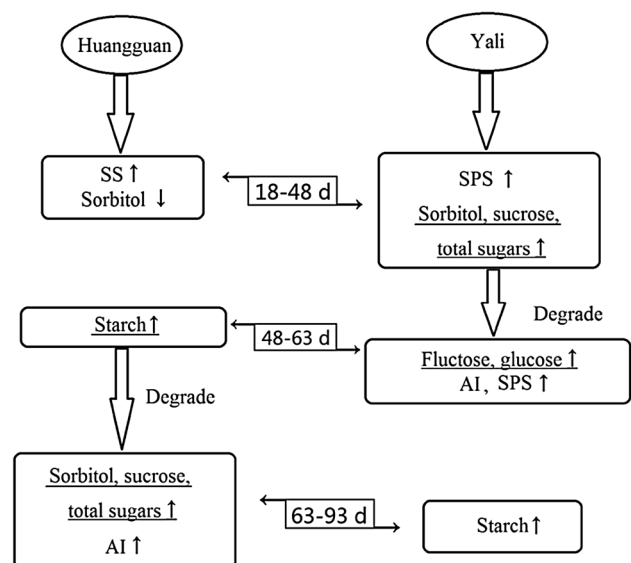


Fig. 4 Speculative model showing the different mechanisms involved in “Huangguan” and “Yali” pears

respectively, resulting in substantial hexose accumulation in mature fruit. We conclude that the incongruence between the SS and SPS gene expressions and enzyme activities in “Yali” pear fruit results in complex transcriptional and translational control. Further investigation is needed to reveal the mechanisms behind the regulation of these genes and to facilitate a better understanding of how the activity of each enzyme depends on or is correlated with other enzymes.

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