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Research Article

Sugar content and expression of sugar metabolism-related gene in strawberry fruits from various cultivars

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Abstract Strawberry (*Fragaria* × *ananassa*) is a globally-cultivated and popular fruit crop, prized for its flavor and nutritional value. Sweetness, a key determinant of fruit quality, depends on the sugar composition and concentration. We selected eight strawberry cultivars based on the fruit soluble solids content to represent high and low sugar content groups. The average soluble solid content was 13.6 °Brix (Okmae, Geumsil, Aram, and Maehyang) and 2.9 °Brix (Missionary, Camino Real, Portola, and Gilgyung53), for the high and low sugar content groups, respectively. Sucrose was the main sugar in the cultivars with high sugar content, whereas fructose was the main component in the low sugar content cultivars. Fruit starch concentration ranged from 3.247±0.056 to 3.850±0.055 g/100g, with a 12% higher concentration in the high sugar content cultivars. Additionally, we identified 41 sugar metabolism-related genes in *Fragaria* × *ananassa* and analyzed the relationship between their transcripts and the sugar accumulation in fruit. *FaGPT1*, *FaTMT1*, *FaHXK1*, *FaPHS1*, *FaINVA-3*, and *FacxINV2-1* were highly expressed in the high sugar content cultivars, while *FapGlcT*, *FaTMT2-1*, *FaPHS2-1*, *FaSUSY1-1*, and *FaSUSY1-2* were highly expressed in the low sugar content cultivars. In general, a greater number of genes encoding sugar transporters or involved in sugar synthesis were highly expressed in the high sugar content cultivars. Contrarily, genes involved in sugar degradation were preferentially transcribed in the low sugar content cultivars. Although gene expression was not perfectly proportional to sugar content or concentration, our analysis of the genes involved in sugar metabolism and accumulation in strawberries provides a framework for further studies and for the subsequent engineering of sugar metabolism to enhance fruit quality.

Keywords Soluble sugar, Glucose, Fructose, Sucrose, *Fragaria* × *ananassa*

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Introduction

Strawberry (*Fragaria* × *ananassa*) is one of the most popular and widely-consumed fruits worldwide. In strawberry production, sweetness, flavor, color, and juiciness are important traits, because they affect consumer and industrial demand. The relationship between sugars and sensory properties, such as flavor or color has been under study for a long time (Alavoine and Crochon 1989; Wozniak et al. 1997). Sweetness is one of the most desirable characteristics in commercially grown strawberries, and is influenced by the amount and composition of sugars accumulated in the fruit (Basson et al. 2010). This quality is primarily determined genetically and environmentally (Kallio et al. 2000; Wang and Camp 2000; Pelayo-Zaldivar et al. 2005; Gündüz and Özdemir 2014). Sugars and the organic acids present in the fruit determine the overall fruit flavor (Kallio et al. 2000; Park et al. 2006). Additionally, sugar content is also dependent on total solids, pH, acidity, and fruit size; furthermore, sugar composition varies with fruit ripeness (Reyes et al. 1982).

Glucose, fructose, and sucrose are the main soluble sugars

accumulated in strawberry fruits (Woodward 1972; Ranwala et al. 1992). These soluble sugars account for approximately 99% of the total fruit sugar content; furthermore, the soluble sugar content significantly increases during fruit development (Makinen and Söderling 1980; Jia et al. 2013). Glucose and fructose concentrations are higher during growth than in the ripe fruits (Forney and Breen 1986), although the amounts of glucose, fructose, and sucrose vary with the degree of ripeness (Kafkas et al. 2007). Several studies have shown qualitative and quantitative variations in the composition of soluble sugars in strawberry cultivars (Basson et al. 2010; Gündüz and Özdemir 2014). Understanding the factors that control carbohydrate partitioning is necessary for improving the efficiency of metabolic engineering. Till date, we do not have enough information on the molecular mechanisms responsible for the control of soluble sugar concentration in strawberries. Until now, molecular approaches have focused on specific sugar-metabolism pathways regulated during ripening (Park et al. 2006), and activities of only some enzymes have been measured, such as sucrose synthase, neutral- and soluble acid invertase, etc. (Hubbard et al. 1991; Ranwala et al. 1992). Nevertheless, the difference in soluble sugar content among different strawberry cultivars remains unclarified.

In this study, we aimed to elucidate the relationship between sugar concentration and gene expression in fully ripened strawberries by analyzing sugar content and sugar concentration in selected strawberry cultivars, which have either a low or high sugar level. We also examined the expression patterns of sugar metabolism-related genes, such as those involved in sugar transport, sugar synthesis, and sugar degradation. The findings of this study may facilitate the future selection of molecular genetic targets to improve carbohydrate accumulation in strawberries.

Materials and Methods

Plant materials

Strawberries were grown in a greenhouse at Chungnam Agricultural Research and Extension in South Korea, from November, 2016 to April, 2017. Cultivars included Missionary, Camino Real, Portola, Gilgyung53, Okmae, Geumsil, Aram, and Maehyang. All fruit samples were collected at maturity and freeze-dried by storing at -55°C. Freeze-dried samples were then stored at 4°C until use for HPLC-ELSD analysis and total RNA isolation.

Preparation of soluble sugars and starch extracts

Sugar content of all cultivars were measured using fresh fruit samples with a Digital Refractometer GMK-703AC (G-won hightech, Korea). Approximately 0.2 g of freeze-dried fruit tissue was dissolved in 20 ml of ethanol (80%) with vigorous stirring for 20 min, followed by sonication for 10 min and further stirring for 5 min. The resulting mixture was centrifuged at 3,000 rpm for 10 min at 4°C, as described by Shanmugavelan et al. (2013). The supernatant solution was filtered through a 0.45-μm filter and used for sugar analysis by HPLC-ELSD. Following ethanol extraction, tissue pellets were re-suspended in 8 ml of sterile distilled water and starch was gelatinized in a water bath at 100°C for 1 h. Gelatinized starch was converted to glucose by the addition of 2 ml 0.5 M sodium acetate buffer (pH 5.2) containing 10 units of amyloglucosidase (Roche Molecular Biochemicals, UK). Samples were incubated at 37°C overnight and then centrifuged at 13,000 g for 5 min. Glucose released from starch was determined by HPLC-ELSD (Souleyre et al. 2004).

HPLC-ELSD analysis

Analysis of sugars by HPLC was performed isocratically using a carbohydrate column (S5 μm, 250 mm × 4.6 mm i.d) using the following HPLC conditions: flow rate = 1.0 μl/min, data rate = 1 pps, run time = 15 min, gain = 1, column heater temperature = 35°C, sample temperature = 5°C, pressure = 50 psi, Nebulizer: heating (90%) and injection volume = 10 μl. An acetonitrile:water (7:3) mixture was used as the mobile phase, as described by Shanmugavelan et al. (2013). For quantification and calibration, a standard solution mixture was prepared by dissolving D(+) fructose, D(+) glucose, and D(+) sucrose in water (HPLC grade) to attain the following five different concentrations: 50, 100, 500, 1,000, 2,500, and 5,000 ppm.

Candidate gene identification of sugar metabolism

The sugar-related genes of *Arabidopsis thaliana* were searched from NCBI GenBank (https://www.ncbi.nlm.nih.gov/). Collected genes were subjected to a tBlastN search against our internal transcriptome database of *Fragaria* × *ananassa* (unpublished data) and GDR database (https://www.rosaceae.org/) to identify their homologues in the strawberry genome. Only resultant sequences with e-value of $<1e^{-100}$ and identity of >30% were considered as orthologous genes. In all, 41 *Fragaria* × *ananassa* sugar metabolism-related genes were selected;

Table 1 Soluble solid contents and composition of sugars (g/100 g) of selected strawberry cultivars. Values (mean ± SD) are average of duplicate samples analyzed individually in triplicate for sugar concentration

	Soluble solid contents (°Brix)	Sugar concentration (g/100 g)				Starch (g/100 g)
		Glucose	Fructose	Sucrose	Total	
Missionary	2.3 ± 0.3	1.027 ± 0.006	2.461 ± 0.007	0.628 ± 0.011	4.116 ± 0.009	3.268 ± 0.088
Camino Real	3.5 ± 0.1	1.460 ± 0.006	2.799 ± 0.011	0.535 ± 0.005	4.794 ± 0.007	3.247 ± 0.056
Portola	2.7 ± 0.3	1.404 ± 0.003	2.623 ± 0.007	0.437 ± 0.005	4.464 ± 0.004	3.357 ± 0.061
Gilgyung53	3.1 ± 0.1	1.481 ± 0.005	2.754 ± 0.010	0.352 ± 0.003	4.587 ± 0.007	3.457 ± 0.079
Geumsil	14.3 ± 0.6	2.742 ± 0.005	3.056 ± 0.006	6.294 ± 0.032	12.092 ± 0.016	3.794 ± 0.132
Aram	13.9 ± 0.7	3.079 ± 0.006	4.802 ± 0.011	7.571 ± 0.021	15.452 ± 0.013	3.850 ± 0.055
Maehyang	13.6 ± 0.6	1.331 ± 0.001	2.236 ± 0.001	4.503 ± 0.007	8.070 ± 0.004	3.725 ± 0.051
Okmae	13.6 ± 0.8	1.687 ± 0.006	2.236 ± 0.010	4.399 ± 0.017	8.322 ± 0.011	3.615 ± 0.077

the gene-specific primers were designed with Primer3 (v. 0.4.0) (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) and used for RT-PCR analysis. The accession numbers of the genes and primer sets used in this study are listed in Table 2 and Supplementary Table 1, respectively.

RNA extraction and RT-PCR analysis

Reverse transcription-polymerase chain reaction was used to analyze the expression of genes involved in sugar metabolism. Total RNA was prepared from freeze-dried fruit samples following the methods of Yu et al. (2012) using an extraction buffer containing 3% CTAB, 100 mM Tris-HCl (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 5% PVP, and 1% β-mercaptoethanol. First-strand cDNA was synthesized from 1 μg isolated total RNA using the ReverTra Ace-α cDNA synthesis kit (TOYOBO, Japan) following the manufacturer's protocol; RT-PCR was performed using the primers listed in Supplementary Table 1.

Results and Discussion

Soluble solid and sugar content analysis in strawberry cultivars

Strawberry (*Fragaria* × *ananassa*) is a globally cultivated and consumed fruit crop valued for its flavor and abundant nutrition. Sweetness is one of the most important quality of strawberry that determines the preference of consumers and industry (Sturm et al., 2003; Gündüz and Özdemir, 2014). Eight strawberry cultivars were selected on the basis of fruit soluble-solids content (°Brix) as representative of low- and high-sugar groups among 60 different strawberry cultivars. The average values were 13.6 and 2.9 °Brix for high and low sugar-content cultivars, respectively (Table 1). The high sugar-content cultivars included Okmae (13.6 ± 0.8 °Brix), Geumsil (14.3 ± 0.6 °Brix), Aram (13.9 ± 0.7 °Brix), and Maehyang (13.6 ± 0.6 °Brix), while the low sugar-content cultivars were Missionary (2.3 ± 0.3 °Brix), Camino Real (3.5 ± 0.1 °Brix), Portola (2.7 ± 0.3 °Brix), and Gilgyung53 (3.1 ± 0.1 °Brix).

Sugar content measured in fruits of all these cultivars are compared in Table 1. Total soluble sugar content in the high sugar-content cultivars was 2.45 times higher than that in the low sugar-content cultivars. It is noteworthy that, among the sugars analyzed here, sucrose showed the most significant difference in concentration between the high- and low sugar-content cultivars. Sucrose content was the highest among that of all the soluble sugars present in the fruits of the high sugar-content cultivars, ranging between 4.399 ± 0.017 and 7.571 ± 0.021 g/100g, while glucose content was the lowest, ranging from 1.331 ± 0.001 to 3.079 ± 0.006 g/100g (Table 1). Sugar content and the ratio of soluble sugar concentration in the high sugar-content cultivars showed a similar trend to that previously reported for the cultivars Maehyang, Seolhyang, Festival, and Sweet Charlie (Shanmugam et al. 2017). In the case of the low sugar-content cultivars, fructose content was the highest (2.461 ± 0.007 g/100g − 2.799 ± 0.011 g/100g), while sucrose content was the lowest (0.352 ± 0.003 g/100g − 0.628 ± 0.011 g/100g) among all the soluble sugars quantitated (Table 1). It has been reported that fructose and glucose are the main sugars in strawberries from the analysis of Festival, Sweet Charlie, Camaraso, Selva, and 12 other cultivars mainly grown in the USA and Europe. (Castro et al. 2002; Sturm et al. 2003; Shanmugam et al. 2017). In this study, two contrasting groups of cultivars differed in their respective sugar composition; sucrose was the main sugar present in the high sugar-content cultivars, while fructose prevailed

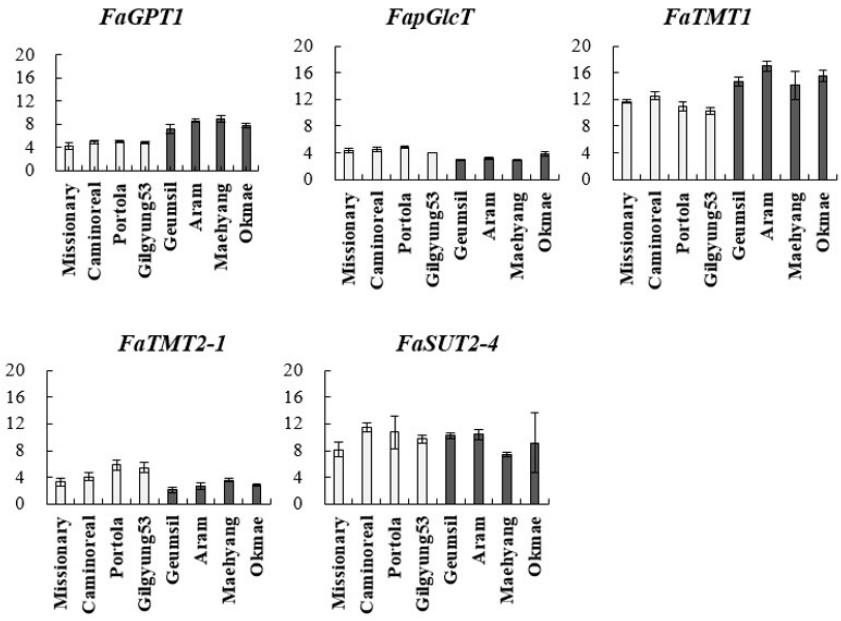

Fig. 1 Relative mRNA expression of putative genes involved in sugar transportation. RT-PCR analysis of different members of the sugar transporter genes in high (gray bar) and low sugar content cultivars (white bar). RNA levels were quantified and normalized to the level of *FaGAPDH*. Values are means ± SD of three samples

in the low sugar-content cultivars. Fruit starch concentration in the eight cultivars examined ranged from 3.247 ± 0.056 contained 12% more starch than the low sugar-content cultivars (Table 1). It has been reported that starch accumulates at the early stages of strawberry maturation and is rapidly degraded as fruits ripen (Souleyre et al. 2004). Our results demonstrated that the extent of starch accumulation, or conversely, the extent of starch degradation in the cultivars under study were different and contributed to the differences recorded in the starch and sugar content among the two groups of cultivars studied.

Identification of sugar metabolism-related genes in *Fragaria* × *ananassa*

Sugar metabolism and accumulation pathways have been extensively characterized in plants (Supplementary Fig. 1). Here we focused on searching genes related with the utilization and transport of the three major sugars quantitated in this study (Table 1). To identify putative sugar-metabolism genes in *Fragaria* × *ananassa*, 26 mRNA sequences related to sugar metabolism were collected as a reference from the *Arabidopsis* genome (Rolland et al. 2006). We obtained a total of 41 transcripts including, 11 sugar transporter genes, 4 hexose-related genes, 9 sucrose-synthesis genes, and 17 sucrose-degradation genes (Table 2). Multiple copies of transcripts were detected for *FaINVG* (invertase G), *FaINVA* (invertase A), *FacwINV2* (cell wall invertase 2), *FacwINV5*, *FaPHS2* (alpha-glucan phosphorylase 2), *FaSUT2* (sucrose transporter 2), *FaSUSY1* (sucrose synthase 1), *FaSUSY2* (sucrose synthase 2), and *FaTMT2* (tonoplast monosaccharide transporter 2), suggesting that *Fragaria* × *ananassa* uses multiple isoforms of these sugar metabolism-related genes, either in a spatial or in a developmental-stage specific manner (Supplementary Fig. 2).

Expression profiling of sugar metabolism-related genes in *Fragaria* × *ananassa*

Aiming to elucidate the expression patterns of sugar metabolism-related genes, we carried out semi-quantitative RT-PCR for sugar transporters, hexose-related genes, sucrose synthesis genes, and sucrose-degradation genes in the two groups of strawberry cultivars identified on the basis of sugar content (Figs. 1-4, Supplementary Fig. 2). Among the 11 sugar-transporter genes, only *FaGPT1* (glucose 6-phosphate/phosphate translocator 1), *FapGlcT* (plastidic glucose translocator), *FaTMT1*, *FaTMT2-1*, and *FaSUT2-4* were expressed in mature strawberries (Fig. 1). The expression of *FaGPT1* and *FaTMT1* were 1.70-fold and 1.35-fold greater, respectively, in the high sugar-content cultivars than in the low sugar-content cultivars. In contrast, the expression of *FapGlcT* and *FaTMT2-1* were 1.39-fold and 1.66-fold greater, respectively in the low sugar-content cultivars. On the contrary, *FaTMT2-2*, *FaSUT2-1* and *FaSUT2-3* were not expressed in either fruit type (Supplementary Fig. 2). Based on the

Table 2 Sugar metabolism related genes identified in *Fragaria* × *ananassa*

Function	*Arabidopsis thaliana*			*Fragaria* × *ananassa*				
	Gene name	Locus ID	CDS length (bp)	Gene name	Gene ID	CDS length (bp)	Identity (%)	E-value
Sugar transporter	*AtGPT1*	At5g54800	1,176	*FaGPT1*	mrna03202.1-v1.0-hybrid	392	100	0
	AtpGlcT	At5g16150	1,599	*FapGlcT*	mrna12375.1-v1.0-hybrid	533	100	0
	AtTMT1	At1g20840	1,959	*FaTMT1*	mrna31477.1-v1.0-hybrid	653	100	0
	AtTMT2	At4g35300	2,394	*FaTMT2-1*	mrna17337.1-v1.0-hybrid	798	100	0
				FaTMT2-2	mrna13020.1-v1.0-hybrid	803	60.4	0
	AtSUC2	At1g22710	1,515	*FaSUT2-1*	mrna27493.1-v1.0-hybrid	505	100	0
				FaSUT2-2	mrna15110.1-v1.0-hybrid	497	73.5	0
				FaSUT2-3	mrna08189.1-v1.0-hybrid	499	71.3	0
				FaSUT2-4	mrna32070.1-v1.0-hybrid	492	70.4	0
				FaSUT2-5	mrna15111.1-v1.0-hybrid	493	69.4	0
	AtSUC4	At1g09960	1,821	*FaSUT4*	mrna26850.1-v1.0-hybrid	607	100	0
Hexose-related	*AtHXK1*	At4g29130	1,449	*FaHXK1*	mrna25718.1-v1.0-hybrid	483	100	0
	AtHXK2	At2g19860	1,497	*FaHXK2*	mrna11313.1-v1.0-hybrid	499	100	0
	AtPGI	At4g24620	1,872	*FaPGI*	mrna12096.1-v1.0-hybrid	624	100	0
	AtPGM	At5g51820	1,854	*FaPGM*	mrna13359.1-v1.0-hybrid	618	100	0
Sucrose-synthesis	*AtPHS1*	At3g29320	3,045	*FaPHS1*	mrna04322.1-v1.0-hybrid	1,015	100	0
	AtPHS2	At3g46970	2,508	*FaPHS2-1*	mrna07968.1-v1.0-hybrid	836	100	0
				FaPHS2-2	mrna29749.1-v1.0-hybrid	1,010	50.1	0
	AtSPP1	At1g51420	1,806	*FaSPP1*	mrna18142.1-v1.0-hybrid	602	100	0
	AtSPP4(3b)	At3g52340	366	*FaSPP4*	mrna30374.1-v1.0-hybrid	122	100	1.56E-19
	AtSPSA1	At5g20280	3,189	*FaSPSA1*	mrna31122.1-v1.0-hybrid	1,063	100	0
	AtSPSA2	At5g11110	3,174	*FaSPSA2*	mrna11606.1-v1.0-hybrid	1,058	100	0
	AtSPSB	At1g04920	3,201	*FaSPSB*	mrna06523.1-v1.0-hybrid	1,067	100	0
	AtSPSC	At4g10120	3,081	*FaSPSC*	mrna31164.1-v1.0-hybrid	1,027	100	0
Sucrose-degradation	*AtSUSY1*	At5g20830	2,421	*FaSUSY1-1*	mrna12940.1-v1.0-hybrid	807	100	0
				FaSUSY1-2	mrna11429.1-v1.0-hybrid	1,526	82.8	0
	AtSUSY2	At5g49190	2,364	*FaSUSY2-1*	mrna07050.1-v1.0-hybrid	788	100	0
				FaSUSY2-2	mrna31666.1-v1.0-hybrid	825	55.6	0
	AtSUSY3	At4g02280	2,544	*FaSUSY3*	mrna11077.1-v1.0-hybrid	848	100	0
	AtSUSY5	At5g37180	5,871	*FaSUSY5*	mrna09290.1-v1.0-hybrid	1,957	100	0
	A/N-INV-G	At1g35580	1,659	*FaINVG-1*	mrna07792.1-v1.0-hybrid	553	100	0
				FaINVG-2	mrna07379.1-v1.0-hybrid	718	78.9	0
				FaINVG-3	mrna08524.1-v1.0-hybrid	602	76.2	0
				FaINVG-4	mrna18695.1-v1.0-hybrid	583	71.1	0
	A/N-INV-A	At1g56560	1,962	*FaINVA-1*	mrna00239.1-v1.0-hybrid	654	100	0
				FaINVA-2	mrna05019.1-v1.0-hybrid	656	72.5	0
				FaINVA-3	mrna22002.1-v1.0-hybrid	670	67	0
	AtcwINV2	At3g52600	1,878	*FacwINV2-1*	mrna23034.1-v1.0-hybrid	626	100	0
				FacwINV2-2	mrna15509.1-v1.0-hybrid	572	56.1	0
	AtcwINV5	At3g13784	1,533	*FacwINV5-1*	mrna06912.1-v1.0-hybrid	511	100	0
				FacwINV5-2	mrna23031.1-v1.0-hybrid	950	74.5	0

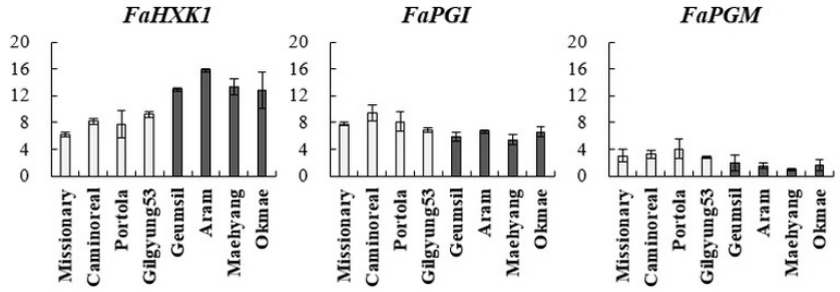

Fig. 2 Relative mRNA expression of hexose-related genes. RT-PCR analysis of different members of the sugar transporter genes in high (gray bar) and low sugar content cultivars (white bar). RNA levels were quantified and normalized to the level of *FaGAPDH*. Values are means ± SD of three samples

expression patterns obtained, *FaTMT1* seemed the main *FaTMT* sugar transporter gene in strawberry fruits. Further, *FaSUT2-4* was the only sucrose transporter gene expressed in the fruits of all eight cultivars under study here, which showed a variable expression pattern in the different cultivars (Fig. 1). As for glucose translocator genes, the expression patterns of *FaGPT1* and *FapGlcT* were mutually opposing, since they encode glucose transporters that transport glucose through the cytosol and chromoplast in opposite directions (Linka and Weber 2005), thereby playing an important role during chromoplast differentiation and fruit ripening by regulating glucose phosphate concentration in the fruit. With respect to sucrose transporters, they are localized in the plasma membrane (Butowt et al. 2003; Rolland et al. 2006; Fettke et al. 2009), indicating sucrose transport from the phloem in the source tissues to the cytosol of cells in the sink tissues; this is especially true for *FaSUT2-4* in strawberries.

Our results revealed two genes regulating hexose conversion (*FaPGI* (phosphoglucoisomerase) and *FaPGM* (phosphoglucomutase)) and two more regulating hexose phosphorylation (*FaHXK1* (hexokinase 1) and *FaHXK2* ((hexokinase 2)) during sugar metabolism (Table 2). *FaHXK2* was not expressed in mature strawberry fruits and the transcript level of *FaHXK1* was 1.74-fold higher in the high sugar-content cultivars (Fig. 2, Supplementary Fig. 2). The expression patterns of *FaPGI* and *FaPGM* showed no significant difference between the two groups of strawberry cultivars under study here (Fig. 2).

Among the 9 sucrose synthesis-related genes, *FaPHS1* (alpha glucan phosphorylase 1), *FaPHS2-1* (alpha glucan phosphorylase 2-1), *FaSPP1* (sucrose-phosphatase 1), and *FaSPSA1* (sucrose phosphate synthase 1) were expressed in strawberry fruits (Supplementary Fig. 2). Transcript level of *FaPHS1* was 1.85-fold higher in the high sugar-content cultivars, whereas *FaPHS2-1* was 2.25-fold higher in the low sugar-content cultivars. *FaSPP1* and *FaSPSA1* showed similar patterns of expression in both groups of cultivars. The expression of *FaSPSA1* was the lowest among the expression of all examined sucrose synthesis-related genes. Higher expression level was detected for *FaPHS1* and *FaSPP1* in the high sugar-content cultivars, and for *FaPHS2-1* and *FaSPP1* in the low sugar-content cultivars (Fig. 3).

In most plants, sucrose is the major carbohydrate imported from source tissues. There are two sources for sucrose accumulation: imported sucrose that has not been metabolized, and newly synthesized sucrose from hexoses (Butowt et al. 2003; Rolland et al. 2006; Fettke et al. 2009). The *SPS* and *SPP* genes contribute to synthesizing sucrose in the cytosol, while *PHS* provides substrate for sucrose synthesis by cleaving glycosidic bonds of heteroglycans in the cytosol (Fettke et al. 2009). In this study, *FaPHS1* showed the clearest difference in expression level between the two groups of cultivars, with the higher expression level in the high-sugar-content cultivars.

To compare the expression patterns of sugar metabolism-related genes between our two groups of strawberry cultivars, we examined the transcript level of sucrose synthases (*SUSYs*) and invertases (*INVs*). Among the 6 *SUSYs*, only *FaSUSY1-1* and *FaSUSY1-2* were expressed in strawberry fruits (Supplementary Fig. 2), with 2.45-fold and 1.41-fold, higher levels, respectively, in the low sugar-content cultivars. *FaSUSY1-2* seemed to be the major sugar synthase gene among the 6 homologs examined. Additionally, expression level of *FaSUSY1-1* was low only in Geumsil and Aram, which accumulated large quantities of hexoses (Fig. 4). As for invertases, *FaINVG-1*, *FaINVA-1*, *FaINVA-2*, *FaINVA-3*, and *FacwINV2-1* were expressed in strawberry fruits; *FaINVG-1* was considered as major gene for sucrose degradation in *Fragaria* × *ananassa*, based on the high transcriptional level expressed in all cultivars, whereas other invertases were only slightly expressed in strawberry fruits with similar levels in both, the high- and low sugar-content cultivars. The expression levels of *FaINVA-3* and *FacwINV2-1* were particularly higher in the high sugar-content cultivars

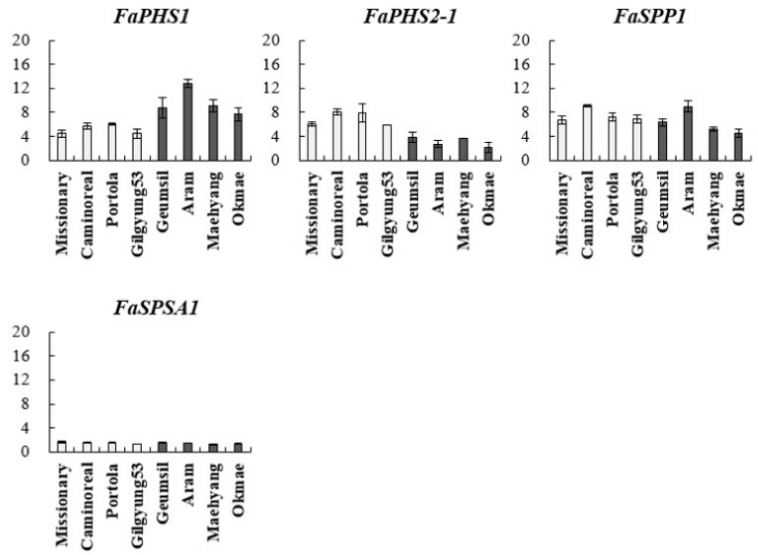

Fig. 3 Relative mRNA expression of sucrose synthesis genes. RT-PCR analysis of different members of the sugar transporter genes in high (gray bar) and low sugar content cultivars (white bar). RNA levels were quantified and normalized to the level of *FaGAPDH*. Values are means ± SD of three samples

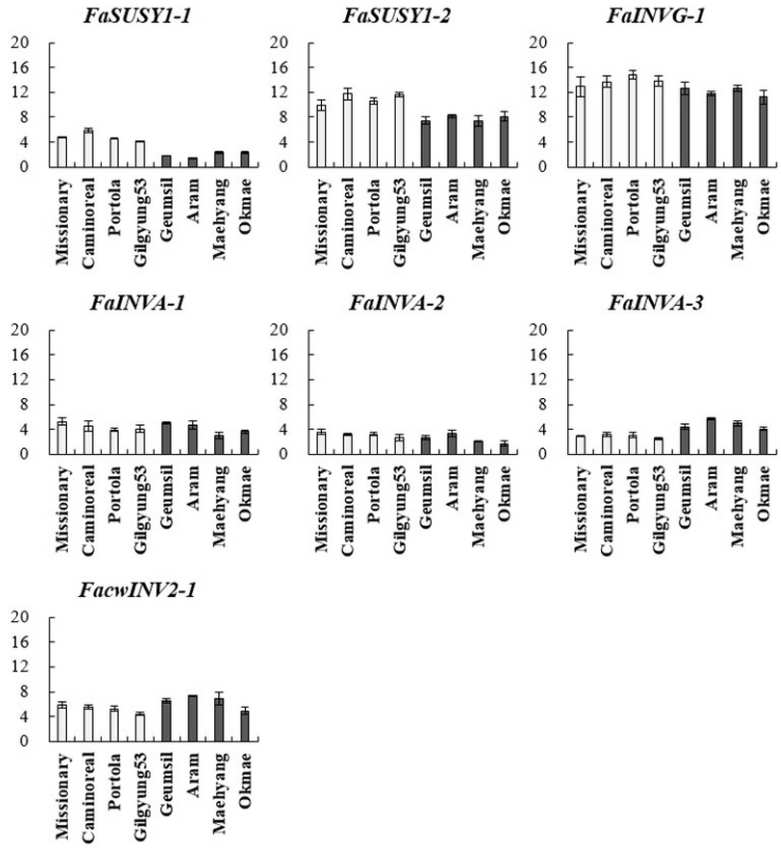

Fig. 4 Relative mRNA expression of putative genes involved in sucrose-degradation. RT-PCR analysis of different members of the sugar metabolism genes in high (gray bar) and low sugar content cultivars (white bar). RNA levels were quantified and normalized to the level of *FaGAPDH*. Values are means ± SD of three samples

(Fig. 4). The concentration and composition of sugars in plant cells are modulated by sugar metabolism. Mainly glucose, fructose, and sucrose are affected by both internal and external factors, such as developmental processes and environmental conditions (Ruan et al. 2010; Dai et al. 2011). Among the various sugars present in strawberry fruits of

the cultivars analyzed in this study, sucrose dominated the sugar profile; thus, genes involved in sucrose metabolism should be considered important candidate genes for application in developing high sugar-content cultivars (Jia et al. 2013; Jia et al. 2016). Sucrose synthase activity was relatively high in the early stages of ripening, but decreased as ripening progressed. Similarly, neutral and soluble acid invertase activities decreased during ripening (Hubbard et al. 1991; Souleyre et al. 2004; Basson et al. 2010). However, some reports have shown that soluble acid invertase activity increased with fruit ripening, which correlated with an increase of glucose and fructose (Ranwala et al. 1992).

Conclusion

In this study, we investigated the relationship between sugar content, sugar concentration, and sugar metabolism-related gene expression by comparing the low- and high sugar-content cultivars in terms of these variables. We measured total soluble sugar concentration, which was proportional to sugar content in strawberry fruits. Among glucose, fructose, and sucrose, sucrose markedly influenced the sweetness of strawberry fruits. Further, sucrose was responsible for the largest difference between the two groups of cultivars under study. From gene expression profiling of sugar metabolism, we found several sugar-related genes, such as *FaGPT1*, *FaTMT1*, *FaHXK1*, *FaPHS1*, *FaINVA-3*, and *FacwINV2-1*, which were highly expressed in the high sugar-content cultivars, suggesting they may play important roles in controlling the accumulation of sugar in strawberry fruits. This work represents a comprehensive analysis of genes involved in sugar metabolism and accumulation in strawberry fruits; our data on their expression profile in ripening strawberry fruits will be helpful for further manipulation and application in the development of controlled sugar content cultivars for research and commercial purposes.

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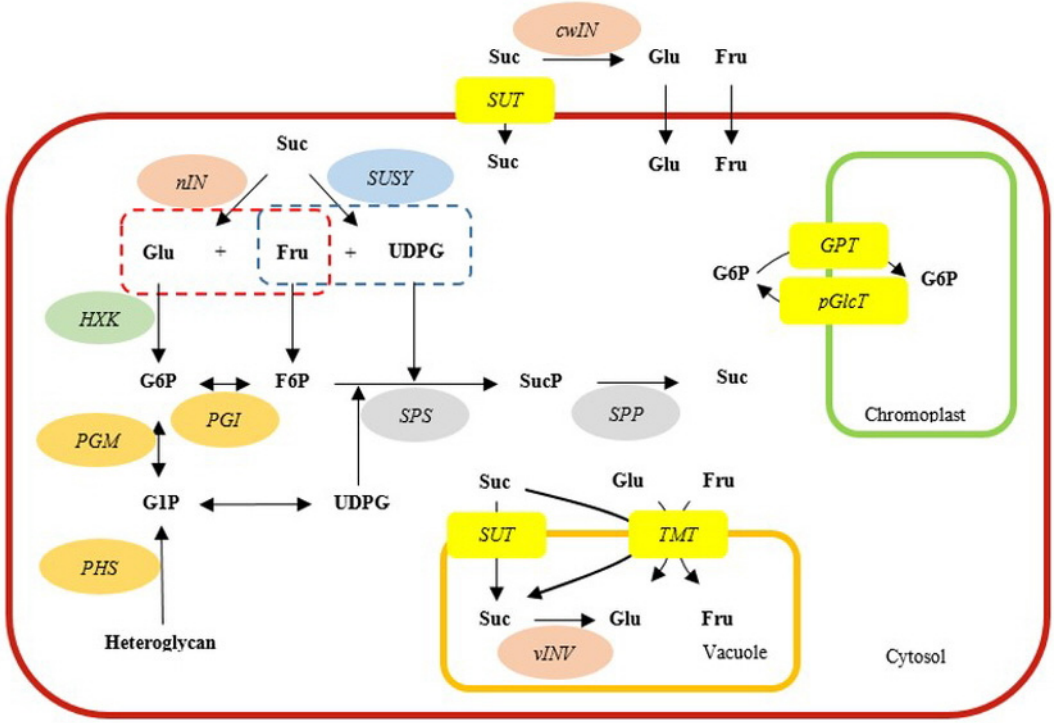
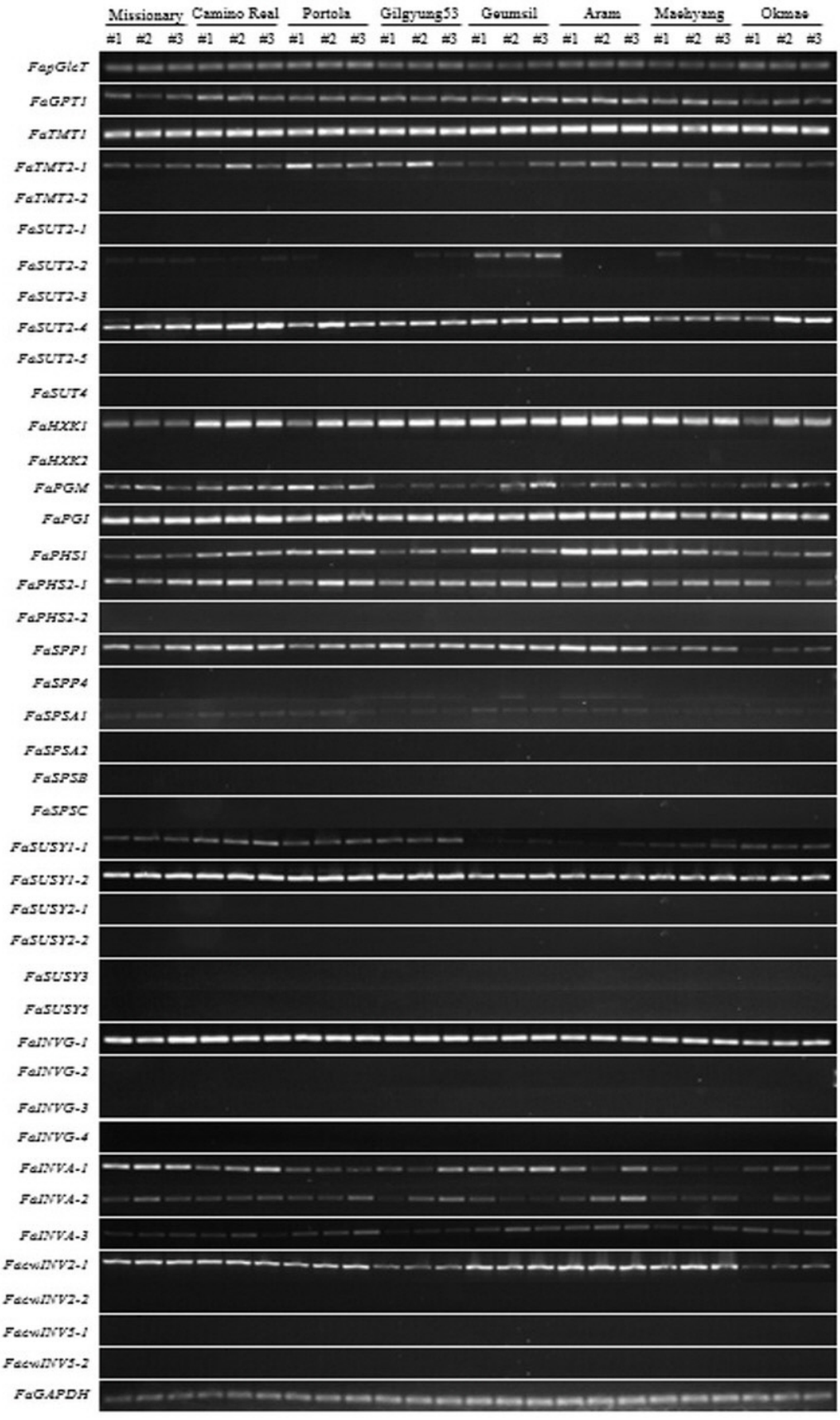
Supplementary Table 1 RT-PCR primers for gene expression involved in sugar metabolism related genes in strawberry (*Fragaria* × *ananassa*)

Gene name	Forward primers (5'→ 3')	Reverse primers (5'→ 3')
FaGPT1	GATTTTTGGAAGGCTCTGTT	AAGACAAATGCCAAGTTTGA
FapGlcT	GCTTCAAGAATCAGAGCAAA	CAAGAAATACAGGCCAATGA
FaTMT1	TCTTTCAAACTTGGGTCTCA	AGTTGGCAATGACAAGAAGA
FaTMT2-1	ACACCTCAAATTCTTGAGCA	TGCATTCACAACACTACCAA
FaTMT2-2	GAAAAAGGGAGGAGTTCAAA	ACCCCATTTATACCAGCAAT
FaSUT2-1	CTCTTTGCTCTCCTGGGTAT	CTTGCATTCTTAGCTGTTGG
FaSUT2-2	AACATGCTTGAACTGGATTG	ACCAGAGTTGCTGCAAAATA
FaSUT2-3	TCACAAAATTTTTCCCTTCA	CTGTTGGAAAGATCGAAAAA
FaSUT2-4	TTCTCATTCTTCATGGCTGT	AGTTCAAGCAAGTGACCAAA
FaSUT2-5	ACAAAAATGCTATGGGAACA	AAGCATTGAGCATTAAACCA
FaSUT4	AGCTTTTGGTTTGATGTTGA	TAGAACGTGGAATAGCCAAG
FaHXK1	AAATGCAGCATATGTGGAAC	GGTGGAACAATATCACCAAA
FaHXK2	GTTTTGTTGAAGATGGCTGA	TCGGAATTCAGTGTAGTGCT
FaPGM	GAAGGTGCGAATAAGATGATAA	GTGAATTCCTTCAGCTTTGA
FaPGI	TCGATTACAGTTTCTGTGGAA	TAAAACCCGCTTTTGAAGA
FaPHS1	CAACATGAAGTTTGCAATGA	TCTTTGCCAACAAGGAAGTA
FaPHS2-1	TGTTTTTGTCCCAAATTACAA	GCATCTAAATAGCCTGCAAA
FaPHS2-2	TTAAGTCAGCTGGGTTTTGA	GTTGATCACTTGGTTTTCCA
FaSPP1	CAAATTTACCCCCAAGAGAT	CGATTTGTGTTGGTAAAACC
FaSPP4	ACCAACCACAACAAAACTTG	TGTCCATTCTACAGAAACCAA
FaSPSA1	TGCATCTGAACAACTTTCAAC	AGTAAATTACATGGCACCTCAA
FaSPSA2	ACAAGTTGCAGCAGATGAAT	AGGCAACAATGTTTGACAAG
FaSPSB	GAAATAAGCTGCCGGATAAT	GCTTCCAAATGGTTTTCTTT
FaSPSC	TGCTCAAGATGATTGACAAGT	TGTTCTTTAGGCCATTCTTTC
FaSUSY1-1	AATTTGGGGAAGTTCTGAAG	AAGTCCAATTCAAGCACAAA
FaSUSY1-2	TTCCACTCTGAAATCGAAGA	GGATTTGCCATTCACAATAA
FaSUSY2-1	ACGATCGAATTCAGAGCATA	TGCCAAGAAATGTTTCAAGT
FaSUSY2-2	ATGATGAAAATGCATTGGAG	TTTGAAATGGAGTGTCCTTG
FaSUSY3	CTCTTATGGCCGATTTCTTT	TTAACCAAATCACGGAACTG
FaSUSY5	AAACAAAGTGCTGAACATGG	ACGAAGAAGCAATTCCAAAT
FaINVG-1	AAAGTCATGAGTGGCGAATA	CTTTCTTGCAATTTGTGGTC
FaINVG-2	AAACTATGAACCACCGGAAT	GAAGCAATATGATCCACCAA
FaINVG-3	ACGATGTGGGTAAGGAATTT	ATTTTCAAAGGCATTTCTCC
FaINVG-4	GGATGATTTGATTGGTGAGA	AGCAATGAGACATTGTTTGG
FaINVA-1	TCCAAAGCAAAATGAAGCTA	GATTCTGCACCAGCATTTTA
FaINVA-2	CGAATCGGAGAGGTTTTAAT	TCACCAAAGGTTTCACATTC
FaINVA-3	ATTCTGCATTACTTGGAGCA	CCAATCAAATAGCCTCCTTT
FacwINV2-1	CAAGCTAACCAAATCAATGC	TGCACTGTCAAAGAAGGTTT
FacwINV2-2	GGGTGTTAAGCATGTTTTGA	AAACATCTCTGCTTTGTCCA
FacwINV5-1	GAAAAGTTGAGAACGAAGCA	CAGTAAACCAAATGGTCCAA
FacwINV5-2	TCTTCAAACTTCCGAACTGA	CATAATGTTGGGACATGCTT
FaGAPDH	TCCATCACTGCCACCCAGAAGACTG	AGCAGGCAGAACCTTTCCGACAG



Supplementary Fig. 1 Sugar metabolism and accumulation in strawberry fruit (Butowt et al., 2003; Fettke et al., 2009; Rolland et al., 2006). Suc, sucrose; Fru, fructose; Glc, glucose; *SUT*, sucrose transporter; *cwINV*, cell wall invertase; *nINV*, neutral invertase; *vINV*, vacuolar invertase; *UDPG*, UDP-glucose; *SUSY*, sucrose synthase; *G6P*, glucose 6-phosphate; *F6P*, fructose 6-phosphate; *HXK*, hexokinase; *PGI*, phosphoglucoisomerase; *PGM*, phosphoglucomutase; *SPS*, sucrose phosphate synthase; *SPP*, sucrose-phosphatase; *PHS*, phosphorylase; *SUT*, sucrose transporter; *TMT*, tonoplast monosaccharide transporters



Supplementary Fig. 2 Relative mRNA expression for genes involved in sugar metabolism