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Research Article

Sugar content analysis and expression profiling of sugar related genes in contrasting Strawberry (*Fragaria* × *ananassa*) cultivars

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Abstract *Fragaria* × *ananassa*, a strawberry evolved from hybridization between *F. virginiana* and *F. chiloensis,* is a globally cultivated and consumed fruit crop valued for its flavor and nutritional value. Flavor and quality of fruits are determined by factors such as sugars and organic acids present during fruit development**.** These characteristics are highly subjective in different genotypes and affected by various environmental factors. In this study, we analyzed contents of major sugar compounds including fructose, glucose and sucrose by HPLC analysis in four cultivars namely, Maehyang, Seolhyang, Festival and Sweet Charlie. We identified 55 genes related to fructose, glucose, sucrose and soluble sugar regulation whose expression were analyzed in four cultivars at three developmental stages of the fruit namely, green, white and ripened stages. Expression of these genes across these progressive fruit developmental stages varied among cultivars. Among the 55 genes, genes FaFru3, FaSuc11 and FaGlu8 revealed differential patterns of expression along developmental stages of the fruit in high and low sugar-containing genotypes, respectively and may be putative candidates for sugar content in strawberries. Expression of genes are discussed with regard to corresponding sugar content in these genotypes. Further analysis and application of these genes may be valuable in developing high sugar containing cultivars via marker-assisted breeding.

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Keywords *Fragaria* × *ananassa*, Sugar content, Sugar-related genes, Expression profiling

Introduction

Fragaria × *ananassa*, the cultivated strawberry evolved by hybridization between *F. virginiana* and *F. chiloensis,* is globally cultivated fruit crop (Molina-Hidalgo et al. 2013; Bestfleisch et al. 2014). It is the rich source of health-promoting phytonutrients (e.g., vitamin C, vitamin B-6, niacin, riboflavin, pantothenic acid, vitamin-E and folate, etc.), flavonoid poly phenolic antioxidants (e.g., lutein, zeaxanthin, and beta-carotene etc.) and minerals (e.g., potassium, manganese, fluorine, copper, iron and iodine) (Wang, Shiow Y., Lin 2000). The consumption of strawberry is known to lower the risk of inflammation, hypertension, cardiovascular diseases and cancer (Basu et al. 2014). Strawberry is thus widely consumed, fresh or in processed forms, making it one of the economically and commercially more important fruit crop and the most studied berries from the agronomic, genomic, and nutritional characteristics (Giampieri et al. 2013). Many conventional and biotechnological approaches were applied to develop the stress tolerance, high quality and flavored strawberry cultivars (Molina-Hidalgo et al. 2013; Bestfleisch et al. 2014).

The sweet flavor and quality of fruits are determined by factors such as sugars and organic acids (Kallio et al. 2000). Due to its inherent chemical composition, strawberry is naturally sensitive fruit rendering the quality of the fruits being affected by environmental factors. Moreover, ripening of strawberry fruits is complex process which includes the participation of a plethora of hormones, sugars and other volatile compounds (Kafkas et al. 2007).

The sweetness, an important sensory quality of the fruit is largely determined by the total soluble sugars and major carbohydrates such as glucose, sucrose and fructose etc.

The contents of these sugars and carbohydrates are variable based on the genotypes and environmental factors (Macías-Rodríguez et al. 2002; Gündüz and Özdemir 2014). Of which, glucose is particularly abundant carbohydrate whereas sucrose is present at low concentration in many strawberry cultivars (Basson et al. 2010; Gündüz and Özdemir 2014).

Sucrose, glucose and fructose are the three major sugars which increases significantly in accordance with fruit development and accounts of 99% of the total sugar content (Jia et al. 2013). Among these three major sugars, sucrose content is highly responsible for fruit development whereas glucose and fructose was not much influenced during fruit development. Sucrose plays an important role in fruit ripening by regulation of ripening-related genes (Jia et al. 2013). In addition, the sucrose and glucose induces the biosynthesis of ABA which is regulating the strawberry fruit development by regulating the ripening related genes (Jia et al. 2013; Jia et al. 2016). Therefore, the soluble sugars and major carbohydrates including sucrose, glucose and fructose are among the major determinants of the growth, development, sweetness and overall quality of the strawberry fruits. Our study thus focuses on broadening the understanding of the roles of sugar regulatory and biosynthetic genes in the developmental process of strawberry fruits via analyzing the contents of sugar and the relative expression of total sugar related genes in contrasting strawberry cultivars.

Materials and Methods

Collection of strawberry cultivars

For this study, fruit materials of four strawberry cultivars namely, Maehyang, Seolhyang, Festival and Sweet Charlie were grown in two different research facility; Suncheon National University and Chungnam Agricultural Research & Extension, South Korea. The sugar content in fruits of four cultivars were measured by Pocket Refractometer PAL-1 (Atago, Japan) in the year 2016 and the data is compared with previous report by Kim al., (2009) as shown in Table 1.

HPLC analysis of sugar content

Fruit samples were collected at different maturation stage and stored at -80°C. The collected samples were powdered and dissolved in distilled water at the ratio of 2 g in 20 mL ratio. Then the solution was filtered through 0.45 μm filter. This filtrate was collected and used for individual sugar analysis by HPLC.

Gene identification and characterization

The sugar related genes were manually identified from Fvesca_V1.0_genemark_hybrid annotation available from the Strawberry garden database (http://strawberry-garden.kazusa.or.jp/). The gene sequences were blasted against the *Fragaria* × *ananassa* draft genome (FANhybrid_r1.2_cds) available from the same database. Conserved functional domains were analyzed for identified genes by using CDD (Conserved Domain Database, http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (Marchler-Bauer et al. 2015) database. The gene description, and gene ontology (GO) were carried by using Blast2Go (https:// www.blast2go.com) for the sugar related genes (Natarajan et al. 2016).

Expression analysis

Samples of leaf and three stages of fruit development; green, white and ripening stages were used for total RNA isolation by using RNeasy mini kit (Qiagen, USA) based on manufacturer's protocol. RNAse free DNase (Qiagen, USA) was added to remove genomic DNA contamination. The cDNA was synthesized from isolated RNA samples by using Superscript III® First-strand Synthesis Supermix kit (Invitrogen, USA). ND-1000 Spectrophotometer and NanoDrop v3.7 software were used for quantification and purity determination (NanoDrop Technologies, USA).

Gene-specific primers were designed by using the primer3 software in coding DNA sequences to perform RT-PCR and Real-time PCR (qPCR). We used the actin as the reference gene for expression analysis (Amil-Ruiz et al. 2013; Galli

Table 1 Sugar content of four strawberry cultivars measured by Pocket Refractometer PAL-1

Cultivars	Fruit Soluble Sugar Content (SSC) (Brix)		
	Kim, et al. (2009)	Chungnam Agriculture and Extension (2016)	Sunchon National University (2016)
Maehyang	12.7	9.1	10.9
Seolhyang	12.8	9.1	10.1
Festival	-	6.1	8.7
Sweet Charlie	6.4	4.3	7.9

et al. 2015). RT-PCR was performed by using 50 ng cDNA (1 μL), 20 pmol primer pairs (2 μL), Emerald master mix (8 L), and H_2O with total volume of 20 μL. Thermal cycler was set at 30 cycles of 60°C for 30 s and 72°C for 45 s, followed by a final extension at 72°C for 5 min. The PCR products were visualized on a 2% agarose gel. For qRT-PCR, 1 μL cDNA with 10 μL iTaq SYBR Green Super-mix with ROX (California, USA) was used with three step amplification (annealing temperature 60°C) and melting peak with three biological replicates. Amplification, detection, and data analysis were carried out using a Light Cycler® 96 Instrument (Roche Diagnostics, United States). Single melting peak was considered for the primer specificity (Shanmugam et al. 2016; Vijayakumar et al. 2016).

Results

Sugar content analysis in different strawberry cultivars

The HPLC analysis was used to analysis the sugar content in different cultivars. HPLC analysis showed Maehyang and Seolhyang as high sugar content cultivar (12.23 and 14.10 g/100g, respectively) whereas Festival and Sweet Charlie as low sugar content (5.05 and 4.78 g/100g, respectively) cultivar based on their fructose, glucose, sucrose and total sugar content (Table 2). Overall, highest total sugar content is present in the cultivar Seolhyang (14.10 g/100g) followed by Maehyang (12.23 g/100g). Among the sugars detected, Seolhyang contained more fructose and glucose (4.22 and 4.30 g/100g, respectively) compared to the other high sugar containing cultivar Maehyang and to low sugar containing cultivars Festival and Sweet Charlie where the content of fructose and glucose ranged between 1.95 -2.41 g/100g (Table 2 and Fig. S1). Unlike, fructose and glucose, sucrose content was the highest in Meahyang (7.80 g/100g) compared to Seolhyang (5.22 g/100g) (Table 2 and Fig. S1). The other sugar, Maltose is detected at very trace level in the high sugar containing genotypes Maehyang and Seolhyang whereas this was not at all detected in low sugar containing genotypes.

Identification of sugar-related genes

In this study, we identified 6 fructose related genes, 16 glucose-related genes, 18 sucrose-related genes and 15 total soluble sugar-related genes (55 in total) from Fvesca_V1.0_genemark_hybrid annotated available from the Strawberry garden database (http://strawberry-garden.kazusa.or.jp/) as shown with their description (Table 3-6). The functional

Table 2 Sugar content in the four Strawberry cultivars measured by HPLC

Cultivars	Sugars content in g/100 g				
	Fructose	Glucose	Sucrose	Maltose	Total
Maehyang	2.41	2.01	7.80	0.007752	12.23
Seolhyang	4.22	4.30	5.22	0.358124	14.10
Festival	2.39	2.07	0.59	Not detected	5.05
Sweet Charlie	2.07	1.95	0.76	Not detected	4.78

g- Gram

Table 3 Fructose-related genes identified in *Fragaria* × *ananassa*

Name	Gene id	CDS length (bp)	Protein length (aa)	Description*
FaFru1	FANhyb_icon00023264_a.1.g00001.1	428	143	pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit beta-like
FaFru2	FANhyb_rscf00002565.1.g00001.1	1755	584	fructose-bisphosphate aldolase chloroplastic
FaFru3	FANhyb_rscf00000635.1.g00002.1	867	288	glutamine--fructose-6-phosphate aminotransferase
FaFru4	FANhyb_rscf00002698.1.g00001.1	1390	462	chloroplast fructose-1,6-bisphosphatase I
FaFru5	FANhyb_rscf00000435.1.g00002.1	1296	431	pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha
FaFru6	FANhyb_rscf00001420.1.g00002.1	1053	350	fructose-bisphosphate aldolase cytoplasmic isozyme-like isoform X1

bp - base pair, aa - amino acid. *BLAST search against NCBI NR database using BLAST2GO.

Table 4 Glucose-related genes identified in *Fragaria* × *ananassa*

Name	Gene id	CDS length (bp)	Protein Length (aa)	Description*
FaGlu1	FANhyb_rscf00000042.1.g00027.1	1647	548	glucose-6-phosphate 1-dehydrogenase chloroplastic
FaGlu2	FANhyb_rscf00000135.1.g00004.1	1449	482	UDP-glucose 6-dehydrogenase 1
FaGlu3	FANhyb_rscf00001884.1.g00003.1	1188	395	glucose-6-phosphate phosphate translocator chloroplastic-like
FaGlu4	FANhyb_rscf00000034.1.g00011.1	1443	480	UDP-glucose 6-dehydrogenase 1
FaGlu5	FANhyb_rscf00000166.1.g00017.1	1581	526	glucose-1-phosphate adenylyltransferase large subunit chloroplastic amyloplastic
FaGlu6	FANhyb_rscf00000097.1.g00020.1	1788	595	glucose-6-phosphate 1- chloroplastic
FaGlu7	FANhyb_rscf00001267.1.g00002.1	1461	486	plastidic glucose transporter 4
FaGlu8	FANhyb_rscf00000021.1.g00018.1	1761	586	glucose-6-phosphate cytosolic
FaGlu9	FANhyb_rscf00000482.1.g00001.1	1566	521	glucose-1-phosphate adenylyltransferase large subunit 1-like
FaGlu10	FANhyb_rscf00002113.1.g00001.1	2424	807	glucose-6-phosphate 1- chloroplastic-like
FaGlu11	FANhyb_rscf00000504.1.g00004.1	1764	587	glucose-6-phosphate 1- chloroplastic
FaGlu12	FANhyb_rscf00000013.1.g00018.1	1443	480	UDP-glucose 6-dehydrogenase 5
FaGlu13	FANhyb_rscf00000040.1.g00031.1	1041	346	probable plastidic glucose transporter 2
FaGlu14	FANhyb_rscf00001636.1.g00003.1	1059	352	UTP--glucose-1-phosphate uridylyltransferase
FaGlu15	FANhyb_rscf00000538.1.g00003.1	1047	348	UDP-glucose 4-epimerase GEPI48-like
FaGlu16	FANhyb_rscf00002773.1.g00002.1	687	228	bifunctional UDP-glucose 4-epimerase and UDP-xylose 4-epimerase 1

bp - base pair, aa - amino acid. *BLAST search against NCBI NR database using BLAST2GO.

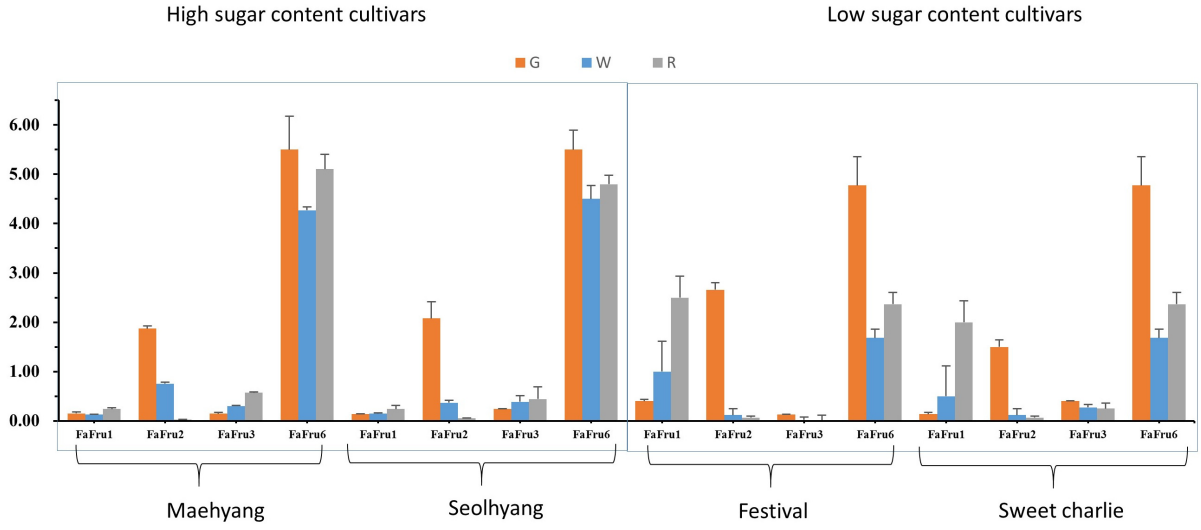

Fig. 1 Expression profiling of fructose related genes in contrasting strawberry cultivars

domains were determined for the identified genes and are shown in (Supplementary Table 1-4). Further, gene ontology and functional analysis were determined for the identified sugar-related genes in strawberry (Supplementary Table 5-8 and Fig. S2).

Expression profiling of Fructose related genes

Among six fructose related genes, expression of four genes were expressed in the four strawberry cultivars (Fig. 1). The other two genes were not expressed and hence, was not included in our results. The results are shown in (Fig. 1). The genes showed variable patterns of expression during different developmental stages in different cultivars. Only the gene FaFru3 showed contrasting patterns of expression between high and low sugar containing cultivars. It's expression increased with the progress in the maturation stages fruit in both the high sugar containing cultivars, but

Table 5 Sucrose-related genes identified in *Fragaria* × *ananassa*

Name	Gene id	CDS length (bp)	Protein length (aa)	Description*
FaSuc1	FANhyb_rscf00001083.1.g00004.1	2271	756	probable galactinol--sucrose galactosyltransferase 1
FaSuc2	FANhyb_rscf00000994.1.g00002.1	1983	659	sucrose transport SUC3
FaSuc3	FANhyb_rscf00000541.1.g00007.1	2520	839	probable sucrose-phosphate synthase 3
FaSuc4	FANhyb_rscf00007596.1.g00001.1	970	322	sucrose synthase 2
FaSuc5	FANhyb_rscf00001056.1.g00003.1	780	259	sucrose transport SUC2-like
FaSuc6	FANhyb_rscf00004885.1.g00001.1	1340	445	sucrose synthase
FaSuc7	FANhyb_rscf00000111.1.g00005.1	3006	1001	probable sucrose-phosphate synthase 1
FaSuc8	FANhyb_rscf00000334.1.g00004.1	2421	806	sucrose synthase
FaSuc9	FANhyb_rscf00000021.1.g00004.1	1488	495	sucrose transport -like
FaSuc10	FANhyb_rscf00000021.1.g00005.1	951	316	sucrose transport SUC2-like
FaSuc11	FANhyb_rscf00000142.1.g00011.1	2211	736	probable galactinol--sucrose galactosyltransferase 2
FaSuc12	FANhyb_icon00036727_a.1.g00001.1	335	111	sucrose transport SUC2-like
FaSuc13	FANhyb_rscf00000011.1.g00010.1	2622	873	probable galactinol--sucrose galactosyltransferase 6
FaSuc14	FANhyb_rscf00000011.1.g00003.1	5379	1792	probable sucrose-phosphate synthase 1
FaSuc15	FANhyb_rscf00001350.1.g00002.1	2991	996	probable sucrose-phosphate synthase 4
FaSuc16	FANhyb_rscf00000207.1.g00012.1	2454	817	sucrose synthase 7
FaSuc17	FANhyb_icon00016073_a.1.g00001.1	569	189	galactinol--sucrose galactosyltransferase-like
FaSuc18	FANhyb_rscf00001543.1.g00001.1	3195	1064	sucrose transport SUC2-like

bp - base pair, aa - amino acid. *BLAST search against NCBI NR database using BLAST2GO.

Table 6 Soluble sugar-related genes identified in *Fragaria* × *ananassa*

Name	Gene id	CDS length (bp)	Protein length (aa)	Description*
FaSug1	FANhyb_rscf00004633.1.g00001.1	1036	344	sugar transport 13-like
FaSug2	FANhyb_rscf00003570.1.g00001.1	1611	536	sugar transport 13-like
FaSug3	FANhyb_rscf00004805.1.g00001.1	955	317	sugar transport 13-like
FaSug4	FANhyb_rscf00000050.1.g00021.1	1473	490	sugar transporter ERD6-like 16 isoform X1
FaSug5	FANhyb_rscf00000071.1.g00017.1	1734	577	UDP-sugar pyrophosphorylase
FaSug6	FANhyb_rscf00000006.1.g00036.1	1431	476	sugar transport 14
FaSug7	FANhyb_rscf00000046.1.g00026.1	1560	519	sugar carrier C
FaSug8	FANhyb_rscf00000069.1.g00020.1	1566	521	sugar transport 10-like
FaSug9	FANhyb_rscf00001892.1.g00002.1	1266	421	sugar transporter ERD6-like 3
FaSug10	FANhyb_icon00015561_a.1.g00001.1	364	121	sugar transporter ERD6-like 7
FaSug11	FANhyb_rscf00007047.1.g00001.1	1112	370	sugar transport 10-like
FaSug12	FANhyb_rscf00005538.1.g00001.1	1506	501	sugar transport 5-like
FaSug13	FANhyb_rscf00004023.1.g00001.1	1506	501	sugar transport 5
FaSug14	FANhyb_icon00014335_a.1.g00001.1	1560	519	sugar carrier C
FaSug15	FANhyb_rscf00000458.1.g00001.1	1461	486	sugar carrier C-like

bp - base pair, aa - amino acid. *BLAST search against NCBI NR database using BLAST2GO.

in contrast, the expression of the gene decreased with fruit maturation stages in low sugar containing cultivars (Fig. 1). The FaFru1 showed lower expression in high sugar content cultivars and it's expression remained same in different stages (e.g., green, white and ripe stages) of fruit development. In contrast, this gene showed increasingly higher expression along with the progressive developmental stages of fruit in low sugar content cultivars. The expression FaFru2 decreased with the different stages of the fruit development in both high and low sugar content cultivars. The gene FaFru6 showed similar patterns of expression in all four cultivars (Fig. 1).

Expression profiling of Glucose related genes

Among 16 glucose related genes, six genes were selected based on their expression in four cultivars (Fig. 2). Like the fructose related genes, the glucose related genes also

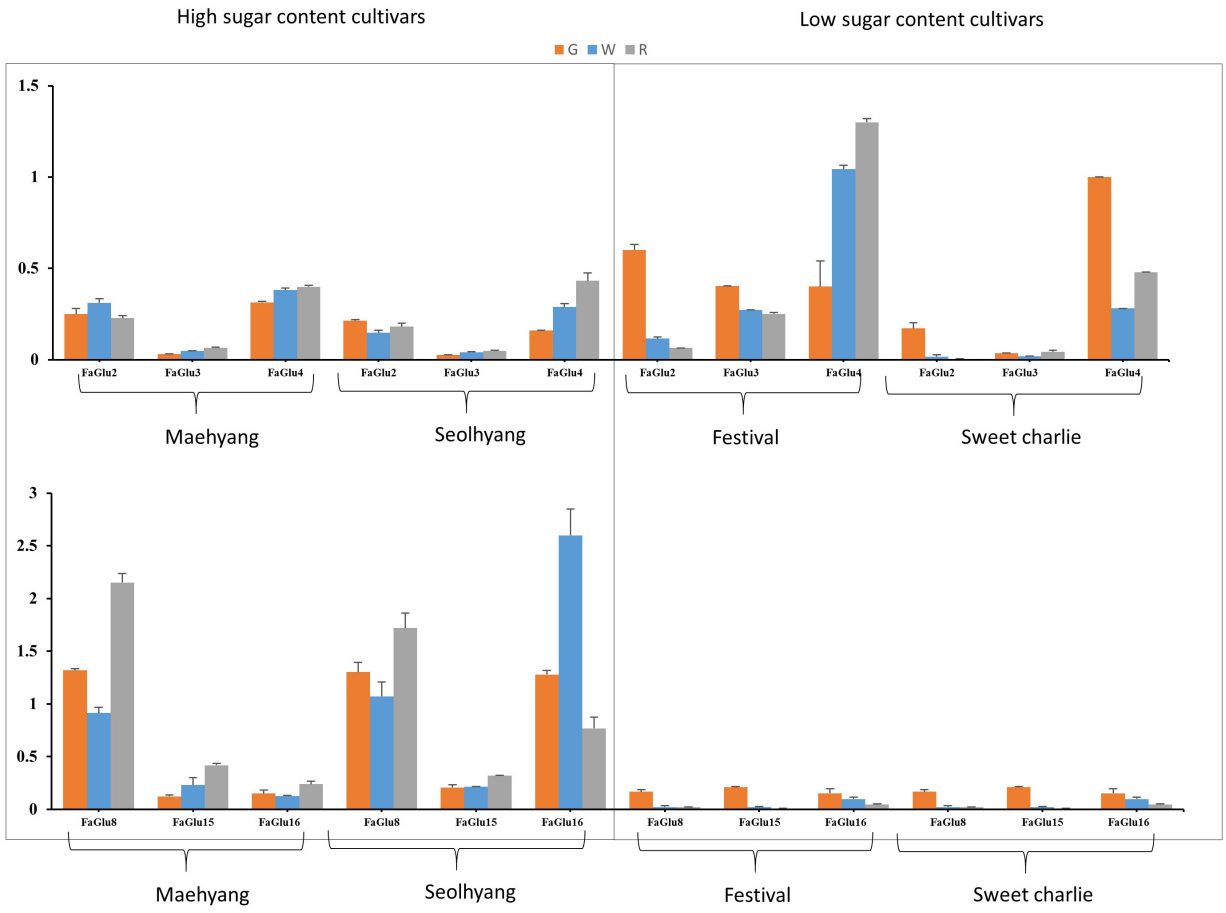

Fig. 2 Expression profiling of glucose related genes in contrasting strawberry cultivars

showed variable expression in different fruit developmental stages of the four studied cultivars (Fig. 2). The gene FaGlu3 showed contrastingly higher and lower expression along the progressive fruit developmental stages in high and low sugar containing cultivars, respectively. Its expression increased in high sugar containing cultivars whereas it decreased in low sugar containing cultivars along the fruit maturation stages. The expression of the gene FaGlu2 decreased with the progressive fruit developmental stages in all four cultivars. The expressions of the genes FaGlu8, FaGlu15 and FaGlu16 were very low in low sugar content cultivars compared to that of in high sugar containing cultivars (Fig. 2).

Expression profiling of Sucrose related genes

Among 18 sucrose related genes, eight genes were considerably expressed in all strawberry cultivars (Fig. 3). However, these genes showed higher expression in high sugar content cultivars compared to the low sugar content cultivars. The genes FaSuc1 and FaSuc11 showed increasingly higher expression whereas the FaSuc7 showed decreasing patterns of expression in high sugar content cultivars along the fruit maturation stages. However, rest of the genes showed unique expression between two high sugar content cultivars (Fig. 3).

Expression profiling of soluble sugar-related genes

Among 15 soluble sugar-related genes, seven were considerably expressed in four cultivars (Fig. 4). The genes FaSug10, FaSug12 and FaSug14 showed contrasting patterns of expression between the high and low sugar containing cultivars where the expression increased with the progress of fruit development in high sugar cultivars whereas decreased in low sugar cultivars (Fig. 4). The genes FaSug2, FaSug5 and FaSug7 showed decreasing patterns of expression along the development of fruit in all four cultivars.

Discussion

Strawberry fruits are mainly preferred for their characteristic flavor and color (besides it's nutritional value) around the world. Sweetness is one of the most important quality traits of strawberry that determines the consumer's pre-

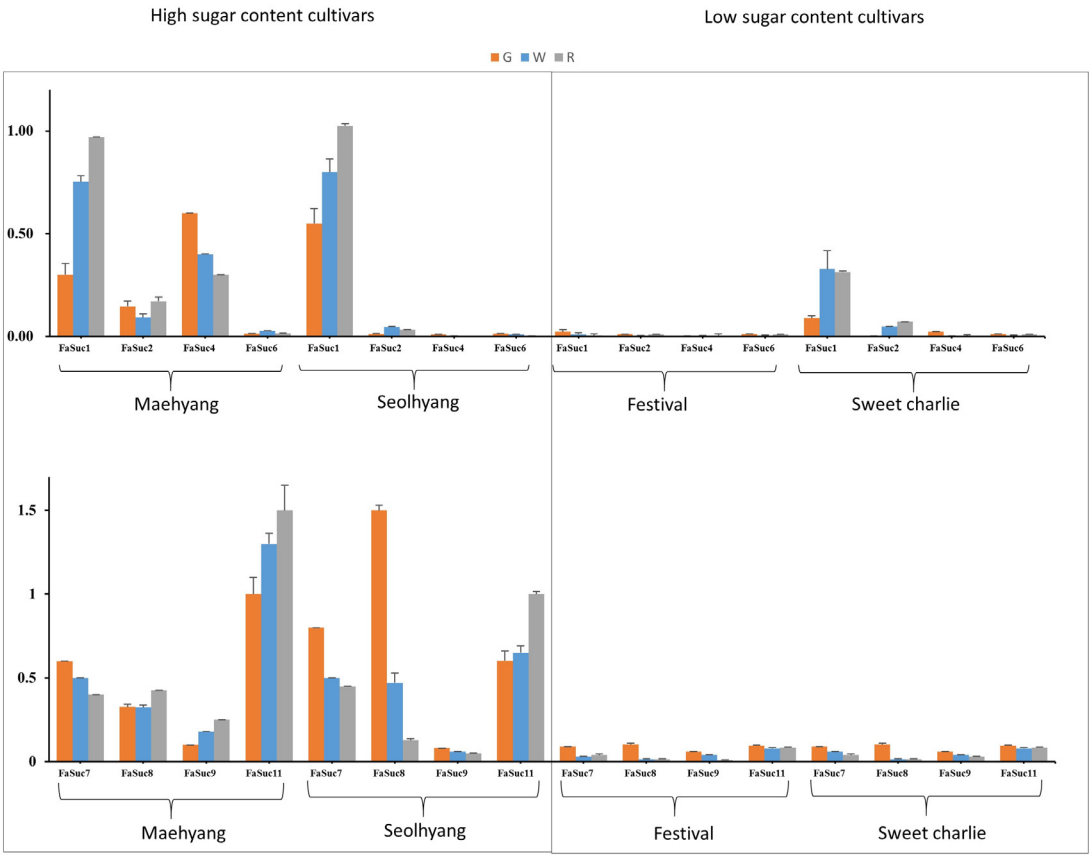

Fig. 3 Expression profiling of sucrose related genes in contrasting strawberry cultivars

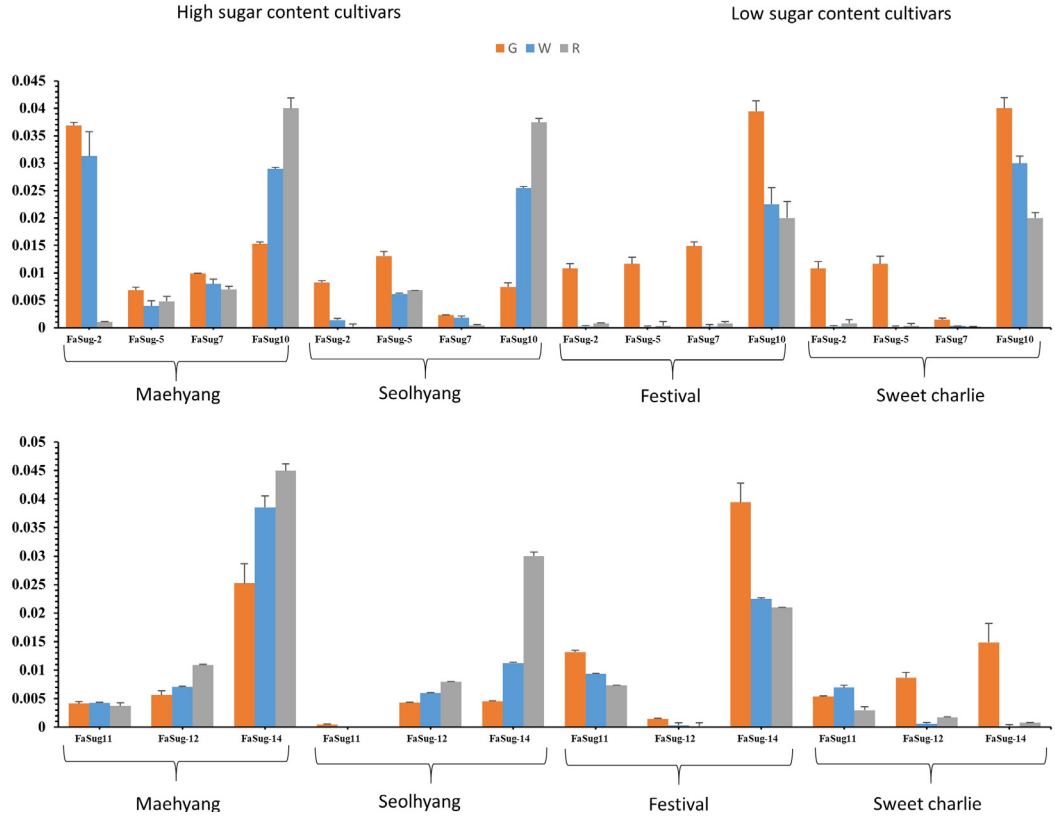

Fig. 4 Expression profiling of soluble sugar related genes in contrasting strawberry cultivars

ference (Sturm et al. 2003; Gündüz and Özdemir 2014). The need for developing high sugar containing strawberry cultivars are thus of paramount importance to breeders. Understanding the role of the associated genes that regulates the biosynthesis of sugar is thus one of the key research areas in a way to identify the novel candidates for potential points of intervention in breeding programs. We analyzed the sugar content in four cultivars namely Maehyang, Seolhyang, Festival and Sweet Charlie and identified that first two are high sugar content and the second two are low sugar containing. This finding is in substantiated with the previous report of Kim et al.., 2009. The sugar profiles of these four genotypes were dominated by sucrose content and there is marked differences among the high vs low sugar content genotypes. For example, sucrose contents in both high sugar content genotypes Maehyang and Seolhyang are almost six times higher than that of the low sugar content genotypes Festival and Sweet Charlie (Table 2). However, no such obvious differences is observed between the low and high sugar content genotypes for Fructose and Glucose content making sucrose as the most important sugar to study the variations in sugar content in strawberry fruit. In strawberry, the variation in sugar and carbohydrate levels across genotypes and fruit developmental stages are diverge which can be pertained to the complexity of the sugar metabolism (Kafkas et al. 2007; Crespo et al. 2010; Mishra et al. 2015). The high sugar content in strawberry cultivars are generally responsible for its characteristic sweet flavor which increases the consumer preference and hence, the market value (Crespo et al. 2010).

Mechanism of fruit ripening is the complex process, which requires numerous fruit ripening and sugar related genes. These genes regulates the sugar metabolism, biosynthesis of hormones and ripening during the fruit development and growth (Jia et al. 2016; Zhongjie et al. 2016). Identifying the genes related to biosynthesis of total soluble sugars, glucose, sucrose and fructose would be helpful to widen our understanding on the variation in the biosynthesis of sugar in different cultivars and to identify the novel candidates as points of interventions for breeding programs. With this focus, we have mined the Strawberry garden database (http://strawberry-garden.kazusa.or.jp/) and identified 55 genome wide sugar related genes. Expression profiling of these genes via quantitative real-time PCR revealed that the expression of these sugar related genes vary during different stages of fruit development in the high and low sugar containing cultivars.

Most of the glucose and sucrose related genes were downregulated or showed lower expression in low sugar content cultivar compared to high sugar content cultivar, which might be responsible for sugar regulation in strawberry fruit in accordance with fruit development (Sturm et al. 2003; Basson et al. 2010; Jia et al. 2013). The expression profiling showed the considerable differential expression of glucose, sucrose and total soluble sugar related between contrasting cultivars based on sugar content. Among the genes involved in fructose biosynthesis, only the gene FaFru3 showed contrasting patterns of expression between high and low sugar-content cultivars across the fruit developmental stage in high and low sugar containing cultivars. The increasing patterns of expression in the progressive fruit developmental stages in the high sugar containing cultivars (and the decrease in the expression of this genes in low sugar containing cultivars) is certainly linked with the higher content of sugar in cultivars Mayhyang and Seolhyang. This makes it one of the candidate for further studying the fructose content in these genotypes. Similarly, the increasingly higher expression in high sugar containing cultivars and decreasing expression in low sugar containing cultivars along the fruit maturation stages of the glucose related gene FaGlu3 makes it the most important candidate for high sugar content in strawberry.

Among the various sugar compounds, sucrose dominated the sugar profile and thus genes that are involved in sucrose content will be very important candidate for any breeding program to develop high sugar containing strawberry cultivars (Jia et al. 2013; Jia et al. 2016). Among the 18 sucrose related genes studied, eight genes showed higher expression in high sugar content cultivars compared to the low sugar content cultivars. The genes FaSuc11 showed increasingly higher expression whereas the FaSuc7 showed decreasing patterns of expression in high sugar content cultivars along the fruit, maturation stages making this gene the most important candidate for sucrose content in these genotypes. The genes thus identified will serve as the target for any strawberry improvement programs for sugar contents.

Conclusion

From this study, we determined the fructose, sucrose and glucose content in contrasting strawberry cultivars. These sugars are responsible for the sweet flavor and quality of strawberry fruit. Among three sugars, sucrose is highly influencing the sweetness of strawberry fruit. Expression profiling of respective sugar related genes are substantiated their role in regulation of different sugar contents in contrasting strawberry cultivars. Expression profiling of respective

sugar related genes substantiated their role in regulation of different sugars in relation with the sugar content. From the expression profiling, we find out some higher differentially expressed genes namely FaFru3, FaSuc11 and FaGlu8. Further, analysis and application of these genes will be helpful for marker-assisted selection followed by development of high sugar content cultivars.

Acknowledgement

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Conflict of Interest

The authors declare that there are no conflicts of interest exists.

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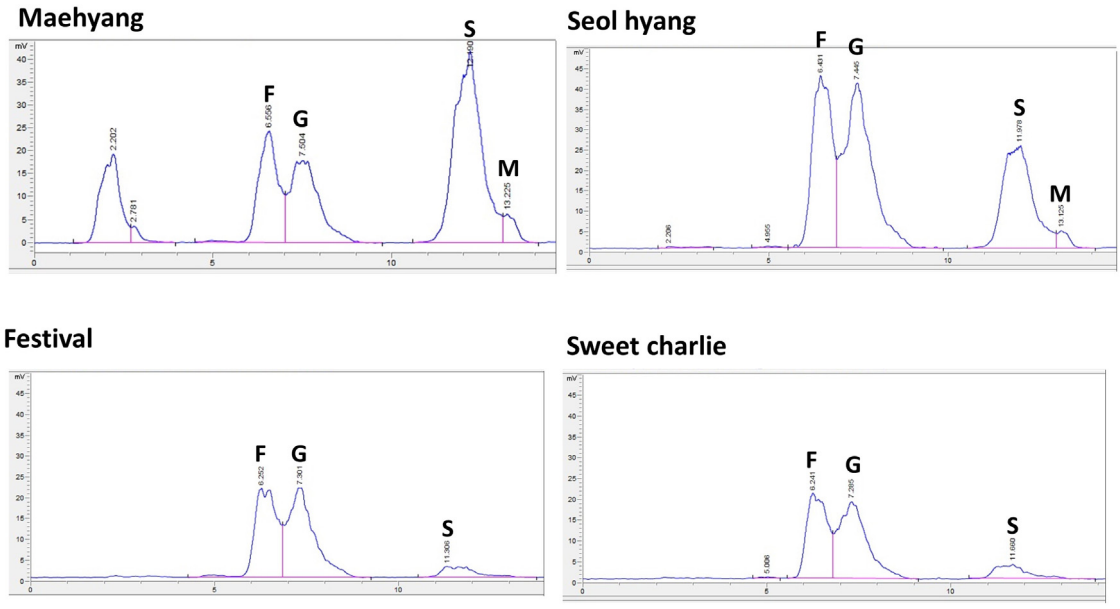

F - Fructose, G - Glucose, S - Sucrose, M - Maltose

Fig. S1 Sugar content analysis of strawberry cultivars by HPLC

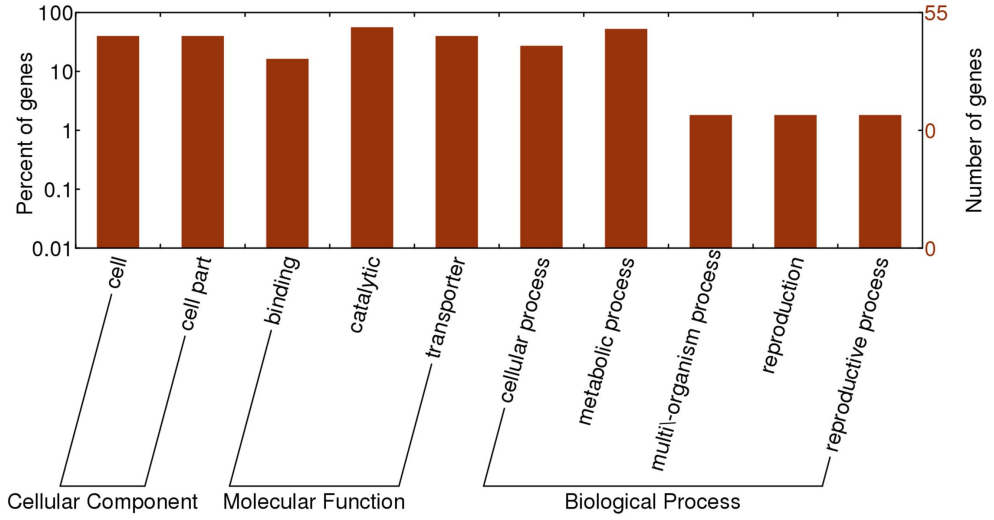

Fig. S2 GO classification of sugar-related genes

Table S1. Functional domains in fructose-related genes

Name	Hit type	From	To	Name
FaFru1	superfamily	3	143	PFK superfamily
FaFru2	superfamily	264	568	ICL_KPHMT superfamily
FaFru3	superfamily	1	288	GlmS superfamily
FaFru4	superfamily	72	462	FIG superfamily
FaFru5	superfamily	8	429	PFK superfamily
FaFru6	specific	3	350	PLN02455

Table S2. Functional domains in glucose-related genes

Name	Hit type	From	To	Name
FaGlu1	superfamily	143	536	G6PD_C superfamily
FaGlu2	specific	1	475	PLN02353
FaGlu3	specific	98	387	TPT
FaGlu4	specific	1	473	PLN02353
FaGlu5	specific	90	526	PLN02241
FaGlu6	superfamily	1	595	G6PD_C superfamily
FaGlu7	specific	106	485	Sugar_tr
FaGlu8	specific	1	576	PLN02649
FaGlu9	specific	85	521	PLN02241
FaGlu10	superfamily	314	804	G6PD_C superfamily
	superfamily	1	300	PLN02193 superfamily
FaGlu11	superfamily	1	584	G6PD_C superfamily
FaGlu12	specific	1	473	PLN02353
FaGlu13	superfamily	1	342	Sugar_tr superfamily
FaGlu14	superfamily	5	352	Glyco_tranf_GTA_type superfamily
FaGlu15	specific	1	346	PLN02240
FaGlu16	superfamily	1	226	NADB_Rossmann superfamily

Table S3. Functional domains in sucrose-related genes

Name	Hit type	From	To	Name
FaSuc1	specific	1	756	PLN02355
FaSuc2	superfamily	70	589	GPH_sucrose superfamily
FaSuc3	superfamily	1	833	PLN00142 superfamily
FaSuc4	superfamily	2	322	PLN00142 superfamily
FaSuc5	superfamily	9	247	GPH_sucrose superfamily
FaSuc6	superfamily	1	441	PLN00142 superfamily
FaSuc7	superfamily	112	996	PLN00142 superfamily
FaSuc8	specific	1	806	PLN00142
FaSuc9	superfamily	13	483	GPH_sucrose superfamily
FaSuc10	superfamily	13	316	GPH_sucrose superfamily
FaSuc11	superfamily	1	725	AmyAc_family superfamily
FaSuc12	superfamily	1	111	GPH_sucrose superfamily
FaSuc13	superfamily	144	873	AmyAc_family superfamily
FaSuc14	superfamily	819	1788	PLN00142 superfamily
	superfamily	91	759	Pnp superfamily
FaSuc15	superfamily	1	992	PLN00142 superfamily
FaSuc16	specific	2	797	PLN00142
FaSuc17	superfamily	1	189	AmyAc_family superfamily
FaSuc18	superfamily	590	1051	GPH_sucrose superfamily
	superfamily	403	558	PKc_like superfamily
	specific	211	295	PAN_AP_plant
	specific	8	47	B_lectin
	superfamily	138	196	S_locus_glycop superfamily

Table S4. Functional domains in soluble sugar-related genes

Name	Hit type	From	To	Name
FaSug1	superfamily	3	322	Sugar_tr superfamily
FaSug2	specific	29	461	Sugar_tr
FaSug3	superfamily	6	291	Sugar_tr superfamily
FaSug4	specific	52	476	Sugar_tr
FaSug5	specific	10	572	PLN02830
FaSug6	specific	30	450	Sugar_tr
FaSug7	specific	26	488	Sugar_tr
FaSug8	specific	28	495	Sugar_tr
FaSug9	superfamily	9	413	Sugar_tr superfamily
FaSug10	superfamily	1	117	Sugar_tr superfamily
FaSug11	superfamily	27	367	Sugar_tr superfamily
FaSug12	specific	28	489	Sugar_tr
FaSug13	specific	9	439	Sugar_tr
FaSug14	specific	26	488	Sugar_tr
FaSug15	specific	27	460	Sugar_tr

Table S5. Gene ontology (GO) of fructose-related genes

Name	No. of Go	GO Names list
FaFru1	5	P:carbohydrate metabolic process; P:generation of precursor metabolites and energy; P:catabolic process; F:kinase activity; P:nucleobase-containing compound metabolic process
FaFru2	5	P:carbohydrate metabolic process; P:generation of precursor metabolites and energy; F:catalytic activity; P:catabolic process; P:nucleobase-containing compound metabolic process
FaFru3	2	P:metabolic process; F:binding
FaFru4	3	P:carbohydrate metabolic process; P:cellular process; F:hydrolase activity
FaFru5	2	P:metabolic process; F:binding
FaFru6	5	P:carbohydrate metabolic process; P:generation of precursor metabolites and energy; F:catalytic activity; P:catabolic process; P:nucleobase-containing compound metabolic process

Table S6. Gene ontology (GO) of glucose-related genes

Name	No. of Go	GO Names list
FaGlu1	3	F:nucleotide binding; P:carbohydrate metabolic process; F:catalytic activity
FaGlu2	3	F:nucleotide binding; F:catalytic activity; P:metabolic process
FaGlu3	2	C:membrane; F:transporter activity
FaGlu4	3	F:nucleotide binding; F:catalytic activity; P:metabolic process
FaGlu5	2	P:biosynthetic process; F:transferase activity
FaGlu6	3	F:nucleotide binding; P:carbohydrate metabolic process; F:catalytic activity
FaGlu7	2	C:membrane; F:transporter activity
FaGlu8	6	P:carbohydrate metabolic process; P:generation of precursor metabolites and energy; F:catalytic activity; P:catabolic process; P:biosynthetic process; P:nucleobase-containing compound metabolic process
FaGlu9	2	P:biosynthetic process; F:transferase activity
FaGlu10	4	F:nucleotide binding; P:carbohydrate metabolic process; F:protein binding; F:catalytic activity
FaGlu11	3	F:nucleotide binding; P:carbohydrate metabolic process; F:catalytic activity
FaGlu12	3	F:nucleotide binding; F:catalytic activity; P:metabolic process
FaGlu13	2	P:carbohydrate metabolic process; F:catalytic activity
FaGlu14	2	P:metabolic process; F:transferase activity
FaGlu15	2	P:carbohydrate metabolic process; F:catalytic activity
FaGlu16	2	C:membrane; F:transporter activity

Table S7. Gene ontology (GO) of sucrose-related genes

Name	No. of Go	GO Names list
FaSuc1	1	F:catalytic activity
FaSuc2	2	C:plasma membrane; F:transporter activity
FaSuc3	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc4	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc5	2	C:plasma membrane; F:transporter activity
FaSuc6	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc7	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc8	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc9	2	C:plasma membrane; F:transporter activity
FaSuc10	2	C:plasma membrane; F:transporter activity
FaSuc11	1	F:catalytic activity
FaSuc12	2	C:plasma membrane; F:transporter activity
FaSuc13	1	F:catalytic activity
FaSuc14	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc15	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc16	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc17	3	P:carbohydrate metabolic process; F:transferase activity; P:cellular process
FaSuc18	4	F:nucleotide binding; P:cellular protein modification process; P:pollen-pistil interaction; F:kinase activity

Table S8. Gene ontology (GO) of soluble sugar-related genes

Name	No. of Go	GO Names list
FaSug1	2	C:membrane; F:transporter activity
FaSug2	2	C:membrane; F:transporter activity
FaSug3	2	C:membrane; F:transporter activity
FaSug4	2	C:membrane; F:transporter activity
FaSug5	2	P:metabolic process; F:transferase activity
FaSug6	2	C:membrane; F:transporter activity
FaSug7	2	C:membrane; F:transporter activity
FaSug8	2	C:membrane; F:transporter activity
FaSug9	2	C:membrane; F:transporter activity
FaSug10	2	C:membrane; F:transporter activity
FaSug11	2	C:membrane; F:transporter activity
FaSug12	2	C:membrane; F:transporter activity
FaSug13	2	C:membrane; F:transporter activity
FaSug14	2	C:membrane; F:transporter activity
FaSug15	2	C:membrane; F:transporter activity