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Original article

Genome-wide identification and analysis of *SAUR* gene family in strawberry (*Fragaria vesca* L.) reveal its potential functions in different developmental stages

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ABSTRACT

Auxin is a plant hormone that is highly associated with various biological processes, especially plant growth, development and fruit ripening. The Small Auxin Upregulated RNA (*SAUR*) genes, whose family is the largest one of early auxin response genes, has received less attention from genome-wide analyzes compared to other gene families. In this study, we successfully conducted a genome-wide analysis of *Fragaria vesca* L. and identified 66 *SAUR* genes. In this paper, we provide important information on the identification of all *SAUR* genes in *Fragaria vesca*, including gene and protein sequences, chromosome mapping, and phylogeny analyzes. Gene expression data from the strawberry eFP Browser demonstrated that *FvSAUR* genes had diversified expression patterns in vegetative tissues. The RT-qPCR analysis demonstrated that 10 selected *SAUR* genes based on eFP strawberry browser could be expressed with expression divergence at least in one of the strawberry organs/tissues tested. Our analysis provides some basic genomic information for the *FvSAUR* genes in strawberry and a foundation for further investigations for deciphering their function during plant development and fruit ripening.

Keywords: auxin, expression analysis, identification, SAUR, strawberry

Introduction

The phytohormone auxins are essential regulators of plant developmental processes, including cell elongation, division, differentiation, root initiation, organ patterning and responses to various stimuli. Auxin mediates these effects at the molecular level by altering the expression of hundreds of genes, including early auxin response gene families, Aux/IAA, Gretchen Hagen3 (*GH3*), and *Small Auxin Up-regulated* RNA (*SAUR*) (Abel & Theologis 1996; Guilfoyle *et al*. 1998; Liscum & Reed 2002). Among these gene families, the *SAUR* is the largest, and the expression of those rapidly and robustly induced by auxin implies that auxin plays a critical role in their transcription (Franco *et al*. 1990). *SAUR*s are also post-transcriptionally

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regulated due to a conserved downstream element (DST) existing in the 3′-untranslated region, which confers highly mRNA instability (McClure & Guilfoyle 1989; Newman *et al*. 1993).

The first *SAUR* gene was identified in elongating soybean hypocotyl sections (McClure & Guilfoyle 1987). Subsequently, homologues of this class have been reported at genome-wide level in many plants, some of those including 80 *SAURs* in apple (Wang *et al*. 2020), 57 *SAURs* in loquat (Gan *et al*. 2020), 65 *SAURs* in watermelon (Zhang *et al*. 2017), 99 and 134 *SAURs* in tomato and potato (Wu *et al*. 2012), and 81 *SAURs* in *Arabidopsis* (Hagen & Guilfoyle 2002). Although many *SAUR* genes have been predicted or identified in many different plant species, only a small number have been functionally characterized (Shin *et al*. 2019). Overexpression of *SAUR19-24* in *Arabidopsis* resulted in increased hypocotyl and leaf size, defective apical hook maintenance, and altered tropic responses, providing clear evidence that *SAUR* genes are important regulators of plant cell expansion (Spartz *et al*. 2012). Ectopic overexpression of *TaSAUR75*, isolated from wheat, enhanced drought and salt tolerance in *Arabidopsis*. Transgenic lines showed longer root structure, higher survival rate, and higher expression level of stress-responsive genes under abiotic stress conditions compared to control plants. In another recent study, overexpression of *AbSAUR1* in *Atropa belladonna* enhanced biomass production by increasing fresh and dry weight (Bai *et al*. 2019).

The first whole-genome sequencing initiative in the Rosoideae was the genome of the woodland strawberry (*Fragaria vesca*, 2n=2x=14), which offers generous advantages for genomic and molecular research of Rosaceae (Shulaev *et al*. 2011). *F. vesca* has become a model plant for understanding the ripening mechanism in non-climacteric fruits where fruit ripening is controlled by abscisic acid (ABA), auxin (IAA), sugar and insensitive to ethylene. (Xie *et al*. 2020).

Characterization of *SAUR* gene families from different plants by formulating better hypotheses regarding physiological and developmental processes is a necessary step. However, as far as we know, no systematic investigation has been reported on the *SAUR* gene family in strawberries. In this study, genome-wide identification of putative *FvSAUR* genes in strawberry was performed to characterize the *SAUR* gene family based on their genomic structures, chromosomal locations, and sequence analyses. Subsequently, the expression profiles of 10 selected *FvSAUR* genes in diverse tissues and ripening stages of fruits were analysed using RT-qPCR. The results of this study will enhance the understanding of the *SAUR* genes as a foundation for future research into the functional roles of *FvSAUR* genes in strawberries.

Materials and methods

Acquisition and identification of FvSAUR genes

The *SAUR* gene sequences of *Arabidopsis* were downloaded from TAIR (The *Arabidopsis* Information Resource (http://www.arabidopsis.org/) and UniProt database (http://www.uniprot.org/) and used as reference sequences. To identify *SAUR* genes in the *Fragaria* genome, downloaded sequences were submitted to the Pfam database (http://pfam.sanger.ac.uk) to obtain the domain architecture of this family. The amino acid sequences of *SAUR* genes were queried in the *Fragaria vesca* genome using Phytozome v13 (https://phytozome-next.jgi.doe.gov/) (Goodstein *et al*. 2012). The Hidden Markov Model (HMM) profiles of *SAURs* were downloaded from PFam database (http://pfam.sanger.ac.uk/) and the HMMER software package was used to verify *SAUR* genes with the best domain e-value cut off as $1e^{-10}$.

Chromosomal localization, sequence and phylogenetic analyzes of FvSAUR genes

Exon/intron information and chromosomal location of *FvSAUR* genes were extracted from the PLAZA (https://bioinformatics.psb.ugent.be/plaza/versions/plaza_v4_5_dicots/) (Van Bel *et al*. 2018), Phytozome v13 (https://phytozome-next.jgi.doe.gov/) (Goodstein *et al*. 2012) and confirmed with NCBI (https://www.ncbi.nlm.nih.gov/). The exon-intron display was constructed according to the Gene Structure Display Server (GSDS, http://gsds.gao-lab.org/) (Guo *et al*. 2007). The location of the *FvSAUR* genes on the chromosome was identified by using MapGene2 (http://mg2c.iask.in/mg2c%5Fv2.1/) (Jiangtao *et al*. 2015). The online PeptideMass (https://web.expasy.org/peptide_mass/) tool was used in analysing to predict the molecular weight and isoelectric point (*pI*) of each *FvSAUR* protein. Predicted subcellular localizations of the *FvSAUR* proteins were determined using the CELLO v2.5 server (http://cello.life.nctu.edu.tw/) (Yu *et al*. 2006).

The MEME (https://meme-suite.org/meme/) is the online tool used to search the motifs of *FvSAURs*. The parameters were set as follows: the site distribution was set to zero or one occurrence per sequence, the number was set to 10, the width was limited to between 6 and 50; and other optional parameters remained default. The MEME motifs were then verified using the Pfam database (http://pfam.sanger.ac.uk/) and the SMART server (http://smart.embl-heidelberg.de/).

Predicted protein-protein interaction network and promoter analyses

The amino acid sequences were used to further analyse the protein–protein interactions of the strawberry *SAUR* proteins. Predicted protein-protein interaction (PPI) networks of *FvSAUR* proteins were analysed using the

STRING v11 (Search Tool for the Retrieval of Interacting Genes; https://string-db.org/) (Szklarczyk *et al*. 2019). To explore *cis*-elements in promoter sequences of strawberry *SAUR* genes, 1000 bp (of genomic DNA sequence upstream of initiation codon (ATG) were downloaded from the Phytozome (https://phytozome-next.jgi.doe.gov/) database. The PlantPAN 3.0 (The Plant Promoter Analysis Navigator) server was employed to identify *cis*-elements related with transcription factor binding sites (TFBSs) in the promoter regions (http://plantpan2.itps.ncku.edu.tw/) (Chow *et al*. 2019).

In Silico gene expression pattern analysis

The transcript relative abundance values of all *F. vesca SAUR* genes from various tissues were obtained from the *F. vesca* transcript abundances datasets (Hawkins *et al*. 2017) in the website of the *F. vesca* electronic fluorescent pictograph browser (eFP) (*F. vesca* eFP bowser: https://bar.utoronto.ca/~asher/efp_strawberry/cgi-bin/efpWeb.cgi). The data were generated from 42 different tissues and stages, and eight RNA-Seq data sets from receptacle parts of ripening fruit of yellow-colored (Yellow Wonder) and red-colored (Ruegen) wild strawberry varieties. In this study, since we focused more on regenerating vegetative tissues (flower, leaf and seedling) and fruit ripening, we only extracted the transcript data of these tissues from the eFP browser. The heatmap was created using 'Clustvis', a web tool to visualize the clustering of the multivariate data tool (https://biit.cs.ut.ee/clustvis/) and all expression values were displayed using the heat map to analyze 'corresponding genes' expression pattern in different tissues.

Plant materials

A *F. vesca* cultivar called 'Ottoman Strawberry' plants was cultivated in a local strawberry producer in Burdur, Turkey. The flower samples and fruits at four different growth stages were harvested at the same time in the month of May 2020. The fruit growth and developmental stages were grouped as follows: Stage 1, small green fruits (S1, 17 days post-anthesis (DPA)); S2, larger green fruits (22 DPA); S3 white fruits (27 DPA); and S4, full red fruits (38 DPA) (Fig. 1). At least three fruits were pooled (at least 10 fruits for S1 stage) and considered as one biological replicate. In total, three biological replicates were harvested from flowers and four different ripening stages. The samples harvested were snap-frozen in liquid nitrogen, and stored at -80 °C for further analysis.

RNA isolation and RT-qPCR

Total RNA was extracted from fine powdered strawberry fruits using PureLink™ Plant RNA Reagent (Thermo Fisher Scientific) in accordance with the manufacturer's instructions. The final concentration of total RNA was quantified by NanoDrop (Epoch Microplate, Biotek) and cDNA was prepared by the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The primers for selected *FvSAUR* genes were designed using primer 3 (http://primer3.ut.ee/) and listed in Table 1. For RT-qPCR analysis, *actin* was used as an internal constitutively expressed gene (Mo *et al*. 2020). The RT-qPCR was performed using CFX96™ Real-Time System (Bio-Rad) using a SYBR® Green Master (Bio-Rad) in the following reaction conditions:

Figure 1. Appearance of cultivar 'Ottoman Strawberry' flower and at four developmental stages.

Table 1. Primers of strawberry *actin* gene (endogenous control) and selected 10 *FvSAUR* genes used for RT-qPCR.

Gene	Forward Sequence	Reverse Sequence
FvSAUR4	ATGGGCGCCTCATATCAGAA	ATGCTCGATCACAACCTCACA
FvSAUR23	GAGTACGGCTTCACCAACCA	GGGAAATGTACCGGACCACC
FvSAUR32	CGACGACGACGACTCAATCA	TTCATCGATTCCGGCTGAGG
FvSAUR35	TGGAAACCAGACAGCTCCAA	TCGCTTCTTCTGGCTCTCTC
FvSAUR36	GGGGAGAGCCAGAAGAAGAGA	GACTCAGCAGCATGTCTCCCA
FvSAUR41	CCCAAAAGGCTATTTCGCAGTC	GGCTTGACTCAGCAAATCCA
FvSAUR44	GGGAAGAGCCAGAAAAAGCGA	AAGGCGTCTTCACTACATGGG
FvSAUR46	TAGGCGAAAACAGAAGCCGA	GTACTCCTCCTCAGCCCTCT
FvSAUR58	GATGACCAATGCCGAGGAGG	ACGTAACGGATCAGTCTCCAC
FvSAUR59	CAAGCAGCGGAGGAGTATGG	AGTGATGCAGTTCTCCCTCTG
Actin	TGGGTTTGCTGGAGATGAT	CAGTAGGAGAACTGGGTGC

2 min at 50 °C, 2 min at 95 °C followed by 35 cycles at 95 °C for 15 s and 60 °C for 30 s.

Gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001). Changes in relative expression levels of the 10 *FvSAUR* genes were checked for statistical significance in accordance with the one-way ANOVA and the means and standard deviation of the replications were compared by the least significant difference (LSD) test at the $P \leq 0.05$.

Results

Identification and classification of SAUR genes in ***F. vesca***

In this study, *SAUR* family genes were identified with a genome-wide scale and the details of those presented in Table 2. After removing the redundant sequences and bioinformatics analysis, 66 putative *SAUR* genes with full-length coding regions were identified and named sequentially from *FvSAUR1* to *FvSAUR66*. Domain analysis showed that all *FvSAUR* proteins have an auxin-inducible domain structure (PF02519). The predicted amino acid length of the strawberry *SAUR* genes ranged from 99 (*FvSAUR41*) to 852 (*FvSAUR22*) with an average of 177.7. The molecular weights of the strawberry *FvSAUR* proteins ranged from 11.047 (*FvSAUR*51) to 94.294 (*FvSAUR*22) kDa. Furthermore, the theoretical isoelectric point (*pI*) of *FvSAUR*s ranged from 4.66 (*FvSAUR58*) to 10.71 (*FvSAUR*66), respectively. Based on the *pI* values, 32 of the *FvSAUR* proteins are basic; three of them are neutral, while 21 of them show acidic character. According to the results obtained from predicted protein localization, we found that most of *FvSAUR* proteins were located in the nucleus (24) and extracellular (18). Moreover, 22 *SAUR* proteins were spread out of mitochondria, cytoplasm and plasma membrane. Interestingly, two *FvSAUR* proteins (*FvSAUR*29 - *FvSAUR*37) were found to be localized in the chloroplast.

Table 2. Details of small auxin up-regulated RNA (*FvSAUR*) gene family in strawberry.

Gene ID	Gene Name	Protein length (aa)	Molecular Weight (Da)	*pI*	Subcellular Localization
FvH4_2g10760	*FvSAUR1*	151	17009.63	9.23	Extracellular
FvH4_2g10770	*FvSAUR2*	312	33965.86	9.14	Extracellular
FvH4_2g10800	*FvSAUR3*	376	20010.83	6.08	Plasma membrane
FvH4_2g10810	*FvSAUR4*	138	15544.91	9.52	Extracellular
FvH4_2g10820	*FvSAUR5*	115	13029.29	10.05	Extracellular
FvH4_6g20761	*FvSAUR6*	168	18467.41	10.39	Extracellular
FvH4_2g10880	*FvSAUR7*	359	39739.72	8.24	Extracellular
FvH4_2g10900	*FvSAUR8*	109	12299.93	6.5	Extracellular
FvH4_7g11280	*FvSAUR9*	167	18775.96	10.27	Extracellular
FvH4_6g36840	*FvSAUR10*	153	17351.15	8.73	Extracellular
FvH4_6g36850	*FvSAUR11*	139	15698.08	8.97	Extracellular
FvH4_6g36860	*FvSAUR12*	185	20800.98	9.11	Extracellular
FvH4_6g35400	*FvSAUR13*	103	11919.00	9.51	Extracellular
FvH4_6g35410	*FvSAUR14*	128	14418.40	5.2	Extracellular
FvH4_5g08800	*FvSAUR15*	186	20458.36	9.38	Extracellular
FvH4_5g08790	*FvSAUR16*	174	19468.51	9.3	Extracellular
FvH4_5g08780	*FvSAUR17*	169	18731.83	9.62	Extracellular
FvH4_5g08770	*FvSAUR18*	212	23350.51	5.35	Cytoplasmic
FvH4_2g02300	*FvSAUR19*	102	11202.99	8.76	Mitochondrial
FvH4_2g02290	*FvSAUR20*	259	30028.03	5.52	Nuclear
FvH4_2g02280	*FvSAUR21*	168	19046.89	6.14	Cytoplasmic
FvH4_2g02250	*FvSAUR22*	852	94293.95	5.98	Nuclear
FvH4_2g38740	*FvSAUR23*	206	23185.81	9.45	Nuclear
FvH4_2g38720	*FvSAUR24*	106	12139.93	6.41	Mitochondrial
FvH4_2g36430	*FvSAUR25*	160	18340.04	8.8	Nuclear
FvH4_6g16370	*FvSAUR26*	109	12250.24	5.43	Cytoplasmic
FvH4_6g16380	*FvSAUR27*	134	15275.53	6.22	Nuclear
FvH4_3g15820	*FvSAUR28*	137	15674.74	8.51	Nuclear
FvH4_5g29950	*FvSAUR29*	166	18512.60	9.77	Chloroplast
FvH4_7g32600	*FvSAUR30*	122	13979.89	6.19	Nuclear
FvH4_1g09280	*FvSAUR31*	238	26399.72	6.21	Nuclear
FvH4_1g09300	*FvSAUR32*	124	13879.66	5.27	Mitochondrial
FvH4_5g22822	*FvSAUR33*	220	24933.33	5.86	Nuclear
FvH4_5g22821	*FvSAUR34*	178	19886.72	6.83	Nuclear

Table 2. Cont.

Gene ID	Gene Name	Protein length (aa)	Molecular Weight (Da)	*pI*	Subcellular Localization
FvH4_3g13730	*FvSAUR35*	166	22373.00	9.83	Plasma Membrane
FvH4_5g22790	*FvSAUR36*	119	13533.31	5.35	Nuclear
FvH4_5g22780	*FvSAUR37*	101	11185.66	5.27	Chloroplast
FvH4_6g10090	*FvSAUR38*	314	29469.35	6.95	Nuclear
FvH4_5g22700	*FvSAUR39*	261	29469.35	6.95	Plasma Membrane
FvH4_5g22690	*FvSAUR40*	192	21715.76	5.85	Nuclear
FvH4_5g22660	*FvSAUR41*	99	10930.35	5.71	Nuclear
FvH4_3g13730	*FvSAUR42*	99	11051.70	7.83	Nuclear
FvH4_5g22620	*FvSAUR43*	200	22653.91	5.39	Plasma Membrane
FvH4_2g10870	*FvSAUR44*	106	12004.93	9.52	Mitochondrial
FvH4_5g22790	*FvSAUR45*	112	12741.52	9.34	Mitochondrial
FvH4_1g11980	*FvSAUR46*	111	12646.56	7.72	Mitochondrial
FvH4_6g19170	*FvSAUR47*	132	14611.73	6.82	Nuclear
FvH4_6g27350	*FvSAUR48*	147	16707.50	9.86	Mitochondrial
FvH4_6g21652	*FvSAUR49*	196	22351.67	8.21	Cytoplasmic
FvH4_7g17340	*FvSAUR50*	196	22412.49	6.2	Cytoplasmic
FvH4_3g13730	*FvSAUR51*	99	11046.64	6.55	Nuclear
FvH4_5g22810	*FvSAUR52*	109	12375.25	9.23	Extracellular
FvH4_4g12051	*FvSAUR53*	336	40013.41	5.89	Nuclear
FvH4_6g33390	*FvSAUR54*	110	12329.43	10.34	Mitochondrial
FvH4_7g19120	*FvSAUR55*	143	16767.86	9.16	Extracellular
FvH4_5g32781	*FvSAUR56*	158	18279.05	9.11	Mitochondrial
FvH4_5g32781	*FvSAUR57*	139	16132.32	9.68	Nuclear
FvH4_6g38640	*FvSAUR58*	282	32426.72	4.66	Cytoplasmic
FvH4_3g31140	*FvSAUR59*	179	20309.22	5.23	Cytoplasmic
FvH4_3g31150	*FvSAUR60*	172	19807.64	7.73	Nuclear
FvH4_5g26890	*FvSAUR61*	120	13627.67	5.16	Nuclear
FvH4_5g26860	*FvSAUR62*	134	14876.14	5.51	Plasma Membrane
FvH4_5g26860	*FvSAUR63*	123	14156.30	5.3	Nuclear
FvH4_3g15390	*FvSAUR64*	145	16121.22	8.68	Nuclear
FvH4_6g17520	*FvSAUR65*	128	14802.92	8.8	Nuclear
FvH4_5g00360	*FvSAUR66*	178	20959.30	10.71	Mitochondrial

Gene structure, conserved motif and chromosomal localization analysis of FvSAUR genes

To obtain more insights into the evolution of the *SAUR* family in strawberry, gene characteristics of all identified *FvSAURs* were analyzed (Fig. 2). The results of the structural analysis showed that the number of exons ranged from 2 to 15. Thirty eight members had no introns. Among the *FvSAUR* genes, 14 had one introns, 12 had two introns, one had three introns and one had sevens introns.

Protein motifs play an important role in the interaction of different modules in transcriptional complexes and are seeming to be closely related to gene classification (Heim *et al*. 2003). Therefore, to reveal the protein structural diversification of *FvSAUR* proteins, 10 conserved motifs were identified by MEME (Fig. 3b). The amino acid sequence length of each motif varied from 8 to 21 amino acids (Fig. 3c). Motifs 1, 2, 3 4, 5 that correspond to *SAUR* (PF02519) domain were the most conserved parts and have been identified in nearly all *FvSAUR* proteins (Fig. 3a). The motifs of *FvSAUR* members within the same subgroups display similar patterns, but a specific biological function of most of these motifs is unknown and remains to be further investigated.

To show the distribution of *SAUR* genes on 7 chromosomes of strawberry, the MG2C (MapGene2Chrom) program was used to map *FvSAUR* genes on the chromosomes. Chromosomal location analysis demonstrates that 66 *FvSAUR* genes were irregularly distributed among the seven *F. vesca* chromosomes (Fig. 4). The number of *FvSAUR* genes on each chromosome has no relationship with chromosome length. Chromosome 5 has the highest number of *FvSAUR* genes (22) while chromosome 4 has the lowest number (2).

Phylogenetic analyses of FvSAUR genes

To explore the evolutionary relationship of the *SAUR* family, the *SAUR* protein sequences of *Fragaria vesca* L. and *A. thaliana* were used to construct the unrooted phylogenetic tree (Fig. 5) The tree showed that 145 *SAUR* genes could be divided into 11 groups, here named as Groups I to XI, which was strongly supported by bootstrap values. Twenty-seven *FvSAURs* were fully assigned to Group I, meanwhile, 21 At*SAURs* belonged to this group. These results show that

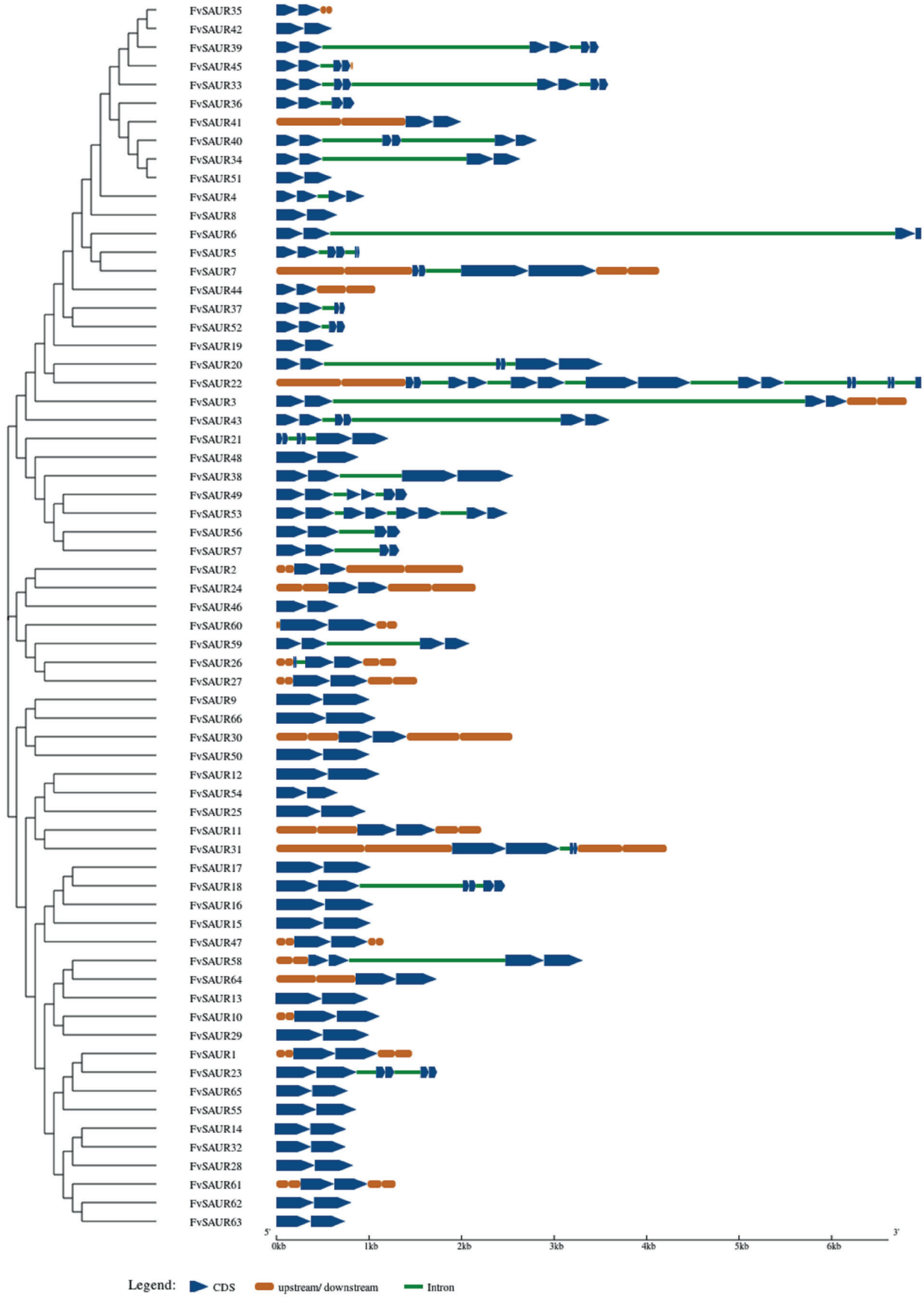

Figure 2. Exon-intron structure of *FvSAUR* genes. Blue boxes indicate exons, orange boxes indicate upstream/downstream, green lines indicate introns.

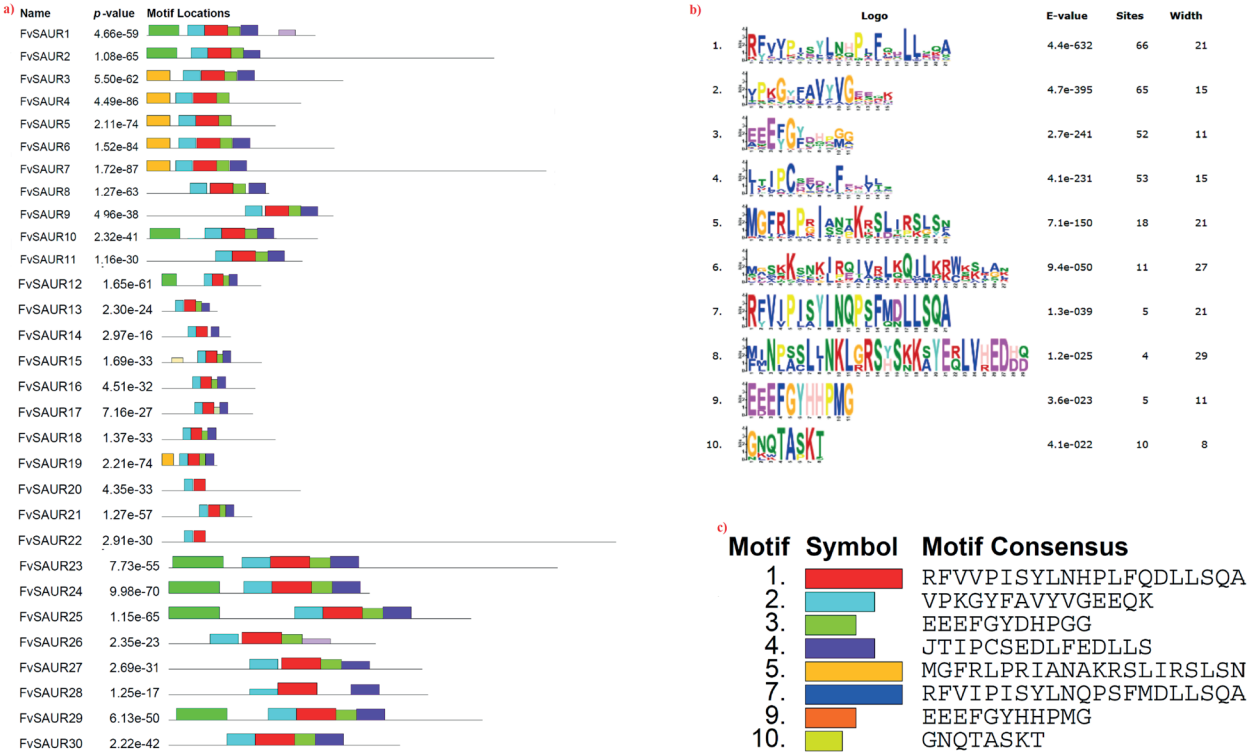

Figure 3. The motif composition of *FvSAUR* proteins. **a**) Distribution of *FvSAUR* protein motifs. **b**) Sequences of *FvSAUR* protein motifs. **c**) The domains found in these *FvSAUR* proteins.

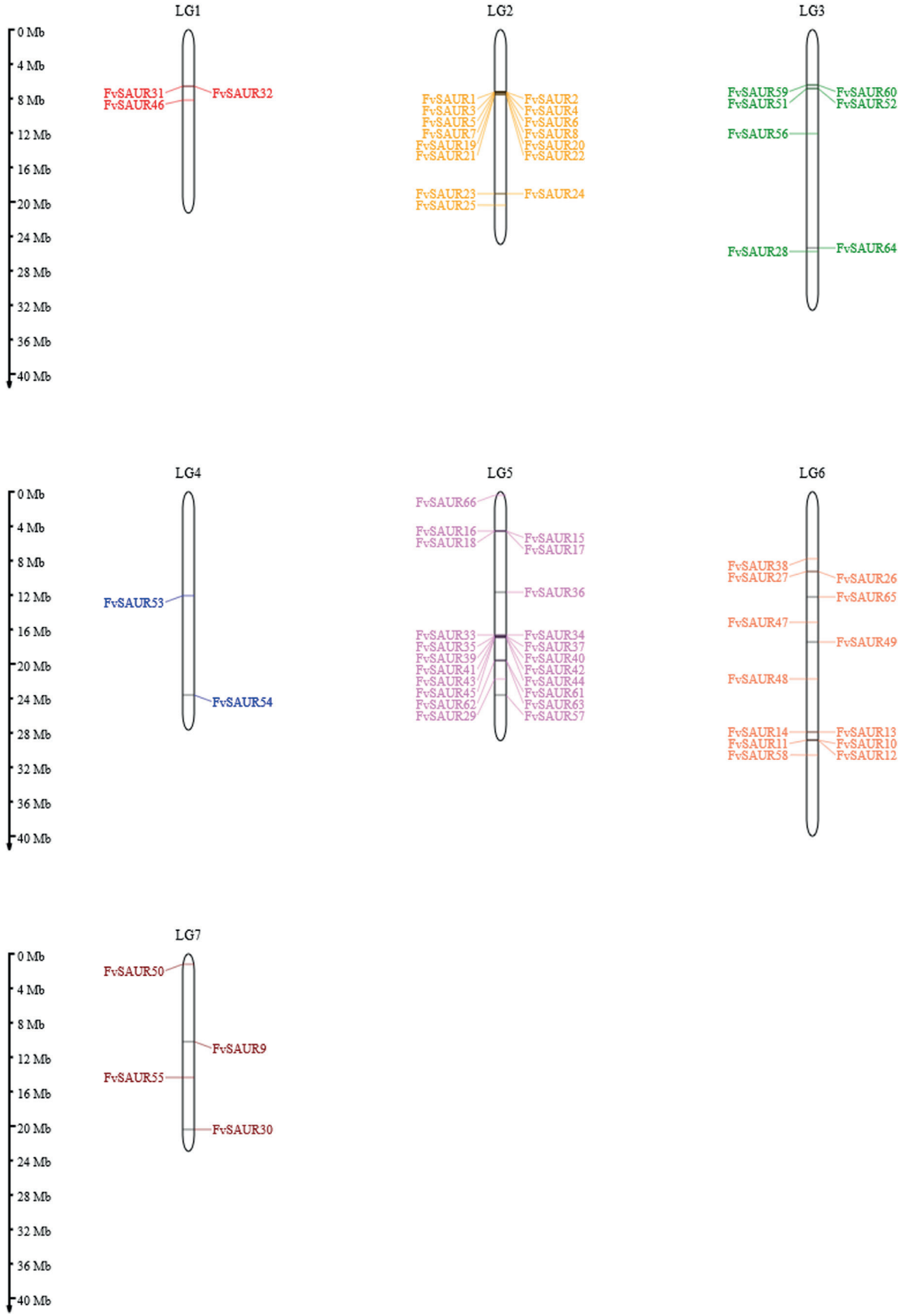

Figure 4. Distribution of *FvSAUR* genes among 7 chromosomes. The scale on the left represents chromosome length. The chromosome number is to the top of each chromosome.

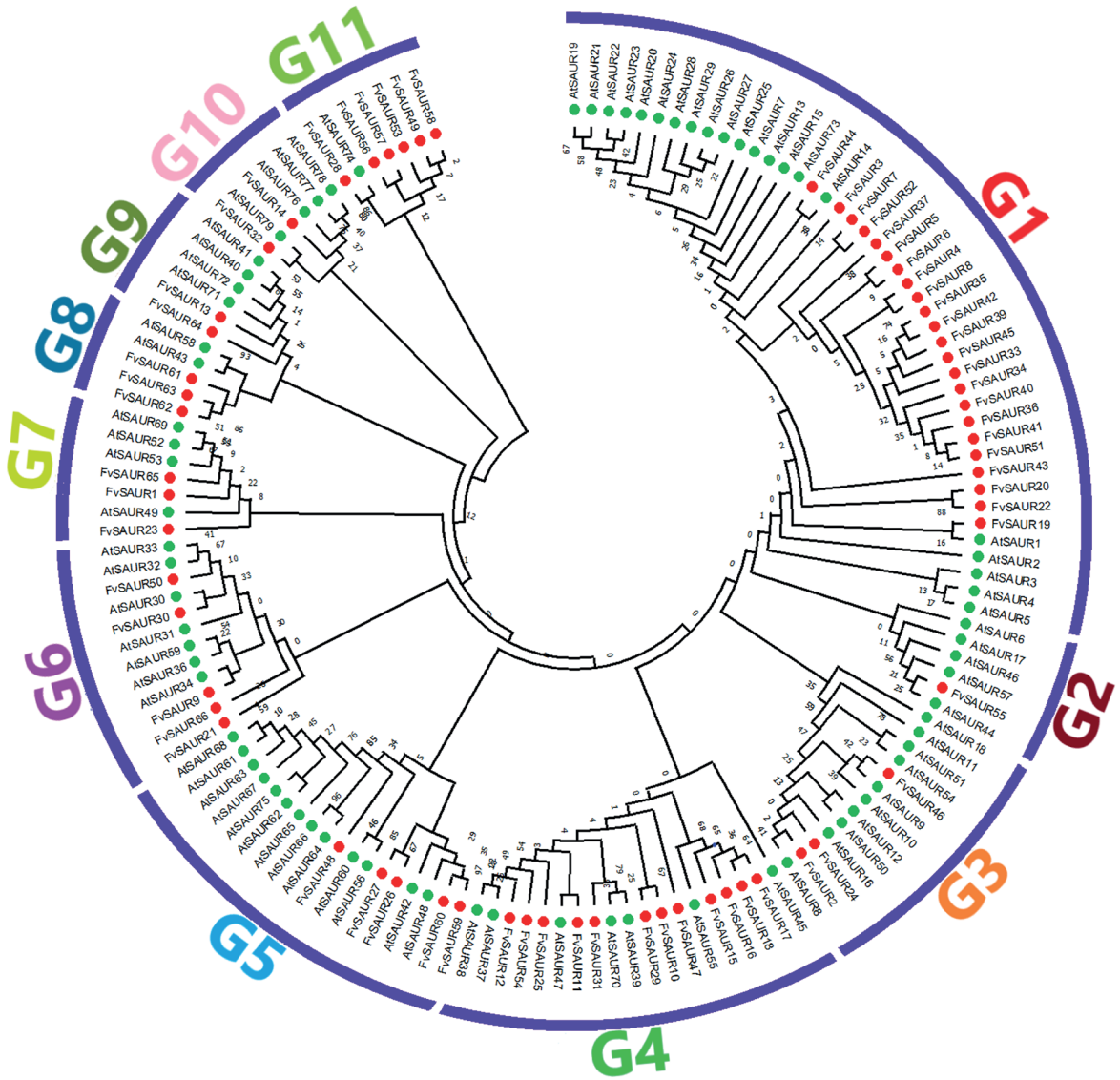

Figure 5. Phylogenetic analysis of *FvSAURs*. The phylogenetic tree was generated using the amino acid sequences of selected *FvSAURs* via NJ method. All tomato *FvSAURs* were classified into 4 groups. Groups I to IV are represented by orange, red, blue, and green, respectively.

genes in this group have a closer evolutionary relationship. Other subgroups have varying numbers of At*SAUR* and *FvSAUR*, which may provide guidance for understanding the relationship between *SAUR* genes in both species.

PPI network and promoter region analysis of FvSAURs

The STRING online database was searched and the corresponding functional PPI networks were reconstructed using the 66 input putative strawberry *SAUR* proteins to explore the functional PPIs (Fig. 6). Among all *FvSAUR* proteins, *FvSAUR*48, *FvSAUR*30, *FvSAUR*32, *FvSAUR*9 stand out as core hub elements that interact very tightly with each other. *FvSAUR*46 stands out as a possible central regulator among these four core hub proteins. *FvSAUR*39, *FvSAUR*14, *FvSAUR*28, *FvSAUR*64, *FvSAUR*15 and *FvSAUR*60 are secondary proteins associated with these core proteins. Interestingly, a direct relationship between *FvSAUR*10, *FvSAUR*26, *FvSAUR*29 proteins has been observed, which could be regarded as crucial nodes for further research. The remaining 53 *FvSAUR* proteins were grouped independently at five different clusters. In order to analyze the complex relationship between *FvSAUR* proteins, in addition to functional analysis, proteins belonging to other gene families should be identified and uploaded to databases for strawberries.

The prediction of *cis*-elements can provide a platform for the spatial and tissue-specific expression of genes. In order to identify putative *cis*-elements in the strawberry *SAUR* promoters, the PlantPAN 3.0 database was used to screen the predicted transcription factors (TF) and their binding sites of the 66 *FvSAUR* genes. A total of 28 type of TFs were detected and the 14 TFs with the highest number of motifs are shown in Table 3. The highest number TF motif was found as 516 for *GATA*, followed by 448 for AP2/ERF,

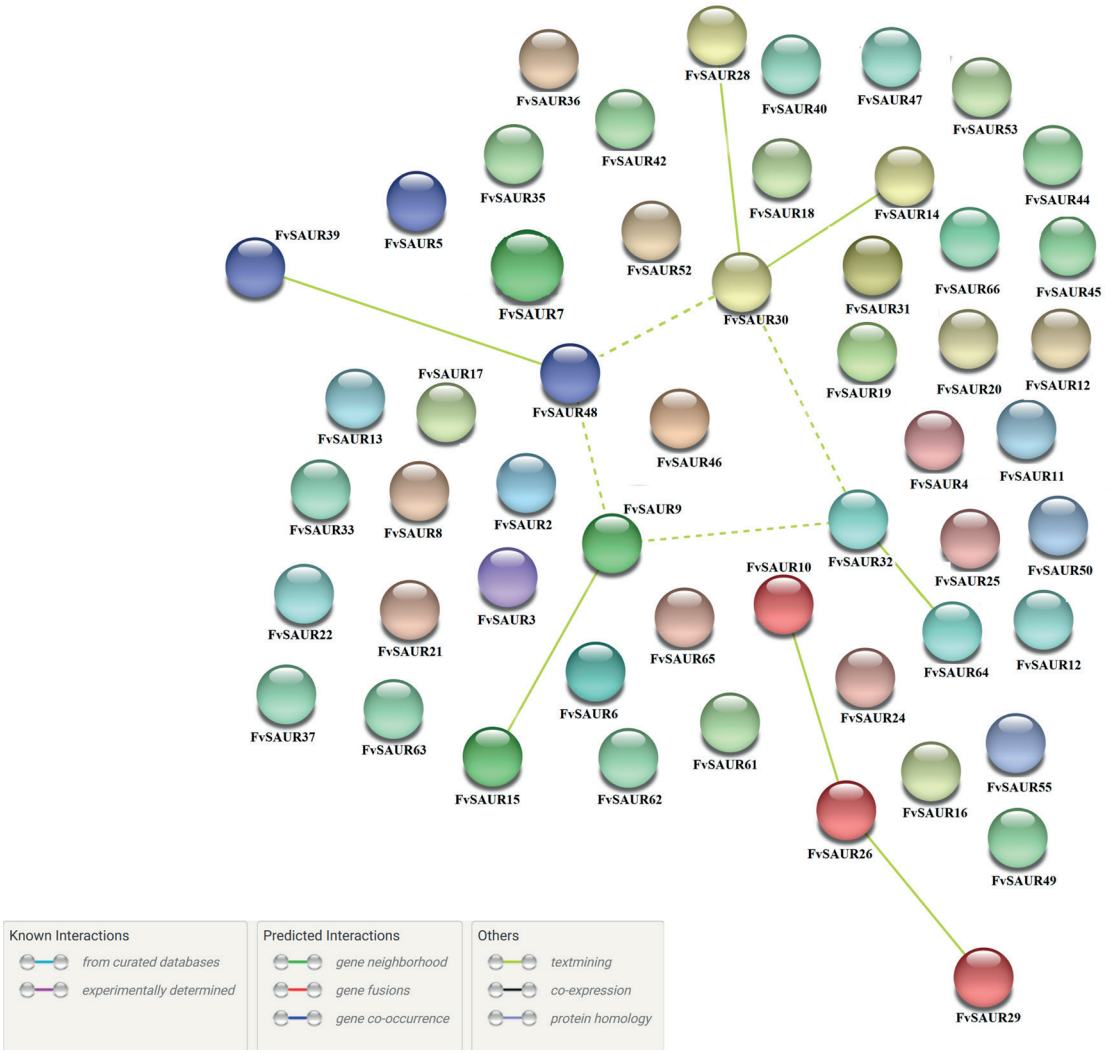

Figure 6. The mapped profile of PPI network was constructed by the STRING online database to probe the functional interactions of *FvSAUR* proteins.

Table 3. Transcription factor binding sites detected in the upstream of promoter regions and total total number in 66 FvSAUR genes.

TF Family	Number of sites	Description	Reference
GATA	516	Regulation of plant developmental and growth processes	An *et al.* 2020
AP2/ERF	448	Ethylene-responsive transcription factor	Guo *et al.* 2016
bZIP	329	Related to plant development, environmental signalling and stress response	Dröge-Laser *et al.* 2018
NF-YB	237	Regulating plant growth, development and participates in various stress responses	Dai *et al.* 2021
Dof	195	Regulating specific biological processes related to plant photosynthesis, growth and development	Shaw *et al.* 2009
Trihelix	183	Regulating plant growth and development involving seeds, embryos stomata and flowers	Kaplan-Levy *et al.* 2012
Homeodomain	178	Regulating maintenance of the stem cell niche in a shoot apical meristem	Lopes *et al.* 2021
SBP	164	Regulating growth, flower development, and signal transduction	Teng *et al.* 2021
HD-ZIP	132	Participate in vascular development, leaf polarity, embryogenesis, meristem regulation and developmental responses to environmental conditions	Ariel *et al.* 2007
WRKY	113	Play crucial roles in plant growth and development, defense regulation and stress responses	Abeysinghe *et al.* 2019
AT-Hook	104	Regulation of growth and development	Favero *et al.* 2020
ZF-HD	95	Play crucial roles in the response to abiotic and biotic stresses	Muthuramalingam *et al.*2018
Dehydrin	87	Participate in salt and osmotic stress signaling pathways.	Luo *et al.* 2019
TBP	85	Play diverse roles in plant growth and development	Mougiou *et al.* 2012

329 for bZIP, 237 for NF-YB, and 195 for Dof. Based on promotor analysis, the presence of too many TF related *cis*-acting elements might support the active roles of the *SAUR* gene family in different developmental stages of plant life cycle activities in *F. vesca*.

In silico expression profiles of FvSAUR genes

Since gene expression patterns provide important clues for understanding the function of genes, we examined the expression of *FvSAUR* genes at seedling, flower, leaf and two ripening stages of two different *F. vesca* genotypes using an integrated transcriptome datasets in flower and early stage of fruit development (Kang *et al*. 2013; Hollender *et al*. 2014), and recent RNA-Seq data set on ripening-stage receptacle (Hawkins *et al*. 2017) (Fig. 7). The electronic expression profiles of 66 *FvSAUR* genes in various organs/tissues were downloaded from the strawberry eFP browser at bar.utoronto.ca. Among those, the transcripts of 15 *FvSAUR* genes were quite low in all detected organs/tissues. In this study, we focus more specifically on the analysis of gene expression in vegetative and reproductive tissues/organs. Therefore, the heatmap indicated that the expression profiles of strawberry *SAUR* genes in seedling, flower, leaf and two ripening stages of two different *F. vesca* genotypes could be divided into four clades (Fig. 7). The genes were highly expressed in Ruegen fruits at white stage (Ruegen F7-4 W), whereas most of them had a quite low expression level at the green stage (Ruegen F7-4 G). Additionally, the highest mRNA levels of 35 genes were detected in flowers. Notably, the transcripts of five genes were specifically detected in the seedlings. Sixteen genes with the highest expression were found in the leaf tissues (Fig. 7). The results indicate that the *FvSAUR* genes are involved in a variety of strawberry plant development and fruit ripening process.

Expression analysis of selected FvSAUR genes

According to the eFP data, the expression level of ten genes with high transcript levels in different tissues and organs were experimentally verified by RT-qPCR. The results mostly confirmed the eFP browser data that all selected genes were differentially expressed among seven strawberry tissues (Fig. 8). The genes *FvSAUR41* and *FvSAUR58* were

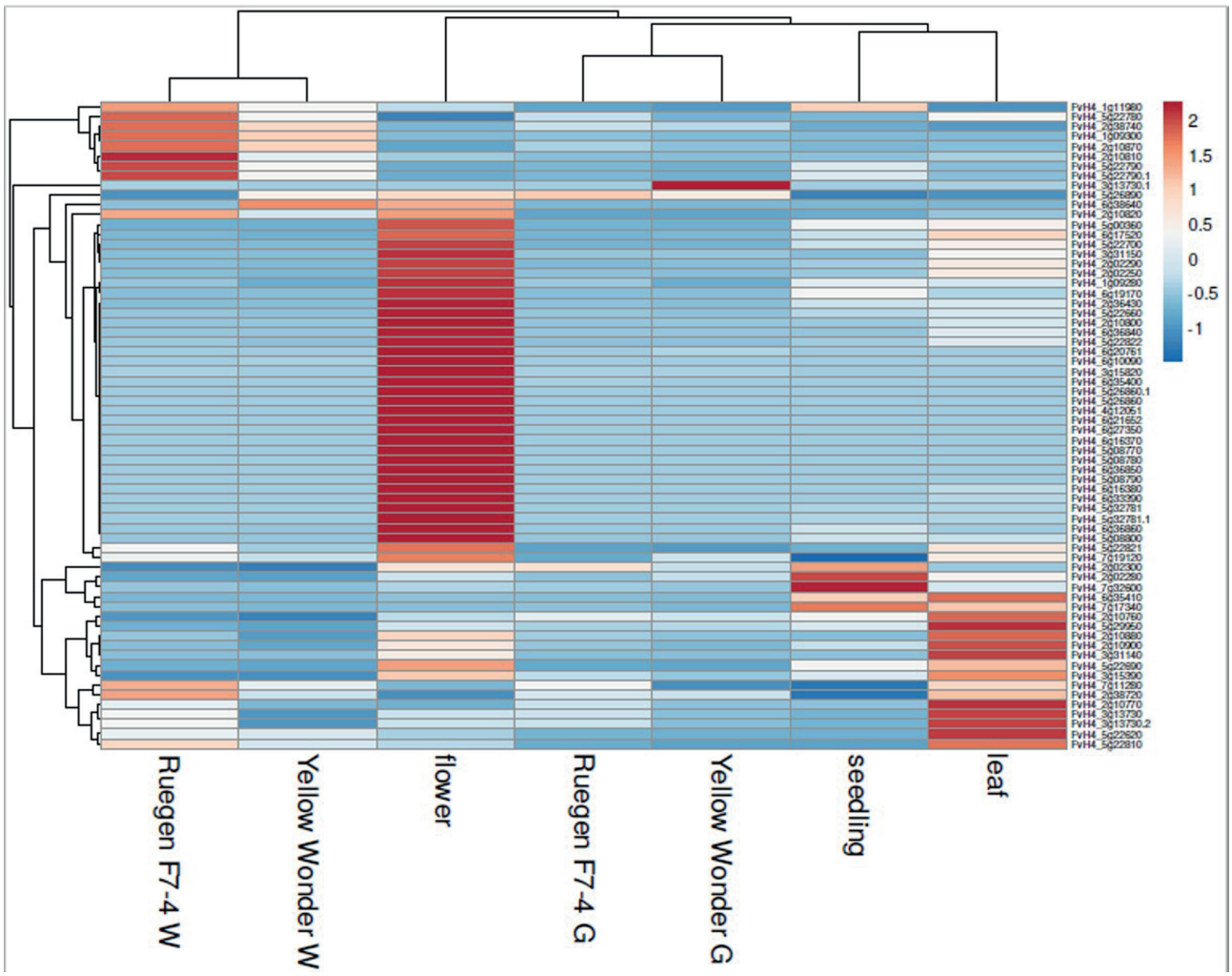

Figure 7. Heat map representation for tissue specific expression and two fruit development stages-related expression in two differet strawberry cultivars. These electronic expression data were downloaded from the strawberry eFP browser at bar.utoronto.ca .

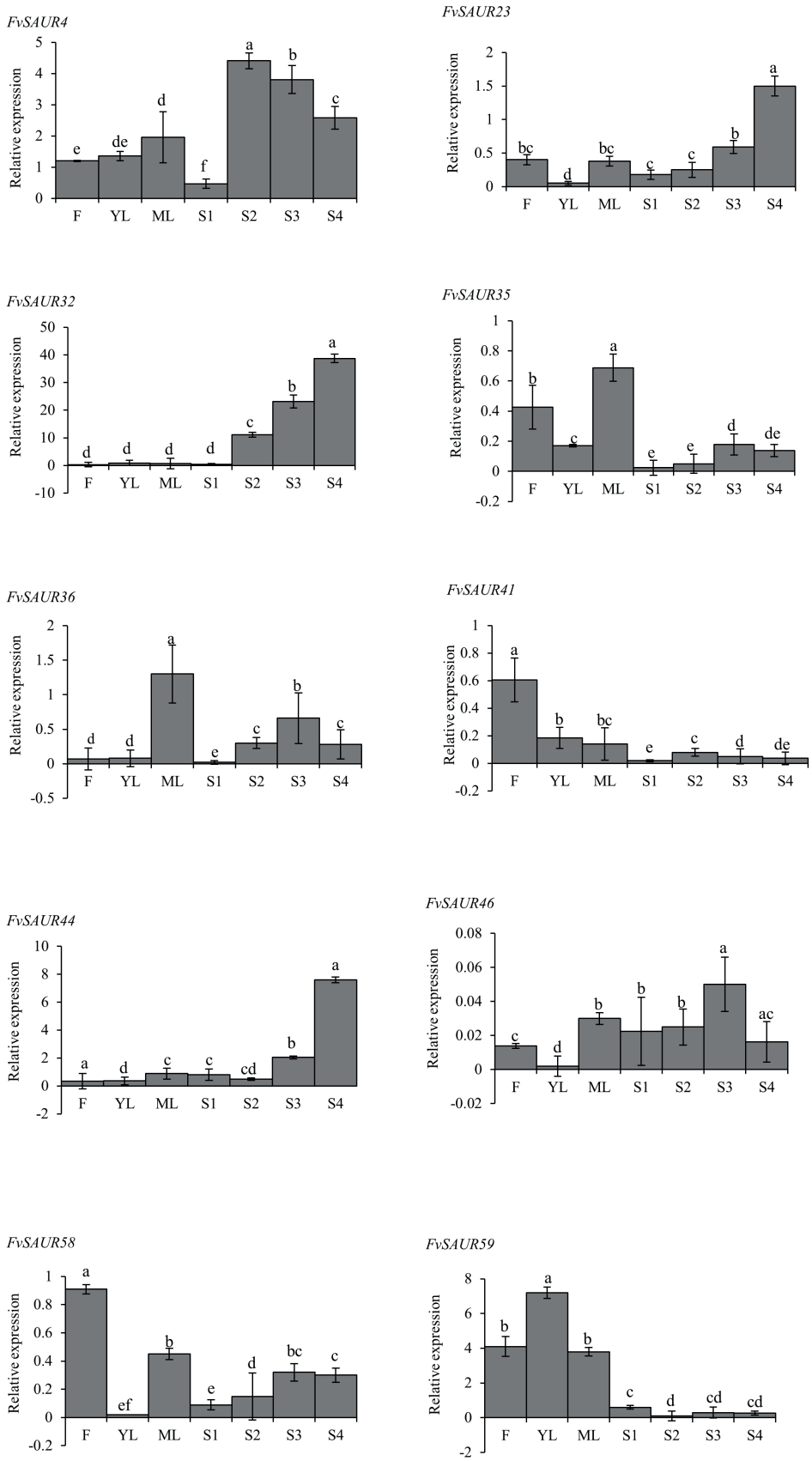

Figure 8. Expression levels of selected 10 *FvSAUR* genes in different tissues/organs of strawberry. F: flower, YL: younf leaf, ML: mature leaf, S1 (small-sized green fruits), S2 (bigger size green fruits), S3 (white-purple fruits), S4 (full ripe fruits). Mean values and SD ± were obtained from three biological and two technical replicates. The bars with different letters indicate significant differences, P≤ 0.05.

mainly expressed in flower tissues. The most expressed genes in young (YL) and mature leaf (ML) tissues were *FvSAUR35*, *FvSAUR36* and *FvSAUR59*. The expression level of *FvSAUR4* was the highest in big green fruits (S2) that was down-regulated in S3 and S4 ripening stages. The expression levels of *FvSAUR23*, *FvSAUR32* and *FvSAUR44* genes increased consistently in strawberry fruits as the ripening progressed.

Discussion

Several research groups have identified and annotated some *SAUR* genes at genome-wide level in *Arabidopsis* (72) (Hagen & Guilfoyle 2002), rice (58) (Jain *et al*. 2006), sorghum (71) (Wang *et al*. 2010) tomato (99), potato (134) (Wu *et al*. 2012), maize (79) (Chen *et al*. 2014), citrus (70) (Xie *et al*. 2015), mulberry (62) (Xing *et al*. 2016), watermelon (65) (Zhang *et al*. 2017) and apple (80) (Wang *et al*. 2020). In the present study, we conducted a genome wide analysis and identified 66 *FvSAUR* genes in the strawberry genome. All the members of the family were predicted to encode the *SAUR* (PF02519) domains. Compared with the *SAUR* gene family in other species, a moderate number of genes was identified in strawberry. The smaller scales of the *SAUR* family might be due to whole genome duplication (Jaillon *et al*. 2009). In the present study, more than a third of the *FvSAUR*s were localized in the nucleus. This feature was found in watermelon (Zhang *et al*. 2017), bamboo (Bai *et al*. 2017), agave (Deng *et al*. 2019), cotton (Li *et al*. 2017), tomato and potato (Wu *et al*. 2012) that is, at least a third of the *SAUR* proteins can be predicted to be localized in the nucleus. However, the functions of *SAUR*s are still unclear in the nucleus (Stortenbeker & Bemer 2019).

By comparing of the 28 regulatory elements in the promoter regions, *GATA* transcription factor (TF) gene family has been identified as the most found *cis*-acting element and this was identified as the most conserved TF from fungi to angiosperms (Gupta *et al*. 2017). *FvSAUR21* (17) has the most, and *FvSAUR47* has the least (0) *GATA cis*-acting elements in their promoter regions. It has been reported that *GATA* TFs play important roles in the regulation of plant photoresponse, chlorophyll synthesis, carbon and nitrogen metabolism, and in the regulation of plant flowering time, leaf extension and other biological processes (Gupta *et al*. 2017). The second most abundant regulatory TF *AP2/ERFs* participates in the hormonal regulation of the stress response in plants (Xie *et al*. 2019). In strawberry, the role of *AP2/ERF* in fruit color and aroma was investigated (Sheng *et al*. 2021). In our analysis, *FvSAUR22* (14) was identified as containing the most *AP2/ERF* element, which makes the gene play an important role in hormonal response in different plant developmental stages. Another high number of TF motifs was found in the leucine zipper (bZIP) TFs which play a vital role in plant development and responses to various stresses (Wang *et al*. 2017). Biochemical and functional analyses have shown that the bZIP family is involved in many major plant biological processes, including plant growth processes such as organ differentiation, flower induction, vascular development, embryogenesis and seed maturation (Abe *et al*. 2005).

In our analysis, *FvSAUR30 and FvSAUR64* (11) have the most and bZIP promoter regions. Therefore, it can be said that especially *FvSAUR30* and *FvSAUR64* would potentially be involved in different plant growth processes. Nuclear factor Y (NF-Y), also called heme activator protein (HAP) or CCAAT-binding factor (CBF), can be found in almost all eukaryotes (Dorn *et al*. 1987). This gene family consists of three subunits, NF-YA, NF-YB and NF-YC. *NF-YB* genes have been shown to be involved in the process of chloroplast biogenesis in rice, fruit ripening in the tomato, grain yield metabolism in wheat. (Li *et al*. 2016; Thirumurugan *et al*. 2008). In our analysis, it was identified that *FvSAUR23* has seven NF-Y binding motifs in their promoter region. Considering all the TF data obtained, it can be suggested that the *FvSAUR* genes may contact various TFs for regulating diverse processes of plant development and play very important roles in hormonal regulations.

Expression Divergence of FvSAUR genes in Different Tissues and Organs

Based on eFP browser data, expression analysis indicated that 51 *FvSAUR* genes in strawberry are predominantly expressed in the seedling, flower, leaf or fruits tissues, whereas the transcripts of 15 genes could not be detected or expressed in very low levels in any strawberry organs. Majority of the genes have expression accumulated in seedling, flower, and leaf tissues indicating that suggesting that *FvSAUR* gene family might play a major role in the reproduction development in strawberry. The functions of *SAUR* genes in dividing tissues, such as cell elongation and cell expansion have been revealed in *Arabidopsis* (Ren & Gray 2015). This statement was functionally supported that *AtSAUR19-24* function as positive effectors of cell expansion by modulating the auxin transport, as *SAUR* gain-of-function and loss-of-function seedlings exhibit increased and reduced basipetal indole-3-acetic acid transport, respectively (Spartz *et al*. 2012). Most of the expression of the genes increased in flower and leaf indicating that the tissues are the more important growing parts of strawberry plants. In other words, more auxin might be produced in the flower and leaf tissues to maintain plant growth. The genes expressed in these organs might share similar functions with *Arabidopsis*.

Eight genes were highly expressed in Ruegen F7-4 that makes a red fleshy receptacle, whereas there were lower expression levels of those genes in Yellow Wonder (YW5AF7) develops yellow fleshy receptacles. Therefore, these genes might be responsible for regulating strawberry fruit development and ripening.

Supporting our results, the expression of a number of *SAUR* genes activated during the development of young fruit in tomato and watermelon (Zhang *et al*. 2017), suggesting that the *SAUR* gene family might play a major role in the reproduction development in different fruits.

Ten *FvSAUR* genes were selected to examine their expression patterns in different strawberry tissues based on their transcript levels in eFP strawberry browser. According to RT-qPCR, *FvSAUR41* and *FvSAUR58* are highly expressed in flowers. In YL and ML tissues, *FvSAUR35*, *FvSAUR36* and *FvSAUR59* showed up-regulation, implying that these genes might be more likely to play critical roles in regulating growing parts. For plants, compared with dormant tissues (such as seed), growing (such as leaf) and developing tissues (fruit developing and maturation) can often produce a large amount of auxin to satisfy the needs for plant growth (Wang *et al*. 2020). Interestingly, *FvSAUR4* and *FvSAUR46* are highly up-regulated in S2 and S3 fruit developmental stages in strawberry fruits, respectively, which might promote fruit growth by regulating cell division. The genes *FvSAUR23, FvSAUR32* and *FvSAUR44* were highly up-regulated specifically in ripe fruits (S4). The expression level of *FvSAUR46* peaked in S3 stage where the red colouration of fruit has just started indicating that *FvSAUR46* could be responsible for the ripening transition phase in which ripening related hormones might just be activated in strawberries. Down-regulation of this gene would possibly delay ripening initiation and decrease softening rate in strawberries. Previous studies have shown that some of the *SAUR* genes could be induced by exogenous auxin (Wang *et al*. 2020). In tomato overexpression of *SlSAUR69* resulted in the premature initiation of ripening and down-regulation of the gene delays the initiation of fruit ripening demonstrated that *SlSAUR69* contributes to the ripening transition in tomato (Shin *et al*. 2019). Based on these results, in order to better understand the role of *FvSAURs*, future work addressing the function and evolution of these genes is necessary in strawberries. Moreover, in future, it would be interesting to functionally characterize especially selected genes by up/down-regulation and to classfy them as positive and negative regulators of plant development and fruit ripening in strawberries.

Conclusion

This study presents a comprehensive analysis of the *FvSAUR* gene family in strawberries. A total of 66 *FvSAUR* genes were identified and the results provided a genomic framework for future characterization of strawberry *SAUR* genes. RT-qPCR analysis demonstrated the existence of the expression of 10 *FvSAUR* genes in different tissues and developmental stages. Our study will serve to better understand the complexity of the strawberry *FvSAUR* gene family and guide future studies for functional analyses.

Declarations

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