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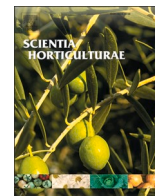
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Heterologous overexpression of strawberry *bZIP11* induces sugar accumulation and inhibits plant growth of tomato

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ABSTRACT

Consumers and breeders value strawberry fruit with high sugar content and sweetness. However, many factors negatively affect this trait. bZIP-S1 subfamily was closely correlated with the carbohydrate metabolism. Moreover, it can regulate plant development and growth, and respond to various stress. In the present study, a total of four bZIP-S1 members were identified in strawberry. Quantitative RT-PCR analysis showed a member of this subfamily, designated as *bZIP11*, most highly expressed in different tissues of strawberry. Especially, it had a significant transcript accumulation during the fruit development and ripening. Constitutive expression of *FvbZIP11* in tomato caused severe plant growth retardation which was accompanied by wrinkled and curly leaves. The leaf yellowish and chlorotic patches were reflected in decrease of chlorophyll. Meanwhile, epidermal cells were smaller in leaves of transgenic plant. In addition, the total soluble solid and soluble sugar content of the fruit was significantly higher than WT during the fruit ripening. Taken together, the heterologous overexpression of strawberry *bZIP11* induced sugar accumulation and hindered plant growth in tomato, which provided more information for improving strawberry fruit quality.

1. Introduction

Transcription factors (TFs) play important roles in plant development and stress resistance by regulating related gene expression, and usually classified into different families on the basis of their conserved DNA-binding structure. It was reported that approximately 100 TF families have been defined in *Arabidopsis* (Pruneda-Paz et al., 2014). Basic leucine zipper (bZIP) family are ubiquitous TFs in plants where they participate in a great deal of physiological and biochemical processes including but not limited to light signaling and photomorphogenesis (Mallappa et al., 2006), flower development and fertility (Zou et al., 2008), seed maturation and germination (Alonso et al., 2009), senescence (Yang et al., 2001) and abiotic stress responses (Wang et al., 2019). bZIP family are characterized by two functionally distinct motifs located on a contiguous α -helix: a basic region for DNA binding and a

leucine zipper for dimerization (Dröge-Laser et al., 2018). Initially, AtbZIPs were systematically divided into ten subfamilies, referred to as group A to I, and S (Jakoby et al., 2002). Subsequently, the classification was further extended to 13 groups (Dröge-Laser et al., 2018). Among these subfamilies, the S group is the largest bZIP cluster and divided into S1, S2 and S3 three subgroups (Ehlert et al., 2006; Jakoby et al., 2002). Particularly, the group S1 has five members, AtbZIP1 (At5g49450), AtbZIP2 (GBF5, At2g18160), AtbZIP11 (ATB2, At4g34590), AtbZIP44 (At1g75390) and AtbZIP53 (At3g62420), and they harbor an unusually long 5' leader containing a highly conserved upstream open-reading frame (uORF) that encodes a sucrose-controlled peptide, which can give rise to sucrose-induced repression of translation (SIRT) and involves in nutrition/energy/stress signaling cascades (Dietrich et al., 2011; Wiese et al., 2004). This structure feature has been used to regulate plant sugar accumulation, amino acid metabolism, stress

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resistance, growth and development. For almost all fruits and some vegetables, sugar content and sweetness increasing is valued. Sagor et al. (2016) proposed a novel strategy that fruit-specific expression of the S1-bZIP main open reading frames (ORFs), without the SIRT responsive uORFs, could produce sweeter tomato fruits. Moreover, group S1-bZIPs could efficiently form specific heterodimers with bZIP members of group C (AtbZIP9, At5g24800; AtbZIP10, At4g02640; AtbZIP25, At3g54620; AtbZIP63, At5g28770) in planta. The S1/C bZIP complex leads to the strong activation of target genes involving stress stimuli and metabolic signals in SnRK1-dependent and -independent way, which facilitates a signal integration in plant transcriptional networks (Dröge-Laser and Weiste, 2018; Ehlert et al., 2006; Weltmeier et al., 2009). It has been demonstrated that sustaining energy homeostasis is important to control plant growth process. Transient energy deprivation will always happen when plant adjust their metabolism to adapt to daily light-dark cycles as well as unpredictable environmental changes (Dietrich et al., 2011; Hartmann et al., 2015; Pedrotti et al., 2018). AtbZIP1 and its heterodimerization partners AtbZIP53, AtbZIP10, and AtbZIP25 can facilitate primary carbohydrate and amino acid metabolism reprogramming to induce salt stress tolerance by ABA-independent signaling module in Arabidopsis root (Hartmann et al., 2015).

In the experiment of sensory perception to strawberry flavor, sweetness intensity which depends on sugar concentration is the strongest driver of overall liking. Especially, the reduction of sucrose would undermine the hedonic effect (Schwieterman et al., 2014). Therefore, creating a sweeter strawberry germplasm is desirable. Given that enormous influence of S1-bZIPs on sugar metabolism, plant growth and development, we identified the S1-bZIP members in strawberry to give a deeper insight into their function.

2. Materials and methods

2.1. Genome-wide identification and sequences analysis of S1-FvbZIPs

The sequence information of Arabidopsis S1-bZIP five members (bZIP1, -2, -11, -44, -53) was obtained from TAIR database (<https://www.arabidopsis.org/>) and were used as query probes to BLAST search against NCBI database (<https://www.ncbi.nlm.nih.gov/>) and *Fragaria vesca* v1.0 genome database (<https://www.rosaceae.org/>). S1-bZIPs were reported to harbor an unusually long 5' leader containing a highly conserved upstream open-reading frame (uORF) that encodes a sucrose-controlled peptide. Hence, uORF of FvS1-bZIPs was isolated and then aligned with uORF of AtS1-bZIPs to further ensure that identified sequences belong to S1-bZIPs in strawberry. Moreover, the evolutionary relationship of S1-bZIP homologues among strawberry, peach, rice, Arabidopsis was analyzed in MEGA v.6.0 according to neighbor-joining (NJ) method with Poisson model and 1000 bootstrap replications, after sequence alignment was performed in ClustalX v.2.0 software. The physical and chemical parameters of FvS1-bZIP proteins were computed using ProtParam tool, and putative subcellular location was predicted in CELLO v.2.5 program. Conserved Motifs of the FvS1-bZIPs were analyzed using the Multiple EM for Motif Elicitation (MEME) web server (<https://meme-suite.org/meme/tools/meme>).

2.2. Tissue-specific expression of S1-FvbZIPs

Strawberry root (R), stem (S), leaf (L), flower (F) and fruits at four different stages (Small green, SG; White, W; Half red, HR; Full red; FR) were collected to examine the tissue-specific expression of S1-FvbZIPs by quantitative real-time RT-PCR (qRT-PCR). The total RNA was isolated using improved CTAB method (Chen et al., 2012). The qRT-PCR assays were performed using 96-well plates on ABI 7300 Real-Time PCR System (Applied Biosystems, USA). 10 µl reaction volume was composed of 5 µl SYBR Green PCR Master Mix (Applied Biosystems, USA), 0.4 µl each primer (final concentration: 0.4 µM), 1 µl diluted cDNA and 3.2 µl

RNase-free water. After incubation of the reaction mixture at 95 °C for 3 min, 40 cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 15 s were performed and then melting curve was inserted, ramping from 65 to 95 °C (increment 0.5 °C/5 s) after the final cycle. Moreover, controls without template were included in each run to check the potential reagent contamination. All primer sequences were listed in Table S1. The calculation method for qRT-PCR was $2^{-\Delta\Delta CT}$.

2.3. Construction of overexpression plasmids

The full-length of *FvbZIP11* main ORF sequence was PCR-amplified using the following primers: 5'-CATGCCATGGATGGCTTCTCTAGTGAACATCC-3' (forward) with a *NcoI* restriction site and 5'-GGACTAGTTCAGTACTGAAACATCTCTGCTGAAG -3' (reverse) with a *SpeI* restriction site. The PCR products were cloned into a modified pGEM-T easy vector (pDAH11) (Promega, USA) to generate the pDAH11-*FvbZIP11* construct for sequencing. The confirmed pDAH11-*FvbZIP11* construct and modified pGSA1403 binary expression vector were simultaneously digested with *NcoI* and *SpeI* (NEB, USA) and then gel-purified. Eventually, the *FvbZIP11* fragment was cloned into modified pGSA1403 vector to form the pGSA1403-*FvbZIP11* overexpression construct.

2.4. Generation of 35S-bZIP11 transgenic tomato plants

The pGSA1403-*FvbZIP11* plasmids were electroporated into *Agrobacterium tumefaciens* strain GV3101. Tomato (*Solanum lycopersicum*) cultivar Ailsa Craig (AC) was used for generation of transgenic plants by the leaf-disk transformation method (Fillatti et al., 1987; Ma et al., 2015). Transgenic plants were screened on MS plates supplemented with 100 mg•L⁻¹ kanamycin. Furthermore, genomic DNA of all the transformants (T0 and T1) was extracted using an improved CTAB method (Murray and Thompson, 1980) and then used as template in PCR reactions together with primers as listed above that targeted against the *FvbZIP11* main ORF sequence. Three positive transgenic tomato T1 lines were selected to analyze the *FvbZIP11* expression level and used to assess other parameters.

2.5. Growth assessment of transgenic tomato lines

T1 tomato lines were grown in pots containing the UC Mix (1/3 Peat, 1/3 Sand, 1/3 redwood compost) (Estrada-Melo et al., 2015) and grown in a greenhouse under routine management. The growth parameters were measured four weeks after planting. The photosynthetic pigments, and epidermal cells of the fourth or fifth leaves from the stem bottom were detected. Approximately 0.1 g leaf powder was homogenized for 24 h at 25 °C in the dark using 10 ml extract solution containing acetone and ethanol (1:1, v/v). After centrifugation for 15 min at 8000 × g, the supernatant was used to determine the chlorophyll and carotenoid content (Arnon, 1949). To observe epidermal cells, leaf imprints were obtained from the same relative position using a leaf surface imprint method (Yu et al., 2008).

2.6. Evaluation of total soluble solids, soluble sugar and titratable acid in transgenic tomato fruits

The content of total soluble solids (TSS) in squeezed tomato juice was measured using a digital PR-100 refractometer (ATAGO, Japan) and expressed as a percentage on the Brix scale. The total soluble sugar content of fruits was measured by the method of anthrone colorimetry and expressed as the percentage of sucrose on a fresh weight (FW) basis. The titratable acid (TA) content was determined by titration method with 0.1 mol/L NaOH solution and expressed as the percentage of citric acid on a fresh weight (FW) basis.

Table 1
List of S1-FvbZIPs identified in *Fragaria vesca*.

Gene ID	Chromosome location	Amino acid length (aa)	MW (kDa)	Theoretical pI	Instability index	Aliphatic index	GRAVY	Subcellular location
gene21832	LG2: 925888..926367+	159	17.97	6.29	66.22	66.98	-0.70	Nuclear
gene15193	LG2: 34440994..34441509+	171	19.14	6.51	58.36	71.40	-0.79	Nuclear
gene14942	LG1: 9334245..9334718-	157	17.85	4.93	44.46	79.49	-0.58	Nuclear
gene02284	LG5: 25840738..25841184-	148	17.19	5.49	60.81	58.65	-0.94	Nuclear

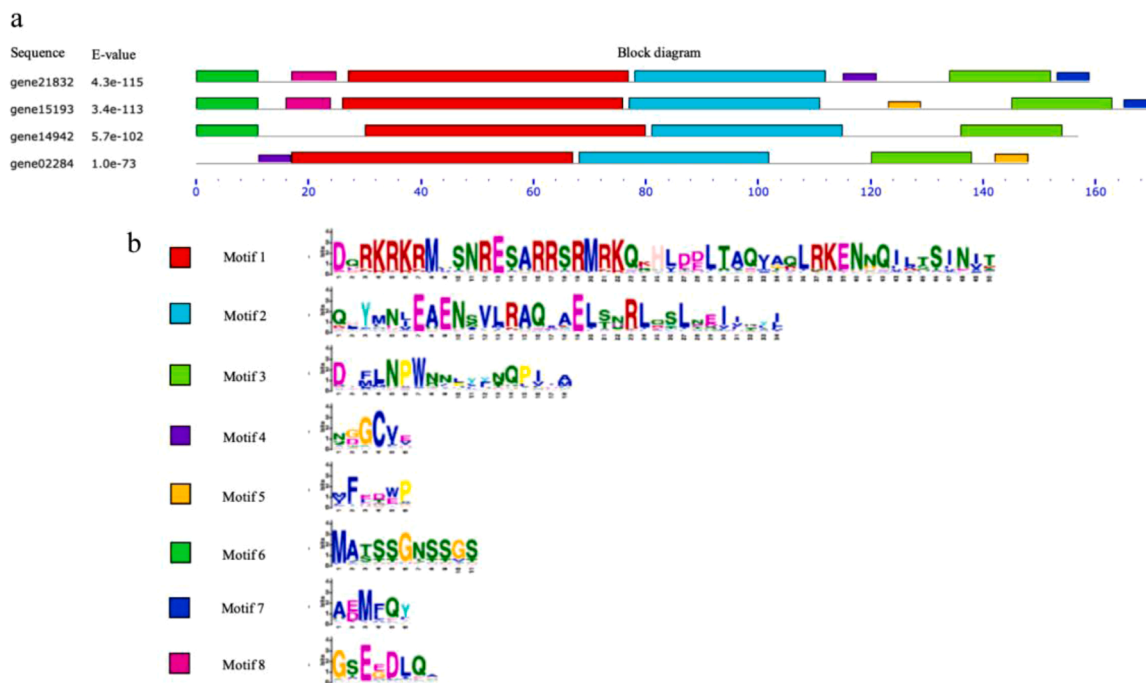


Fig. 1. Conserved motifs of S1-bZIP proteins in strawberry. (a) The distribution of conserved motifs in S1-bZIP proteins. Different color boxes represent eight putative conserved motifs, respectively. (b) The specific sequence of conserved motifs in S1-bZIP proteins. Names of all family members and E-values are shown on the left side. The blue dotted line scale represents the length of amino acid. The height of a letter indicates its relative frequency at the given position in the motif.

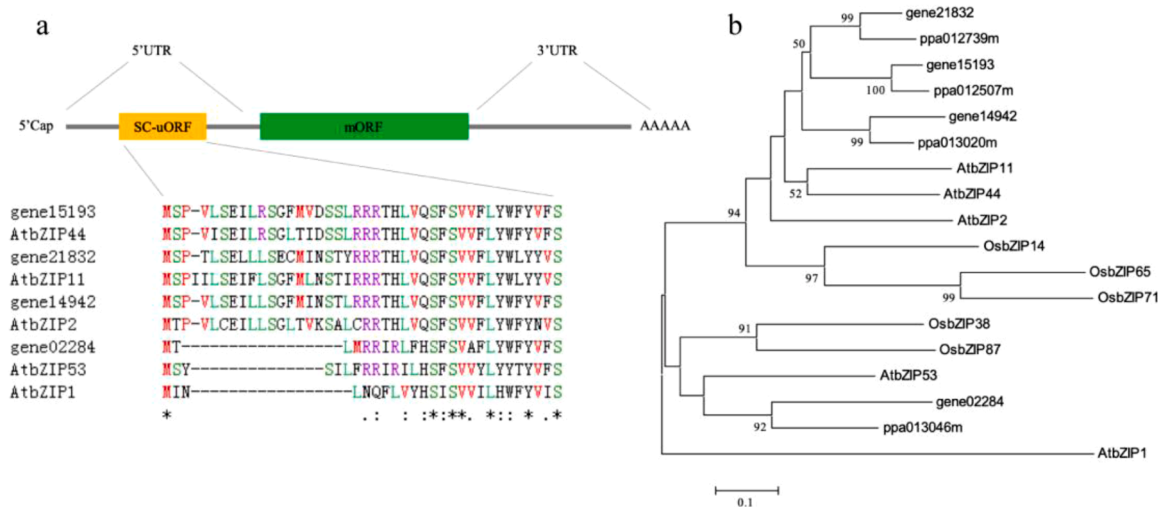


Fig. 2. Identification of S1-FvbZIPs from strawberry. (a) Schematic representation of the structure of S1-FvbZIP genes with their conserved uORFs aligned to the homologous sequences from Arabidopsis. (b) Phylogenetic relationship of S1-FvbZIPs with other closely related bZIP sequences from Arabidopsis, rice, peach based on the neighbor-joining method.

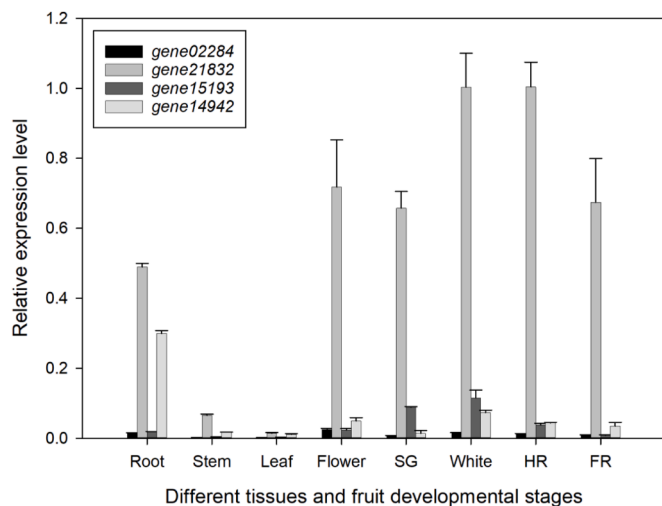


Fig. 3. Expression pattern of *S1-FvbZIP* genes in different tissues and fruit developmental stages. SG, small green; HR, half red; FR, full red.

3. Results

3.1. Sequence properties and conserved motif of *S1-FvbZIPs*

A total of four *FvbZIP-S1* genes (gene21832, gene15193, gene14942, gene02284) were identified in *Fragaria vesca* v1.0 genome database. As shown in Table 1, two members of gene21832 and gene15193 were mapped to chromosome 2, while gene14942 and gene02284 were respectively distributed to chromosome 1 and 5. The deduced amino acid lengths varied from 148 to 171 aa, and the predicted molecular weights (MW) ranged from 17.19 to 19.14 KDa. The gene21832 and gene15193 had the similar theoretical isoelectric point (pI) around 6.5, and the pI of gene14942 and gene02284 were 4.93 and 5.49, respectively. Instability indexes above 40 indicated that all *FvbZIP-S1* proteins were unstable. Aliphatic indexes defined as the relative volume occupied by aliphatic side chains were predicted between 58.65 and 79.49. The identified proteins with negative GRAVY (grand average of hydropathy) values within -0.58 and -0.94 were therefore hydrophilic. Subcellular localization results showed all of them were located in nuclear. To further investigate the structural diversity and predict function of *FvbZIP-S1* proteins, a total of eight conserved motifs were identified using online MEME tool (Fig. 1a). All members shared motif 1–3. Motif 1 was annotated as bZIP-basic domain. (Fig. 1b).

3.2. Conserved uORF and phylogenetic analysis of *S1-FvbZIPs*

Each *S1-bZIP* harbored a sucrose controlled upstream open reading frame (SC-uORF). Here, SC-uORFs of four *S1-bZIP* sequences in strawberry were isolated and aligned with those in Arabidopsis (Fig. 2a). The results showed that SC-uORFs were relatively conserved. The length of three SC-uORFs in gene15193, gene21832 and gene14942 were longer than that in gene02284. To analyze phylogenetic relationship of *S1-bZIP* proteins in strawberry, rice, peach, and Arabidopsis, the full-length amino acid sequences were aligned to construct an unrooted phylogenetic tree based on the neighbor-joining method (Fig. 2b). Phylogenetic tree was categorized into three distinct branches. *AtbZIP1* was singly formed to be a branch. *AtbZIP53* together with *OsbZIP38*, *OsbZIP87*, *ppa013046* and gene02284 clustered into a group, while others classified into another branch. The strawberry gene21832 were referred to as *FvbZIP11* as a consequence of it clustering with Arabidopsis homologous *bZIP11*-related TFs (*AtbZIP2*, -11, -44).

3.3. Expression profiling of *S1-FvbZIPs* in different organs

To explore the tissue-specific expression levels of these four *S1-FvbZIPs* in strawberry, strawberry root, stem, leaf, flower and different stage fruits were collected for qRT-PCR (Fig. 3). The results showed that four members could be detected in all tissues, but their expression patterns differed. Obviously, four *S1-FvbZIPs* had relatively lower transcript abundance in stem and leaf. The gene21832 was the most highly expressed gene among four *S1-FvbZIP* members in various tissues. In particular, its transcript was mainly presented during fruit development and ripening. The gene02284 had the lowest expression level in these tissues, and it most highly expressed in flower in comparison with other tissues. The gene14942 had a very significant transcript accumulation in root, while the gene15193 mainly expressed during the fruit development process and had the similar change tendency to gene21832 (increase and then decrease).

3.4. Molecular identification of transgenic tomato lines

As expected, the positive transgenic plants could be successfully detected the target fragment of *FvbZIP11* (Fig. 4a). Gene expression analysis by qRT-PCR showed that the transcript of *FvbZIP11* were only displayed in young leaves from three independently transformed T1 lines (L6, L8, and L9) in contrast to wild type (WT). Of these, L6 had the highest expression level and the L8 had the lowest transcript abundance (Fig. 4b).

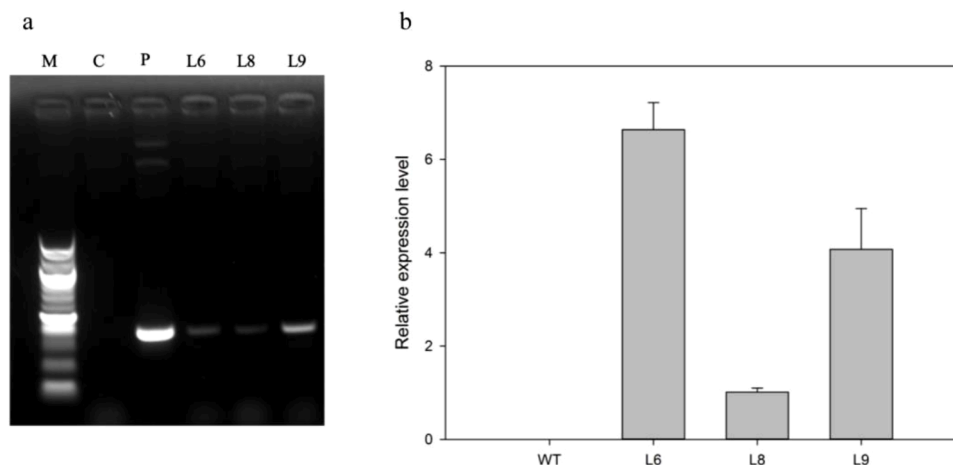


Fig. 4. Molecular analysis of *FvbZIP11* in transgenic plants. (a) Genomic DNA-PCR analysis of *FvbZIP11* in WT and transgenic lines. M, marker; C, WT; P, the plasmids with *FvbZIP11*; L6, L8, L9, transgenic lines. (b) The transcript level of *FvbZIP11* in leaves of WT and transgenic lines.

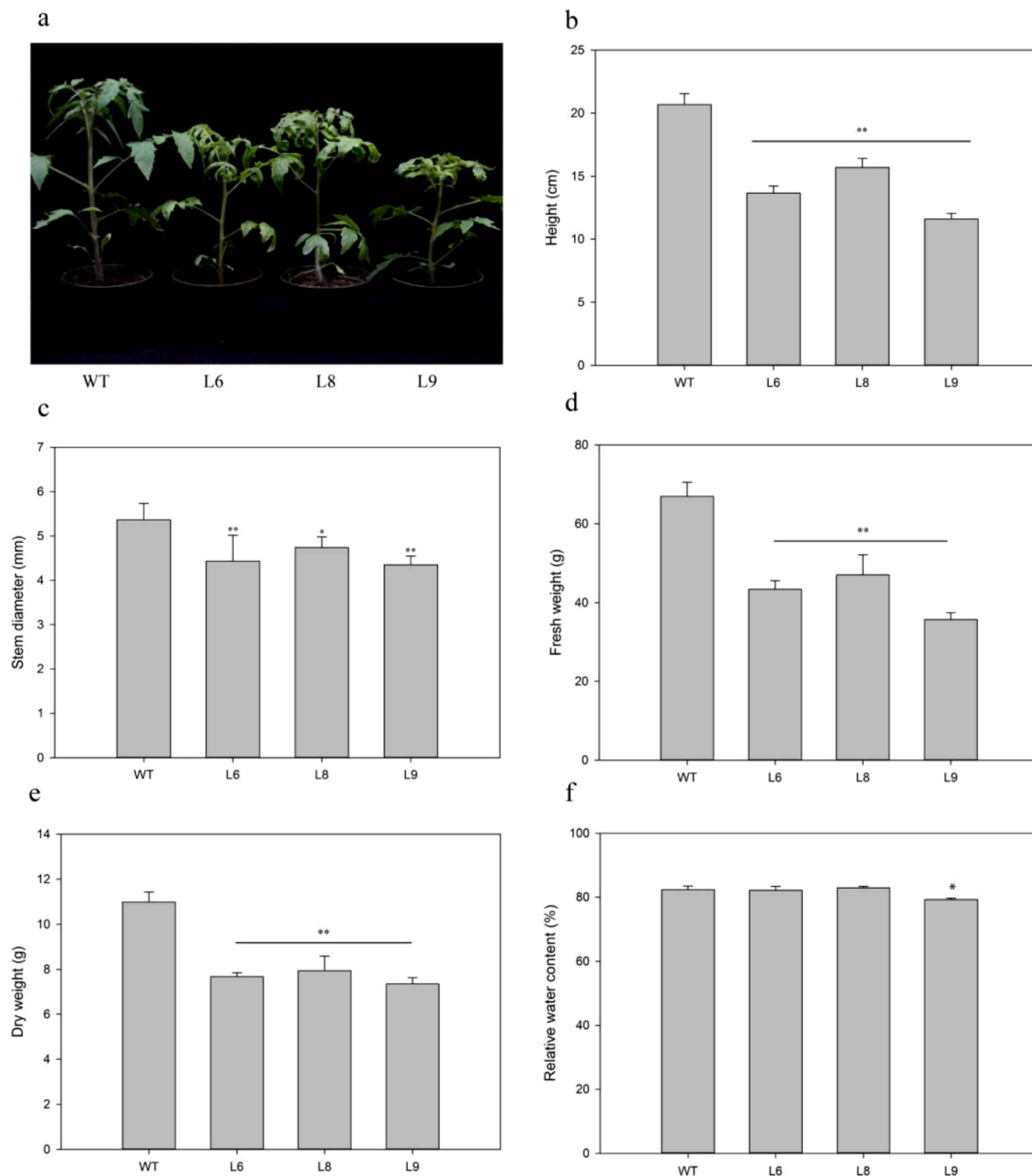


Fig. 5. The phenotype (a), height (b), stem diameter (c), fresh weight (d), dry weight (e), and relative water content (f) of transgenic lines and WT. Each value in the histogram represents mean \pm standard error from three biological experiments ($n = 3$). Asterisks indicate significant differences based on one-way analysis of variance in SPSS 23.0 followed by the Dunnett t -test (asterisk, $P < 0.05$; double asterisk, $P < 0.01$).

3.5. Morphological differences between wild type and transgenic tomato lines

The growth of transgenic plants was significantly retarded. The values of plant height, stem diameter, fresh and dry biomass of above-ground part were dramatically lower than wild type, but the relative water content was almost the same (Fig. 5). Transgenic plants displayed the curly and wrinkled leaf, accompanied by a significant decrease of the chlorophyll (Chl) a and b, while the carotenoid almost had no remarkable difference. Besides, overexpressing plants exhibited anatomical changes in the leaves, including smaller epidermal cells (Fig. 6).

3.6. Analysis of total soluble solid, soluble sugar and titratable acid content in fruits

As is shown in Fig. 7a, b, the contents of total soluble solid (TSS) and soluble sugar (SS) showed an upward trend in both transgenic tomato

and WT during fruit development. Moreover, the contents of TSS and SS in transgenic tomato were significantly higher than WT at different fruit developmental stages except small green or 20-day post anthesis (DPA). The titratable acid (TA) content in all samples increased firstly and peaked at 40DPA, and then decreased. No significant difference was detected in TA content between transgenic tomato and WT during the fruit development except 30DPA (Fig. 7c). The SS/TA ratio in transgenic tomato was noticeably higher than WT during the fruit development (Fig. 7d), indicating that *FvbZIP11* may increase SS/TA ratio by promoting the production of soluble sugar.

4. Discussion

The basic leucine zipper (bZIP) transcription factor family is one of the largest and most diverse TF families. It has been widely identified or predicted in plants, as the massive genome sequences are released, including rice (Nijhawan et al., 2008), wheat (Li et al., 2015b), maize

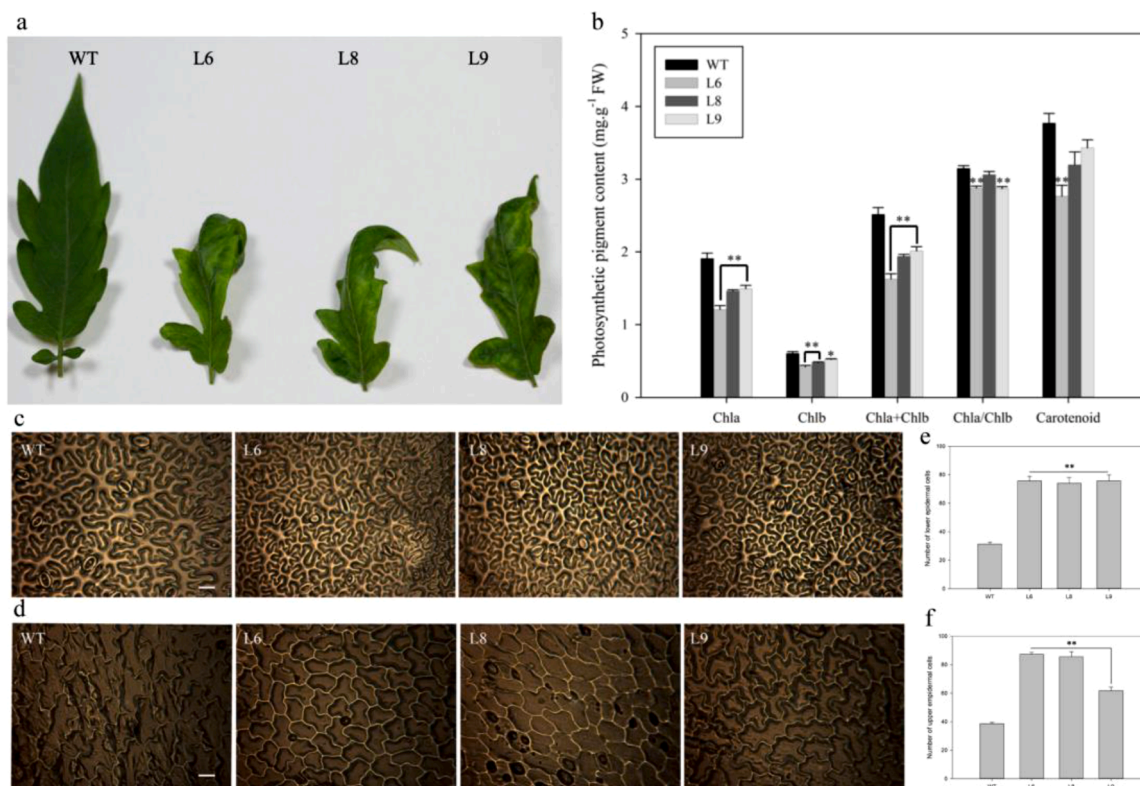


Fig. 6. The phenotype (a), photosynthetic pigments (b), lower epidermal cells (c, e) and upper epidermal cells (d, f) of transgenic plant and WT leaves. Each value in the histogram represents mean \pm standard error from three biological experiments ($n = 3$). Asterisks indicate significant differences based on one-way analysis of variance in SPSS 23.0 followed by the Dunnett t-test (asterisk, $P < 0.05$; double asterisk, $P < 0.01$). Chla, chlorophyll a; Chlb, chlorophyll b. Epidermal imprint images and cell numbers of the transgenic plants and WT were observed at the same magnifications.

(Wei et al., 2012), barley (Pourabed et al., 2015), Arabidopsis (Jakoby et al., 2002), tomato (Li et al., 2015a), cucumber (Baloglu et al., 2014), grapevine (Liu et al., 2014), tea (Xue et al., 2018), watermelon and melon (Unel et al., 2019), three rosaceae species of strawberry, peach and apple (Wang et al., 2015), etc. Extensive studies have provided strong evidences that bZIP TFs are involved in many different biological processes that range from the response to various types of stress to the control of the nitrogen/carbon balance, the regulation of genes involved in the seed protein storage and others (Corr ea et al., 2008). bZIPs family can be further classified into several subfamilies. Among these members, the S1 branch belonging to bZIP S subfamily is very special. They harbor an unusually long 5'-leader region corresponding to a highly conserved sucrose controlled upstream open reading frame (SC-uORF), which affects translation of bZIP protein (Dietrich et al., 2011; Wiese et al., 2004). This feature has been attracting much attention in exploring S1-bZIP function. It has been documented that S1-bZIPs played an important role in plant growth and development, especially affecting accumulation of metabolites (sugar, amino acid, etc.) which is very vital for fruits (Sagor et al., 2016). However, the bZIP S1 genes were just deeply studied in some model plants, like Arabidopsis, tobacco and tomato, and very little is known about them in strawberry and other fruit crops.

This study characterized gene14942, gene21832, gene15193, gene02284 four S1-bZIP members from strawberry at the genome-wide level, which were respectively distributed on chromosomes 1, 2, and 5. The results showed that all FvbZIP-S1 were unstable proteins, consistent with the previous report (Wang et al., 2017). Moreover, they were predicted to be nuclear-localized. It was related to the basic region of 16 amino acid residues with an invariant N-x7-R/ K motif in bZIP family (Jakoby et al., 2002). Obviously, the FvbZIP-S1 had highly conserved SC-uORFs in their long 5' UTRs, indicating they were involved in sucrose sensing such that the mORF translation was inhibited if the amount of

cellular sucrose increases and vice versa. The conserved uORFs take up only a fairly small portion of all uORFs in eukaryotic transcripts, but it is a key element for gene expression at post-transcriptional level (Jorgensen and Dorantes-Acosta, 2012; Tran et al., 2008). It has been reported that they can regulate the translation of the downstream mORFs through a specific metabolite, i.e. sucrose (Thalor et al., 2012), polyamines (Hanfrey et al., 2005), and phosphocholine (Alatorre-Cobos et al., 2012), in a feedback manner. However, there is little information about the mechanism that uORFs are sensitive to small molecules. The circumstantial proofs somewhat hint that uORFs possibly act as the receptor for the metabolites (Von Arnim et al., 2014). The phylogenetic analysis showed strawberry S1 FvbZIPs had a closer correlation with peach and Arabidopsis S1-bZIPs than those of monocotyledonous rice, indicating the genes were established before the corresponding taxonomic lineages diverged. Moreover, those homologs clustered in the same group were possible to be involved in similar functions.

Four S1-FvbZIP genes were expressed in all of the samples analyzed, but they had the different transcript abundances, suggesting they may possess functional divergence and redundancy for strawberry growth and development. Notably, the expression level of *gene21832* kept greatly high during fruit development and other tissues, so we speculated the *gene21832* regulated metabolism involved in fruit ripening or quality, and affected plant growth. Since Arabidopsis bZIP11-related TFs was the closest homolog of *gene21832*, it was denoted as *FvbZIP11* to reflect this homology. The previous studies have illustrated that S1-bZIPs could be induced by different external elicitors and had the organ-specific feature (Lee et al., 2006; Weltmeier et al., 2009). *AtbZIP11* transcripts are abundant in flowers and roots, but fairly low in leaf tissues; i.e., photosynthetic organs, indicating the gene plays a role in carbohydrate-consuming (i.e. sink) tissues. *AtbZIP11* transcript levels are upregulated by both light and sugars, which contribute to photo-assimilates availability as a result of photosynthesis. When carbohydrate

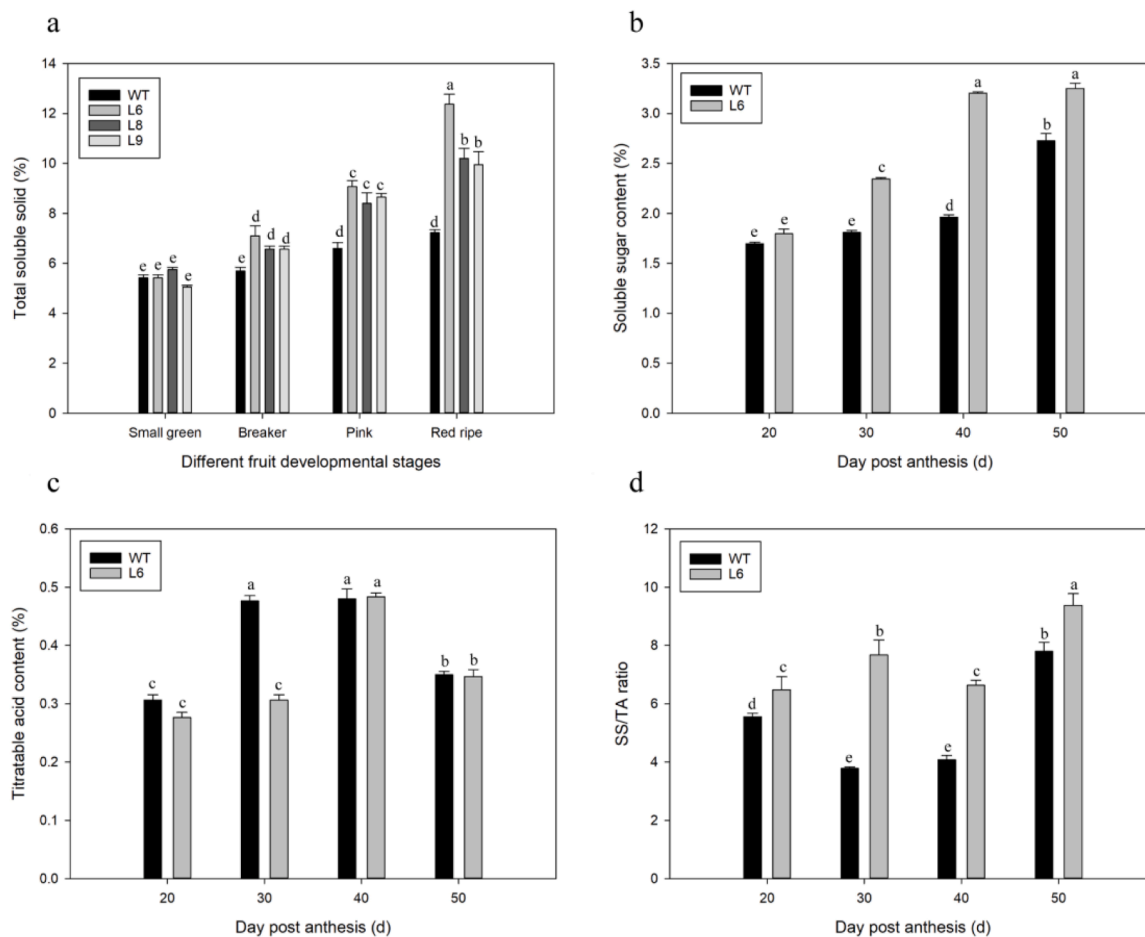


Fig. 7. The total soluble solid (a), soluble sugar (b), titratable acid (c) and SS/TA ratio (d) in fruits. Each value in the histogram represents mean \pm standard error from three biological experiments ($n = 3$). Lowercase letters indicate significant differences based on one-way analysis of variance in SPSS 23.0 followed by the Duncan-test ($P < 0.05$).

supply is sufficient, sucrose-mediated repression of *AtbZIP11* translation would be initiated. In this way, *AtbZIP11* activity could keep carbohydrate homeostasis in plant (Rook et al., 1998a; Rook et al., 1998b). In contrast, the expression levels of *AtbZIP1* and *AtbZIP53* were induced after extended night treatment and repressed by sugars application (Dietrich et al., 2011). In strawberry, *bZIP11* (gene21832) was induced by red and blue light, while *bZIP53* homologue (gene02284) was depressed. In addition, sucrose treatment did not significantly affect *bZIP53* at transcriptional level (Chen et al., 2020). These findings indicated that bZIP S1 members differentiated in response to some factors. All of bZIP S1 transcriptional factors have the SIRT-responsive uORFs, so researchers proposed a novel SIRT-bZIP technology to enhance sweetness especially for some plant species rich in sucrose (Sagor et al., 2016). In our study, strawberry *bZIP11* overexpressing in tomato indeed increased the TSS and SS content and SS/TA ratio, which provided an applicable method for improvement of strawberry and other fruit quality in the future. However, constitutive overexpression of *FvbZIP11* caused a growth impairment, which have been observed in tobacco (Thalor et al., 2012), Arabidopsis (Alonso et al., 2009), banana (Shekawat and Ganapathi, 2014). An explanation of this phenotype was that *bZIP11* presumably severely affected carbohydrate partitioning via a mechanism that might include direct regulation to cell-wall invertase and sucrose transporter expression (Wobbles, 2004). To avoid growth impairment, Sagor et al. (2016) overexpressed *SlbZIP1* and *SlbZIP2* under the control of the fruit-specific E8 promoter. The growth and morphology of the resulting transgenic tomato plants were comparable to those of wildtype plants. Most fruit-specific promoters currently

available have been isolated from tomato, but these promoters probably are inappropriate to be used in non-climacteric fruits, like strawberry (Nardi et al., 2016). Hence, identification of a suitable promoter could facilitate the function analysis of a specific gene involving fruit development and help to specifically improve fruit quality.

5. Conclusion

The present work identified four members of S1-bZIP transcription factors at genome-wide level and revealed their sequence properties, conserved motif, uORF structure and phylogenetic relationship by systematical bioinformatics. The expression pattern analysis among different tissues and fruit developmental stages showed S1-bZIP genes probably had functional divergence and redundancy for strawberry growth and development. The *gene21832*, referred to as *FvbZIP11* was the most highly expressed gene among four S1-*FvbZIP* members in various tissues. In particular, its transcript was mainly presented during fruit development, indicating *FvbZIP11* played an important role in strawberry fruit ripening. Constitutive expression of *FvbZIP11* in tomato caused a plant growth impairment, but significantly increased the fruit TSS and SS content and SS/TA ratio. These findings provided valuable information in developing strategies to improve strawberry and other fruit quality.

CRedit authorship contribution statement

Yunting Zhang: Writing – original draft, Software. **Shanlin Li:**

Resources, Data curation. **Yan Chen:** Visualization, Formal analysis. **Yongqiang Liu:** Validation. **Yuanxiu Lin:** Visualization. **Mengyao Li:** Investigation. **Yan Wang:** Formal analysis. **Wen He:** Visualization. **Qing Chen:** Writing – review & editing. **Yong Zhang:** Software. **Ya Luo:** Writing – review & editing. **Xiaorong Wang:** Writing – review & editing. **Cai-Zhong Jiang:** Conceptualization, Supervision. **Haoru Tang:** Conceptualization, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Supplementary materials

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