The tomato CONSTANS-LIKE protein SlCOL1 regulates fruit yield by repressing SFT gene expression

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Abstract
Background: CONSTANS (CO) and CONSTANS-LIKE (COL) transcription factors have been known to regulate a series of cellular processes including the transition from the vegetative growth to flower development in plants. However, their role in regulating fruit yield in tomato is poorly understood.

Result: In this study, the tomato ortholog of Arabidopsis CONSTANS, SlCOL1, was shown to play key roles in the control of flower development and fruit yield. Suppression of SlCOL1 expression in tomato was found to lead to promotion of flower and fruit development, resulting in increased tomato fruit yield. On the contrary, overexpression of SlCOL1 disturbed flower and fruit development, and significantly reduced tomato fruit yield. Genetic and biochemical evidence indicated that SlCOL1 controls inflorescence development by directly binding to the promoter region of tomato inflorescence-associated gene SINGLE-FLOWER TRUSS (SFT) and negatively regulating its expression. Additionally, we found that SlCOL1 can also negatively regulate fruit size in tomato.

Conclusions: Tomato SlCOL1 binds to the promoter of the SFT gene, down-regulates its expression, and plays a key role in reducing the fruit size.

Keywords: Constans-like, Flowering, Fruit, SINGLE-FLOWER TRUSS, Tomato, Yield

Background
Tomato is one of the most important vegetable crops cultivated worldwide. It also serves as a model plant for research on fruit development and fruit ripening. Breeding for high yield has been one of the ultimate goals for crop breeders. Inflorescence architecture is the main determinant of flower number and crop yield [1]. With the increasing demands for tomato, higher standards for the high-yield tomato varieties have been put forward. Therefore, a better understanding of the key genes that regulate tomato fruit yield is very important for commercial production.

FLOWERING LOCUS T (FT) has been shown to be a key protein at the convergence of several signaling pathways and serves as the key flowering initiation signal, i.e. the florigen, in Arabidopsis. The function of FT as the flowering inducer is conserved among plant species [2–5]. SINGLE-FLOWER TRUSS (SFT), the tomato ortholog of FT, regulates primary flowering time, sympodial habit, and inflorescence development [6, 7]. Tomato sft mutant plants produce flowers later than the wild type, and the inflorescences revert to indeterminate vegetative branches or become a single flower, and the yield of the mutant is significantly decreased [6, 7]. Several regulatory factors of FT have
been identified. The trimeric Nuclear Factor-Y (NF-Y) complexes, comprising CO/NF-YB/NF-YC, bind to the CCAAT DNA element of the FT gene promoter and regulate flowering time [8, 9]. Arabidopsis CONSTANS promotes FT gene expression, accelerating flowering in the long day condition [10, 11]. In rice, Heading date 1 (Hd1), the ortholog of Arabidopsis CONSTANS, promotes flowering under short-day conditions, but delays flowering under long-day conditions by regulating the expression of the rice FT ortholog, Heading date 3a (Hd3a) [12]. Thus, more transcription factors involved in regulating the expression of SFT need to be explored in tomato.

CONSTANS is a B-box (BBX) protein, originally identified in Arabidopsis thaliana [13]. There are 32 BBX family members in Arabidopsis, which can be divided into five structural groups, based on the number and sequence features of the B-box domain and the presence or absence of a CCT domain [14]. CONSTANS has been identified as a mediator of the circadian clock in controlling the flowering time in Arabidopsis [4, 5, 10, 11]. CONSTANS-LIKE (COL) genes have been studied in many other plant species. Overexpression of COL5 can induce flowering in short-day grown Arabidopsis [15]. On contrary, overexpression of COL9 delays flowering by reducing the expression of CO and FT in Arabidopsis [16]. OsCOL3, a rice CONSTANS-LIKE gene, controls flowering time by down-regulating the expression of FT-like genes under short-day conditions [17]. OsCOL13 functions as a negative regulator of flowering downstream of OsphyB and upstream of Ehd1 in rice [18]. In tomato and tobacco, overexpression of COL1 and COL3 has resulted in late-flowering phenotypes [19]. However, it remains unknown if COL1 participates in direct regulation of SFT gene expression in tomato.

Previous studies on CO and COL proteins have been focused mainly on their roles in mediation of the circadian clock and flowering time in plants. Here we show that SICOL1 binds to the promoter of SFT and negatively regulates its expression. Suppression of SICOL1 gene expression in transgenic tomato lines increased the flower and fruit numbers and the size of fruits. On the other hand, its overexpression in transgenic tomato lines resulted in increase in the number of vegetative inflorescences, decrease in the numbers of flowers and fruits, and reduction in the size of fruits. Furthermore, yeast one-hybrid experiments and GUS reporter assays showed that SICOL1 can directly bind to the cis-regulatory elements of the SFT promoter. These findings provide new insight on how SICOL1 negative regulates tomato fruit yield.

Results
Expression patterns of SICOL
SICOL1 (Solytc02g089540), also referred to as SIBBX3, is the ortholog of Arabidopsis CONSTANS (CO) protein. SICOL1 has an ORF of 1176 bp, encoding a protein of 391 amino acid residues that contains two B-box domains and a CCT domain. The B-box is a conserved 88-amino acid region and the two B-box domains span the region of the amino acid residues (34–297). The CCT domain is a conserved 45-amino acid region (964–1098). Gene expression analysis showed that SICOL1 was expressed in all tested tissues, with the highest expression in the mature leaves and flowers (Fig. 1A). Analysis of GUS staining in ProSICOL1::GUS transgenic line 10 plants 90 days post anthesis (dpa) showed high expression of SICOL1 in the flowers (SAM) and flowers, and very low in the stems, young leaves and fruits (Fig. 1B-C).

The subcellular localization of SICOL1 protein was determined using confocal laser scanning microscopy. Bioinformatics analysis indicated that a nuclear localization signal is present in the same region where the B-box and CCT domain reside. The nuclear marker protein Ghd7 [20] was fused with the cyan fluorescent protein (CFP) for the identification of the nucleus. We found that the SICOL1-GFP protein was localized exclusively to the nucleus and its green fluorescence fully overlapped the cyan fluorescence of Ghd7-CFP, when co-expressed in the N. benthamiana protoplasts (Fig. 2). In contrast, the free GFP fluorescence was distributed throughout the cell (Fig. 2B). Thus, SICOL1 is a nuclear protein, which is consistent with its function as a transcription factor.

Regulation of inflorescence morphology and fruit numbers
To better understand the function of SICOL1, we generated SICOL1 RNA interference (RNAi) and overexpression (OE) tomato transgenic lines. Three independent lines (OE-5, OE-6 and OE-8, and RNAi-1, RNAi-10 and RNAi-17) from each transformation were selected for further analysis (Fig. S1A). The transgenic tomato lines were morphologically distinguishable from the wild-type plants under normal growth conditions. Eight weeks after germination, the average number of sympodial units under the first inflorescence was about 8 in the SICOL1-RNAi lines, as compared to 12 in the WT plants. The number of flowers and fruit yield were also increased in the SICOL1-RNAi lines. On the other hand, SICOL1-OE lines produced about 16 leaves in the primary shoot which was significantly more than that of the WT plants. The fruits of the SICOL1-OE lines were scattered on the inflorescences (Fig. 3A, C). The numbers of flowers and fruits over the entire growth season was also examined. The transgenic plants produced 46–130 flowers and 1–40
fruits in the \textit{SlCOL1}-RNAi lines and 14–66 flowers and 0–18 fruits in the \textit{SlCOL1}-OE lines. In contrast, WT plants produced 50–110 flowers and 1–30 fruits (Fig. 3D, E). The total yield of fruit was increased approximately 37% in the \textit{SlCOL1}-RNAi lines and reduced approximately 42% in the \textit{SlCOL1}-OE lines as compared to the fruit yield in the WT plants (Fig. 3B). These results illustrated that \textit{SlCOL1} plays a major role in the regulation of flowering time, flower and fruit number and yield in tomato.

\textbf{SlCOL1 represses the expression of the flowering gene \textit{SFT}}

Tomato \textit{sft} mutant inflorescences revert to indeterminate vegetative branches or become a single fertile flower, thus, the mutant plant has much fewer flowers and a much lower fruit yield than WT [6, 7]. The tomato \textit{SlCOL1} overexpression transgenic and \textit{sft} mutant plants were phenotypically similar. Their flower and fruit numbers were significantly reduced (Fig. 3D, E), and as a result, their fruit yield was reduced as well (Fig. 3A-B).

\textbf{CONSTANS activates \textit{FT} transcription through binding to the CO-responsive (CORE, CCACA) and CCAAT-box elements in the \textit{FT} gene promoter in \textit{Arabidopsis}} [8, 21–23]. Gene expression analysis revealed that the \textit{SFT} (Solyc03g063100) transcripts were lower in the \textit{SlCOL1}-OE lines but higher in the \textit{SlCOL1}-RNAi lines when compared with those in the WT plants (Fig. S1B). Thus, \textit{SlCOL1} gene appears to serve as the core transcription factor that regulates the expression of the \textit{SFT} gene in tomato.

\textbf{SICOL1 negatively regulates \textit{SFT} expression by directly binding to the regulatory \textit{cis}-elements in the \textit{SFT} promoter}

We searched the 2.5 kb promoter region of \textit{SFT} and found three CCACA and four CCAAT sequences (Fig. 4A). To examine if \textit{SlCOL1} could bind to these \textit{cis}-elements and drive gene expression, we first selected five \textit{SFT} promoter fragments that contained different combinations of the conserved \textit{cis}-DNA elements, including SFT1 (no \textit{cis}-element), SFT2/3/4 (different combinations of the two \textit{cis}-elements) and SFT5 (only one CCACA \textit{cis}-element) (Fig. 4B). Three constructs (SFT2/3/4) were found to confer the antibiotic resistance in the presence of 10–20 mM AbA when \textit{SlCOL1} was co-expressed. In contrast, the
constructs that contained no cis-element (SFT1) or only one cis-element (SFT5) could not rescue the yeast cell growth in the presence of 10 mM AbA (Fig. 4B), suggesting that the cis-elements of the SFT promoter were required for the SICOL1 transcription factor to drive the resistance gene expression, and one cis-element (CCACA) was not sufficient to allow the resistance gene expression in this Y1H system. In order to examine the minimum cis-elements that were required for the AbA resistance gene expression, we selected six SFT promoter fragments that contained either the CCACA motif (in SFT2–1 and SFT3–2) or the CCAAT element (in SFT2–2, SFT3–1, SFT4–1, and SFT4–2) from SFT2, SFT3, and SFT4 (Fig. S2). Four constructs (SFT2–2, SFT3–1, SFT4–1, and SFT4–2) were found to confer the antibiotic resistance in the presence of 10 mM AbA, when SlCOL1 was co-expressed. In contrast, the constructs that contained the CCACA motif alone (in SFT2–1 and SFT3–2) could not rescue the yeast cell growth in the presence of 10 mM AbA (Fig. S2). These results suggest that the CCAAT cis-element was necessary and sufficient for the binding of the SICOL1 transcription factor to the SFT promoter.

To test whether SICOL1 could regulate the expression of the SFT gene in planta, we co-expressed 35S–SICOL1 and ProSFT-GUS constructs in tobacco leaves. Our result showed that the GUS reporter (ProSFT-GUS) alone was able to express in tobacco leaves (Fig. S3), suggesting that the host cells had endogenous transcription factors that could drive the reporter gene expression. This background level of the reporter expression was not affected by the co-expression of the empty vector of pHELLS-GATE8 in tobacco leaves (Gate8 + ProSFT-GUS, Fig. 4C left). However, when the 35S–SICOL1 construct was used to replace the empty pHELLS-GATE8 vector, the GUS staining became much weaker (SICOL1 + ProSFT-GUS, Fig. 4C middle), suggesting that co-expression of SICOL1 repressed the GUS expression driven by the SFT gene promoter. These results indicated that SICOL1 acts as a transcriptional repressor of the SFT gene in planta.

**SICOL1 negatively regulates fruit size in tomato**

The total yield of fruit was significantly increased in the **SICOL1**-RNAi lines and reduced in the **SICOL1**-OE lines as compared to the fruit yield in the WT plants (Fig. 3B). In addition to the reduction in the number of fruits, we found the average of fruit weight was 27 to 31% higher in the RNAi lines and 18 to 26% lower in the overexpression lines than the WT plants (Fig. 5A–B). The length and diameter of the fruits were also reduced in the **SICOL1**-OE plants and increased in the **SICOL1**-RNAi plants.
relative to the WT (Fig. 5C). These results illustrated that SlCOL1 also plays a major role in the regulation of fruit size in tomato.

**SlBBX24 functions to regulate tomato fruit size**

The fruit size was significantly increased in tomato sft mutant plants as compared with WT [7]. In the SlCOL1-OE plants, the fruit size was significantly decreased (Fig. 5). Therefore, we believe that SlCOL1 regulates fruit size in tomato not directly through regulating SFT gene expression. Tomato SlBBX24 (Solyc06g073180), as a CONSTANS-LIKE protein, has two B-BOX domains and interacts with SlCOL1 [19, 24]. For these reasons, we tested whether SlBBX24 may regulate tomato fruit development. SlBBX24 expression was determined using real-time RT-PCR on total RNA extracted from various tomato organs. The transcripts of SlBBX24 were detected in all tissues tested, with the highest expression level in leaves and flowers, which is similar to the expression patterns of SlCOL1 (Fig. S4).

To better understand the function of SlBBX24, we next generated SlBBX24 overexpression (OE) and CRISPR/
Fig. 4 (See legend on previous page.)
cas9 (CR) tomato lines. Three lines of each transformation experiment were selected for further analysis, including OE-2, OE-3 and OE-9 from the overexpression lines and CR-1, CR-2 and CR-3 from the CRISPR/cas9 transformation. We found that the *SlBBX24* gene could affect the fruit size based on our transgenic functional analysis (Figs. 6A, S5). Overexpression of the *SlBBX24* significantly reduced the fruit size (Fig. 6). However, no significant phenotype in fruit size and other plant morphological traits was observed in the three CR-*sibbx24* lines as compared to those in the WT plants. We tested the fruit weight and fruit length and diameter of transgenic lines and WT plants and found that fruits of the overexpression lines were smaller than those of the WT plants (Fig. 6B-C). These results suggest that SIBBX24 may interact with SICOL1 to form a heterodimer of transcription factor and plays a role to regulate fruit size in tomato.

In this work, we found that the transcript levels of *SICOL1* and *SFT* were not affected in *SIBBX24*-OE lines as compared with their expression levels in WT (Fig. S6). We also found that the transcript levels of *SIBBX24* were not affected in *SICOL1*-RNAi lines as compared with that in WT (Fig. S7, right). These results showed that SIBBX24 may not regulate flowering time in tomato, but participates in fruit size regulation by interacting with SICOL1.

**Discussion**

The BBX transcription factor family is known to be involved in a wide range of cellular processes, including the resistance to abiotic stresses [25–27], control of the...
circadian clock [28] and regulation of flowering time [17, 29]. Several BBX genes have been shown to play key roles in the regulation of flowering time and flower development in different plant species. Within a plant species, several BBX genes are known to participate in flowering regulation through different mechanisms [12, 15, 16, 19, 30, 31]. As the first identified BBX protein, CONSTANS is known to activate FT transcription through binding to the CORE (CCACA) and CCAAT-box cis-elements in the FT promoter in Arabidopsis [8, 21–23, 31]. Hd1, the rice ortholog of CO, also regulates the expression of Hd3a, the rice ortholog of Arabidopsis FT, by binding to the CORE (CCACA) DNA element of the Hd3a promoter [12, 32–34]. In this study, we demonstrated that tomato SICOL1 regulates flower time, flower number and yield by binding to the SFT gene promoter, repressing its expression (Figs. 3, 4 and S1B). These results illustrate that CO and its orthologs play conserved roles in flowering regulation through binding to the FT promoter to regulate its expression.

CO and its homologs have been shown to regulate the expression of downstream target genes by modulation of DNA methylation. In Arabidopsis, overexpressing CO can change the chromatin status in the FT locus, such as a decrease in binding of LIKE HETEROCROMATIN PROTEIN1 (LHP1) and an increase in the acetylation of H3K9 and K14 [31]. In addition, Nuclear Factor-Y (NF-Y) can interact with CO to modulate H3K27me3 levels of the SOC1 promoter and regulate the transcription of SOC1 in Arabidopsis [35]. In rice, the DTH8 (NF-YB) transcription factor plays a critical role in mediating the Hd1 regulation of Hd3a transcription in photoperiodic flowering through its interaction with Hd1 to shape epigenetic marks. The DTH8-Hd1 module enhances H3K27 trimethylation at Hd3a and represses Hd3a expression in long day conditions, but reduces the H3K27me3 levels at Hd3a and enhances Hd3a expression in short day conditions [12]. In our previous study, we have illustrated that NF-YBs bind to the CCAAT element of the CHS1 promoter and regulate the levels of H3K27me3 at the CHS1 locus during tomato fruit ripening. Suppression of the expression of NF-YB significantly reduces the expression level of CHS1 and leads to the development of pink-colored fruits with colorless peels [36]. Previous studies have revealed that CONSTANS may replace NF-YA in the NF-Y complex to form a trimeric CO/NF-YB/NF-YC complex [19, 21]. Therefore, we hypothesized that SICOL1 represses the expression of SFT possibly through regulating the levels of H3K27me3 at the SFT promoter by interacting with the NF-Y complex.

The BBX gene family comprises 29 members in tomato and can be divided into five structural groups based on the number and sequence features of the B-box domain and the presence or absence of a CCT domain [24]. In this study, we found that down-regulation of the expression of SICOL1 by RNAi led to drastic phenotypes of flower development, while knocking out SICOL1 by CRISPR/cas9 did not display any visible phenotype in plant growth and reproduction (Fig. S8). This implies that there could be redundancy in the BBX genes. Sequence analysis indicated that SICOL2 (Solyc02g089500) and SICOL3 (Solyc02g089520) share high similarities with SICOL1, and they are grouped into the same branch in the BBX family. We assume that SICOL2 and SICOL3 may play redundant roles with SICOL1 in the regulation of flowering time and fruit yield. In fact, the expression levels of SICOL2 and SICOL3 were both reduced in the SICOL1-RNAi lines (Fig. S1A).

Our previous studies have shown that overexpression of SIBBX20 results in transgenic tomato plants with smaller leaves and plant size as compared with those of the WT plants [37]. The fruit size has also been found to be reduced in the SIBBX20-OE lines. This implies that BBX genes from different groups of the BBX gene family may play a similar function in regulating organ size in tomato. In the present work, the SIBBX24 gene was shown to regulate the tomato fruit size as well. Moreover, the fruit size of SIBBX24 overexpression lines was found to be smaller than that of WT (Fig. 6). SIBBX20 and SIBBX24 genes belong to the same branch in the BBX family [24]. It is interesting to point out that SIBBX20 and SIBBX24 are not grouped to the same branch with SICOL1 in the BBX family [24]. In this work, the transcript levels of SIBBX20 and SIBBX24 were not affected in SICOL1-RNAi lines (Fig. S7, left). These results imply that SIBBX20 and SIBBX24 may exert their biological functions in the regulation of fruit size through interacting with SICOL1 or through regulating the expression of other genes. It is also likely that SICOL1, SICOL2, SICOL3, SIBBX20 and SIBBX24 all play a role, either uniquely or redundantly, in regulating fruit size in tomato.

**Conclusion**

Based on our findings, we propose a model in which SICOL1 controls the tomato yield traits by regulating the expression of SFT, and regulates tomato fruit size by modulating the expression levels of downstream genes (Fig. 7). There are at least two distinct pathways: SICOL1 may act as a transcriptional repressor that controls the production of fruit by down-regulating SFT expression (Fig. 7); and SIBBX24 and SICOL1 may control tomato fruit size by regulating the expression levels of downstream genes (Fig. 7). Thus, the fine tuning of the expression of SICOL1 will have the potential for improving tomato fruit yield (fruit number and size) and a better
understanding of this pathway may eventually lead to similar genetic improvements in other crops.

Materials and methods

Plant materials and growth conditions

The tomato (Solanum lycopersicum) variety Ailsa Craig (AC, LA2383A) was used as the wild-type (WT) control and for genetic transformation experiments in this study. The seeds of AC were originally obtained from the Tomato Genetics Resource Center, UC Davis, USA (https://tgrc.ucdavis.edu/, accession number LA2383A) with permission. WT (AC) and transgenic lines were grown in nutrition pots in a greenhouse on the campus of Huazhong Agriculture University in Wuhan (30.4 °N, 114.2 °E), China. Nicotiana benthamiana and tomato plants were grown in an environmentally controlled room at 22 °C with a photoperiod of 16 h light/8 h darkness.

RNA isolation and gene expression analysis

Total RNA was extracted from various tissues of the transgenic lines or WT plants using the TRIZOL reagent (Invitrogen, USA). Complementary DNAs (cDNAs) were synthesized using an M-MLV reverse transcriptase kit (Toyobo, Japan). The LightCycler480 SYBR Green I Master Kit (Roche Applied Sciences, Germany) was used for qPCR analysis. Three biological replicates from each genotype were carried out and analyzed for statistical differences. The Actin gene (BT013524, Solyc11g005330) was used as the internal control. The primer sequences used in real-time PCR are listed in Table S1.

Vectors constructs and tomato transformation

The full-length ORF and RNAi fragments for SICOL1 and SIBBX24 were amplified from tomato cDNA using the KOD-Plus DNA polymerase (Toyobo, Japan) and cloned into the effector vector pHELLSGATE8. The CRISPR/cas9 (PTX041) vector targeted two sites in the first exon of the ORF of SIBBX24 were designed at CRISPR-PLANT (http://www.genome.arizona.edu/crispr/CRISPR search. html). The sequences of primers used in these experiments are listed in Table S1. The vectors were introduced into the Agrobacterium tumefaciens strain C58. This strain was used for plant transformation in tomato Ailsa Craig (AC) as described previously [38]. Genomic DNA was extracted from transgenic plants using the CTAB method as described by Murray and Thompson (1980). The genomic DNA was analyzed using PCR-based markers to identify transgenic plants. The transgenic materials (SICOL1-OE, SICOL1-RNAi, CR-bbx24 and SIBBX24-OE) have been deposited in Key Laboratory of Horticultural Plant Biology, Ministry of Education, Huazhong Agricultural University (Hubei, China).

Yeast one-hybrid assay

The yeast one-hybrid (Y1H) assay was used to test whether COL1 could bind to the SFT promoter. The full-length SICOL1 ORF sequence was amplified from tomato cDNA and cloned into pGADT7 (Clontech). Five promoter fragments (−2528 to −2246 bp, −289 to −0 bp, −2087 to −1791 bp, −862 to −501 and −519 to −0 bp relative to the translation initiation codon of the SFT) were amplified from tomato genomic DNA and cloned into pAbAi (Clontech). The transformed yeast strains were picked and diluted in 0.9% NaCl to an OD₆₀₀ of 0.1, and 2 μL of the suspension was spotted on a SD/−Leu medium, with or without aureobasidin A (AbA, Clontech). The plates were incubated for 3 to 7 days in an incubator at 30 °C.

GUS staining

For GUS staining in tobacco, the full-length ORF of SICOL1 and was amplified and cloned into the effector vector pHELLSGATE8. The cauliflower mosaic virus (CaMV) 35S promoter was used to drive gene expression in pHELLSGATE8 vector. The 2.53-kb promoter region of SFT was amplified and cloned into the effector vector pHELLSGATE8 (with the GUS gene, but without the 35S promoter). A. tumefaciens GV2260 was separately transformed with the effector and reporter vectors. For GUS staining in tomato, a DNA fragment of 3013 bp from the SICOL1 promoter region was amplified by PCR and cloned into the effector vector pHELLSGATE8 (with the GUS gene, but without the CaMV35S promoter). Agrobacterium tumefaciens strain C58 was transformed with the vector. This strain was used for plant transformation in tomato Ailsa Craig (AC) as described previously [38]. Transgenic tomato seedlings, floral buds, and developing...
fruits at different stages were selected for GUS staining. The selected seedlings and tissues were incubated at 37°C for 24h in staining buffer (100mM sodium phosphate, pH 7, 0.1% Triton X-100, 0.1% N-laurylsarcosine, 10mM Na2EDTA, 1 mM K3Fe (CN)6, 1 mM K4Fe (CN)6, and 0.5 mg mL-1 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid), followed by washing with 70% (v/v) ethanol. The expression of the *GLUS* gene was quantified using qRT-PCR. All primers used for the construction of the vectors are listed in Table S1.

**Transient expression in tobacco protoplasts and microscopy**

The *SICOL1* CDS without the stop codon was amplified by PCR and fused to the 5′ end of the open reading frame encoding GFP in pCAMBIA 1302, which uses the CaMV 35S promoter to drive gene expression, generating 35S:SICOL1-GFP. Ghd7-CFP was used as the marker for the nucleus. Tabaco leaf protoplasts were prepared and transient transcriptional activation was assayed as described previously [39]. Fluorescence from the transformed protoplasts was imaged using a confocal laser scanning microscope (Leica TCS SP2). The pertinent primer sequences are listed in Table S1.

**Statistical analysis**

Statistical analyses were conducted using SigmaPlot, Excel and the SPSS (IBM, SPSS 22) software. Comparisons between pairs of the groups were performed using the Student’s *t*-test. Statistically significant differences were categorized into two groups: *P* < 0.05 and *P* < 0.01.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12870-022-03813-4.

**Additional file 1: Fig. S1.** Transcript levels of SICOL1, SICOL2, SICOL3 and SFT in SICOL1 transgenic and WT plants. A-B Quantitative RT-PCR analysis of SICOL1, SICOL2 and SICOL3 expression (A) and SFT expression (B) in the young leaves of the WT tomato and three representative lines each of SICOL1-OE and SICOL1-RNAi. Asterisks indicate statistically significant differences. ***, *P* < 0.01.

**Additional file 2: Fig. S2.** Yeast-one-hybrid (Y1H) analysis of SICOL1 binding to the different core sequences of the SFT promoter. Six constructs containing six different promoter fragments (SFT2–1 to SFT4–2) were used in Y1H assays. The bait vectors, SFT2–1 to SFT4–2, and the SICOL1-containing prey vector were introduced into the yeast strain Y1H Gold. The enhanced resistance to antibiotic aureobasidin A (AbA) indicated an interaction between the bait and prey vectors. Co-transformation of the bait vectors, SFT2–1 to SFT4–2, with either pGADT7 or pGADT-Rec2–53 served as negative and positive controls, respectively.

**Additional file 3: Fig. S3.** GAL4/UAS-based analysis on ProSFT-GUS.

**Additional file 4: Fig. S4.** Transcripts levels of SIBBX24 in different tomato organs. R, roots; S, stems; Y1, young leaves; M, mature leaves; F1, flower buds; F3, unfold flowers; F5, fold flowers; fruits at SDPA, 1SDPA and 2SDPA, 5, 15 and 25 days post anthesis, respectively; MG, mature green stage fruits; BR, breaker stage fruits; B+4, four days after breaker stage fruits; RR, red ripe stage fruits. All samples were collected from plants nine weeks after planting.

**Additional file 5: Fig. S5.** Quantitative RT-PCR analysis of SIBBX24 transcript levels in young leaves of SIBBX24-OE lines, WT, wild-type tomato plants; OE-2, OE-3, and OE-9, three representative lines from the SIBBX24-overexpression (*SIBBX24-OE*) experiment. Asterisks indicate statistically significant differences. ***, *P* < 0.01.

**Additional file 6: Fig. S6.** Quantitative RT-PCR analysis of SICOL1 and SFT transcript levels in young leaves of SIBBX24-OE lines. A-B WT, wild-type tomato plants; OE-2, OE-3, and OE-9, three representative lines from the SIBBX24-overexpression (*SIBBX24-OE*) experiment.

**Additional file 7: Fig. S7.** Quantitative RT-PCR analysis of SIBBX20 and SIBBX24 expression in young leaves of transgenic tomato plants. WT, wild-type tomato plants; OE-5, OE-6, and OE-8, three representative lines from the SICOL1-overexpression (*SICOL1-OE*) experiment; R-1, R-10 and R-17, three representative lines from the SICOL1-RNAi plants.

**Additional file 8: Fig. S8.** Flowering time and fruit yield phenotype of CR-slc01 transgenic tomato plants. A Schematic illustration of the two sgRNA target sites (red arrows) in SICOL1. Black arrows represent the location of the primers that were used for PCR-based genotyping. B Verification of the CR-slc01 mutant alleles by DNA sequencing analysis. The red font indicates sgRNA target sequences. The black boxes indicate protosparase-adjacent motif (PAM) sequences. C Quantitative RT-PCR analysis of SFT expression in the young leaves of the WT tomato and three representative lines of CR-slc01. D Number of nodes under the first inflorescence in the WT tomato and three representative lines of CR-slc01 eight weeks after planting. E Total fruit yield of the WT tomato and three representative lines of CR-slc01. F Mean values of fruit weights from the CR-slc01 transgenic and WT tomato plants.

**Additional file 9: Table S1.** Sequences of primers used in this study.

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**Authors’ contributions**

J.Z., L.C. and Z.Y. planned and designed the research. L.C., F.Z., J.W., C.Z., D.Z., S.G., C.Z., J.Y., Z.Y., B.O.Y., T.W. and Z.Y. performed the experiments. L.C., F.Z. and J.W. participated in generating and screening all the transgenic materials. L.C., F.Z. and J.W. performed the field statistical data experiment. L.C., F.Z. and D.Z. performed the statistical analyses; L.C., F.Z. and C.Z. performed the field statistical data experiment. S.G. and C.Z. participated in the transgenic and physiological analyses; L.C., F.Z. and C.Z. participated in the transgenic and physiological analyses; L.C., F.Z. and C.Z. participated in the transgenic and physiological analyses; L.C., F.Z. and C.Z. participated in the transgenic and physiological analyses.

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**Availability of data and materials**

The gene sequences used in our experiments are available from the Sol Genomics Network databases using the following accession numbers: SICOL1, Solyc02g089950; SICOL2, Solyc02g089950; SICOL3, Solyc02g089950; SIBBX24, Solyc01g110180; SlBBX20, Solyc02g089950; SlBBX24, Solyc06g073180; SlActin; Solyc11g005330 and SlCOL2, Solyc02g089950.
Declarations

Ethics approval and consent to participate
The wild-type tomato (Solanum lycopersicum) variety used in this study is tomato variety Ailsa Craig (AC; LA2383A) originally obtained from the Tomato Genetics Resource Center, UC Davis, USA (https://tgrc.ucdavis.edu/, accession number LA2383A) and used for genetic transformation experiments. The high generation tomato seeds were preserved in our laboratory. All the transgenic materials (SICOL1-RNAi, SICOL1-1/2/3, ProSLCOL1::GUS, CR-slcol1, SBBX24-1/2-3, CR-sbbx24) were generated in the Ailsa Craig (AC; LA2383A) background. Transgenic materials (SICOL1-RNAi-1/10/17, SICOL1-1/2-5/6/8, ProSLCOL1::GUS-10, CR-scol1-1/2/3, SBBX24-1/2-3/9, CR-sbbx24-1/2/3) and wild-type (AC; LA2383A) tomato plants were grown and deposited in the Key Laboratory of Horticultural Plant Biology, Ministry of Education, Huazhong Agricultural University (Hubei, China). The current study complies with relevant institutional, national, and international guidelines and legislation for experimental research and field studies on plants. The authors declare that they have no conflict of interest.

Consent for publication
Not applicable in this study.

Competing interests
The authors declare no competing financial interests.

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