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Constitutive expression of full-length or partial of *SOC1* genes for yield enhancement in tomato

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Manipulating the expression of flowering pathway genes holds potential for regulating tomato fruit productivity. *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) is a MADS-box gene that serves as a key integrator in the flowering pathway. In this study, two full-length *SOC1* genes cloned from maize (*ZmSOC1*) and soybean (*GmSOC1*), along with a partial *SOC1* gene from blueberry (*VcSOC1K*, containing the K-domain), were individually transformed into tomato for constitutive expression. Phenotypically, the expression of *VcSOC1K* and *ZmSOC1*, but not *GmSOC1*, led to early flowering. Most transgenic lines carrying any of the three constructs exhibited a significant increase in fruit number per plant, with gains of 84-161% for *ZmSOC1*, 72-135% for *GmSOC1*, and 55-96% for *VcSOC1*K. Notably, compared to non-transgenic controls, all three constructs enhanced fruit yield per plant to varying degrees, including *ZmSOC1* by 81-169%, *GmSOC1* by 60-112%, and *VcSOC1*K by 52-88%, primarily through enhanced branching. At the transcriptomic level, comparative analysis of *GmSOC1* revealed the broader impact of the transformed genes. The increased expression of *CLF* and *EZA1* appears to explain the unchanged flowering time of the *GmSOC1* transgenic plants, while the repressed expression of *DWARF* genes likely contributes to enhanced branching. Additionally, numerous genes associated with biotic and abiotic stress tolerance displayed differential expression. These findings demonstrate that constitutive expression of either full-length or partial *SOC1* has the potential to enhance tomato fruit production by modulating multiple pathways, at least at the transcript levels.

KEYWORDS

brassinosteroids, flowering time, lycopersicon esculentum, MADS-box gene, plant architecture, soybean SOC1, yield enhancement

1 Introduction

The MADS-box gene family encodes transcription factors that are present in all eukaryotic organisms, playing essential roles in animals, plants, and fungi (Alvarez-Buylla et al., 2000; Gramzow et al., 2010; Gramzow and Theissen, 2010). In plants, these genes are crucial for a wide range of physiological and developmental processes (Colombo et al.,

2008). While MADS-box proteins have been extensively studied for their roles in regulating flower development, emerging research indicates their involvement in fruit development, embryo establishment, vegetative organ development, and stress resistance. These findings highlight the significance and functional diversity of this gene superfamily in plant development (Busi et al., 2003; Ito et al., 2008; Ehlers et al., 2016).

The plant MADS-box family includes type I and II genes (Masiero et al., 2011; Gramzow and Theissen, 2013). Type I MADS box factors are crucial regulators of plant reproduction; they play key roles in the development of the female gametophyte, embryo, and endosperm development (Masiero et al., 2011). Type II genes have been extensively studied and further classified into MIKC^{C}- and MIKC*-type two subgroups (Henschel et al., 2002). MIKC^{C}-type genes are the most thoroughly investigated members of the plant MADS-box family and encode proteins containing four distinct domains: M, I, K, and C. The M (DNA-binding) domain is the most conserved and is shared across all MADS-box genes. The I (intervening) domain, while less conserved, facilitates the specificity of DNA-binding dimer formation. The K (keratin-like) domain is structurally conserved and mediates protein–protein interactions, while the C (C-terminal) domain, the least conserved, is involved in ternary complex formation and transcriptional activation (Henschel et al., 2002). MIKC*-type genes are believed to have originated from ancestral MIKC^{C}-type genes through either elongation of the I region or truncation of the K box (Henschel et al., 2002; Kwantes et al., 2012; Liu et al., 2013). These genes are crucial for pollen development (Verelst et al., 2006; Liu et al., 2013). This structure-function relationship highlights the critical role of MADS-box genes in regulating diverse developmental processes, including fruit development, embryogenesis, vegetative organ formation, and stress responses.

MIKC^{C}-type MADS-box (MIKC^{C}) contribute floral organogenesis and flowering transition (Yanofsky et al., 1990; Weigel and Meyerowitz, 1994; Becker and Theissen, 2003). Among these, *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) serves as a conserved floral activator and integrator within the plant flowering pathway (Parenicova et al., 2003; Liu et al., 2009; Seo et al., 2009; Lee and Lee, 2010). Due to the diverse functions of the SOC1 protein in regulating plant development, its expression can be genetically engineered to improve crop yield, as evidenced in blueberry (*Vaccinium corymbosum*) (Song and Chen, 2018), leaf mustard (*Brassica juncea* cv. Varuna) (Tyagi et al., 2019), soybean (*Glycine max*) (Han et al., 2021), and maize (*Zea mays*) (Song and Han, 2021; Song et al., 2021).

Crop yield, such as in tomato, is influenced by genetic background, environmental conditions, and management practices. The potential for crop yield is genetically determined through the interactions of multiple genes and complex gene networks (Bhandari et al., 2023). Strategically, genetic improvement can be achieved by incorporating genes for biotic resistance and abiotic stress tolerance through breeding to protect yields, while modifying key genes related to growth and development can further enhance productivity (Bailey-Serres et al., 2019).

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop and a valuable source of essential nutrients and phytochemicals, including vitamin C, lycopene, and antioxidants (Raza et al., 2022). Enhancing yield is a top breeding priority for fresh-market tomato (Bhandari et al., 2023). This can be achieved through either traditional breeding or genetic engineering (Krieger et al., 2010; Cui et al., 2022). However, since yield is a trait controlled by multiple genes, both approaches present significant challenges. Although numerous genes have been functionally characterized in tomato, only a few, particularly those involved in flowering pathways, including four tomato *SOC1* genes, have shown potential for increasing yield (Krieger et al., 2010; Wang et al., 2021; Cui et al., 2022; Zahn et al., 2023). Yet the specific effects of these genes on yield remain unexplored.

Due to concerns that overexpression of the tomato *SOC1* gene may lead to dosage-related effects, this study investigated the constitutive expression of two heterologous *SOC1* genes, *GmSOC1* from soybean (*Glycine max*) and *ZmSOC1* from maize (*Zea mays*), along with a truncated derivative of blueberry (*Vaccinium corymbosum*) *SOC1* lacking the M domain (*VcSOC1K*), to assess their potential for improving tomato yield.

2 Materials and methods

2.1 Constructs and plant transformation

The ZmSOC1 and GmSOC1 protein sequences were used as queries to conduct BLAST searches against the tomato proteome in Phytozome v13. The identified tomato SOC1 and SOC1-like proteins were subsequently selected for phylogenetic analysis (Figure 1).

A 696-bp *ZmSOC1* (also known as *ZmMADS1*), identical to the sequence derived from HQ858775.1 in GenBank, was cloned using polymerase chain reaction (PCR) from the cDNA of the maize inbred line B104 (Han et al., 2021). The corresponding protein sequence matches NP_001105152.1 in GenBank (Figure 1). The fragment was inserted into a modified pCAMBIA1300 vector between a CaMV 35S promoter and a CaMV poly(A) signal at the *Kpn*I and *Xba*I restriction sites (Figure 1).

Similarly, a 630-bp *GmSOC1*, identical to the sequence derived from NM_0011249448.2 in GenBank, was cloned from the cDNA of the soybean cultivar Thorne. The fragment was inserted into the same modified pCAMBIA1300 vector between a CaMV 35S promoter and a CaMV poly(A) signal at the *Kpn*I and *Xba*I sites (Figure 1). The corresponding protein sequence is identical to NP_001236377.1 in GenBank (Figure 1).

The *VcSOC1K* gene was previously cloned into the T-DNA region of the binary vector pBI121, positioned between the CaMV 35S promoter and the Nos terminator for constitutive expression (Song and Chen, 2018). In this study, the *VcSOC1K* expression cassette was excised from pBI121 using *Hin*dIII and *Eco*RI digestion and subsequently ligated into the T-DNA region of a *Hin*dIII- and

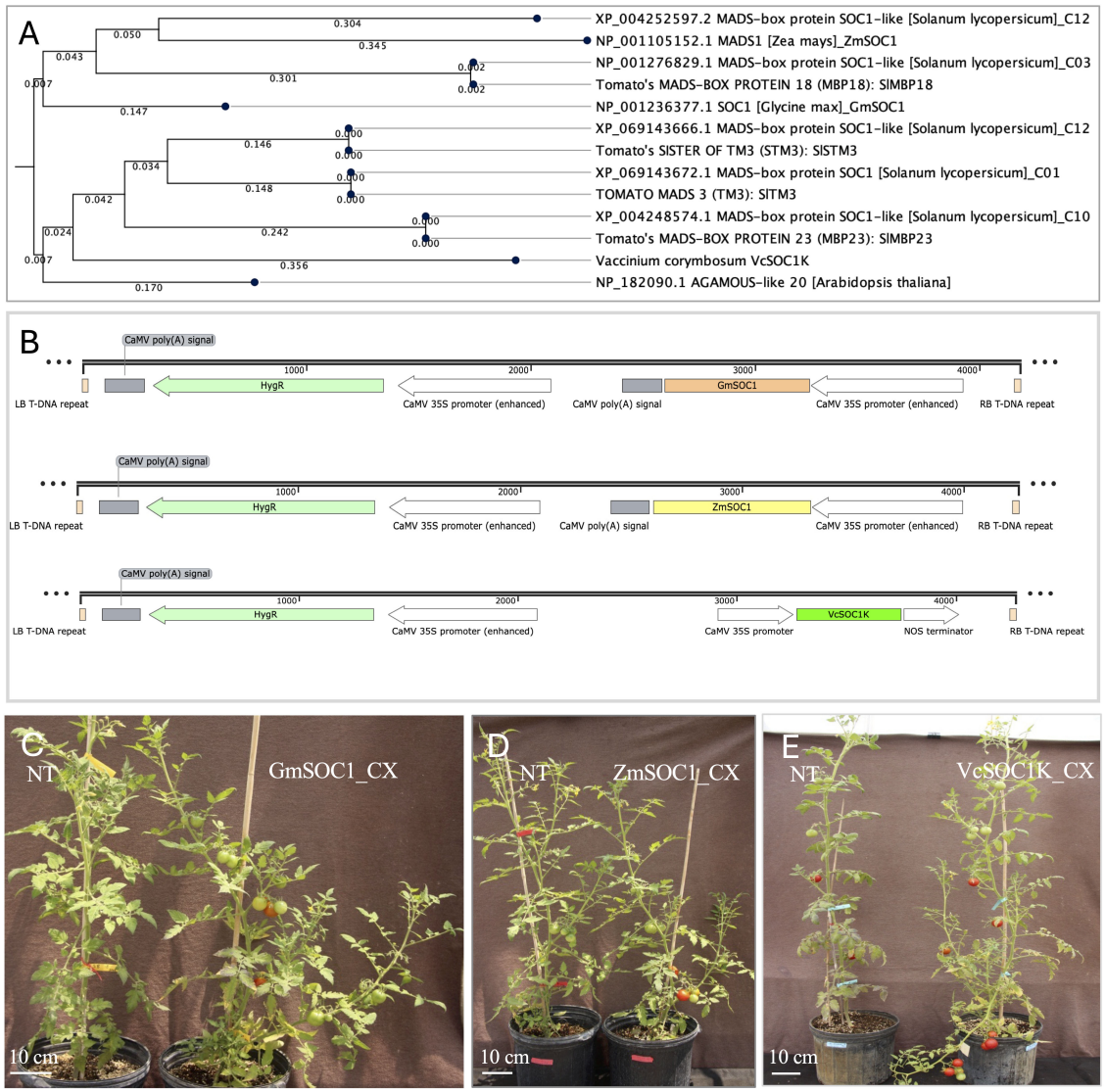

FIGURE 1
Genes and constructs used for generating transgenic tomato. **(A)** Phylogenetic analysis of five tomato SOC1 and SOC1-like proteins from four chromosomes. Protein sequences of TOMATO MADS3 (SlTM3), SISTER OF TM3 (SlSTM3), SlMBP18. and SlMBP23 are from Zahn et al., 2023. C01, C03, C10, and C12 are chromosome numbers. The analysis reveals similarities to *Arabidopsis* SOC1, soybean GmSOC1, maize ZmSOC1, and blueberry SOC1 K-domain (VcSOC1K), using the Neighbor Joining method with the Jukes-Cantor protein distance metric and 500 bootstrap replicates. **(B)** T-DNA regions of three pCAMBIA1300-derived constructs containing *GmSOC1*, *ZmSOC1*, and *VcSOC1K* genes. HygR: the hygromycin B resistance gene. LB and RB: the left and right borders, respectively. CaMV: Cauliflower mosaic virus. **(C)** Constitutive expression of *GmSOC1* (GmSOC1-CX) in T_0 plants and non-transgenic (NT) regenerants. **(D)** Constitutive expression of *ZmSOC1* (ZmSOC1-CX) in T_0 plants and NT regenerants. **(E)** Constitutive expression of *VcSOC1K* (VcSOC1K-CX) in T_0 plants and NT regenerants.

*Eco*RI-digested pCAMBIA1300 (PC1300) vector. This ensures that all three constructs share the same binary vector backbone (Figure 1).

All three constructs were confirmed by sequencing the target genes and subsequently transformed into *Agrobacterium tumefaciens* strain EHA105. For tomato transformation, indeterminate tomato (*Lycopersicon esculentum*) 'Ailsa Craig' was used. Seeds were sterilized in a 2.5% (v/v) sodium hypochlorite solution and germinated to produce cotyledons. Non-inoculated cotyledons were cultured on antibiotic-free regeneration medium to produce non-transgenic (NT) regenerants, which were used as controls. The regeneration medium consisted of Murashige and Skoog (MS) basal salts (Murashige and Skoog, 1962), Gamborg B5 vitamins (Gamborg et al., 1968), 2.85 µM zeatin riboside, 2.86 µM indole-3-acetic acid (IAA), 30 g/L sucrose, and 6 g/L agar. Transformation was carried out following established protocols (Danial et al., 2021). Regeenrated shoots approximately 2–3 cm in length were excised and transferred to 30 mL MS medium for rooting. For NT plants, the rooting medium lacked antibiotics, whereas for transgenic plants, it was supplemented with 10 mg/L hygromycin, 250 mg/L timentin, and 250 mg/L cefotaxime.

2.2 Transplanting, phenotyping, and genotyping of the transformants

After rooting, T_0 plantlets were transplanted into 4-inch plastic pots containing water-soaked Suremix Perlite planting medium (Michigan Grower Products Inc., Galesburg, MI). The plants were covered with plastic bags and placed in a growth room maintained at 25 °C with a 16/8-hour light/dark photoperiod. Over a two-week period, the plastic bags were gradually removed to acclimate the plantlets. Once acclimated, the plants were repotted into one-gallon pots and transferred to a greenhouse.

For each construct, approximately 10–20 T_0 transformants were cultivated in the greenhouse. Each transformant, derived from a separate explant, was considered an independent transgenic line. At the time of repotting into one-gallon pots, transformed and non-transformed plants of similar size, 3–5 lines per construct and non-transformants, were selected. These comparable plants were used as representatives for phenotyping of T_0 plants. First-generation (T_1) seeds were harvested separately from each T_0 plant.

For phenotyping T_1 plants, 20–30 seeds from each of three selected T_0 transgenic lines per construct were germinated in soil. Ten plants from each transgenic line were transferred to one-gallon pots and grown in a greenhouse.

Phenotypic assessments for each plant included measuring the time and height of the first flower appearance, recording the time of the first mature fruit appearance, counting the total number of branches and fruits harvested, and weighing all harvested fruits. Photographs were taken to document phenotypic variations.

For genotyping of the T_0 and T_1 plants, genomic DNA was extracted from young leaves using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1987). PCR was performed to detect the hygromycin phosphotransferase (*hpt*) transgene and the full-length sequences of *GmSOC1*, *ZmSOC1*, and *VcSOC1K* using the primers listed in Supplementary Table S1.

2.3 RNA sequencing and quantitative reverse transcription PCR analysis

Three groups of T_1 plants, derived from three T_0 transgenic lines containing *GmSOC1*, were selected for RNA sequencing and transcriptome analysis. Each group of T_1 plants was segregated into transgenic and NT plants. For each group, two newly formed mature leaves from all transgenic plants before their flowering were pooled together to create one biological replicate for transgenic plants, while leaves from NT plants were similarly pooled to form one biological replicate for NT plants. In total, three transgenic samples and three NT samples were collected, immediately frozen in liquid nitrogen, and stored at -80°C for RNA isolation.

Total RNA from each sample was isolated using a modified CTAB method (Chang et al., 1993) and further purified with the RNeasy Mini Kit (Qiagen, Valencia, CA, United States). To eliminate any residual DNA, on-column DNase digestion was performed using the RNase-Free DNase Set (Qiagen, Valencia, CA, United States). RNA quality was assessed with the High Sensitivity RNA ScreenTape system (Agilent Technologies, Santa Clara, CA, United States). All RNA samples used for sequencing and RT-qPCR analysis had RNA integrity number equivalent scores exceeding 5.0.

The RNA samples were sequenced using the Illumina NovaSeq 6000 platform (150 bp paired-end reads) at the Research Technology Support Facility at Michigan State University (East Lansing, Michigan, United States). The quality of the sequencing reads was evaluated using the FastQC program, focusing on per-base quality scores. Each of the six biological samples yielded 19–21 million paired reads (MR), with average quality scores exceeding 35, ensuring suitability for transcriptome analysis.

A transcriptome reference was assembled from approximately 120 million paired reads (MR) obtained from all six NT and transgenic lines using Trinity v2.15.1 (Haas et al., 2013). This reference was used for differential expression analysis. Transcripts identified as differentially expressed (DETs) with a false discovery rate (FDR) below 0.05 were selected for further analysis of various pathway genes. The transcriptome reference was annotated using Trinotate/4.0.2.

Pathway genes for nine phytohormones in *Arabidopsis*, including auxin, cytokinin, abscisic acid (ABA), ethylene, gibberellin (GA), BRs, jasmonic acid, salicylic acid, and strigolactones, were retrieved from the RIKEN Plant Hormone Research Network. Similarly, sugar pathway genes in *Arabidopsis* were identified. These hormone, MADS-box, and sugar pathway genes from *Arabidopsis* were used as queries to perform BLAST searches against the transcriptome reference, and isoforms with e-values less than -20 were identified for transcriptome comparisons. Flowering pathway genes in *Arabidopsis* and cereals (Walworth et al., 2016) were used to analyze flowering-related differentially expressed transcripts (DETs) identified in this study. Gene networks of overrepresented gene ontology (GO) terms for the selected DETs were constructed using Cytoscape 3.10.3.

Six selected DEGs were further analyzed through RT-qPCR using using the SYBR Green system (LifeTechnologies, Carlsbad, CA). A tomato *ACTIN* gene served as the reference gene to normalize the RT-qPCR results. All primers used in the analysis are included in Supplementary Table S1. The same RNA samples used for RNA sequencing, including three biological samples and three technical replicates, were used for the RT-qPCR analysis. Fold changes were calculated using $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct_{TARGET} - Ct_{NOM})_{transgenic} - (Ct_{TARGET} - Ct_{NOM})_{non\text{-}transgenic}$.

2.4 Data analysis

Data were processed using Microsoft Office Excel. Graphing and statistical analysis were generated in R (version 4.4.0) using the ggplot, annova, and emmeans packages. Fisher's Protected Least Significant Difference test was used to determine statistical significance at $p < 0.01$ and $p < 0.05$ levels.

3 Results

3.1 Phylogenetic analysis of tomato's SOC1 and SOC1-like proteins

The tomato proteome in Phytozome v13 includes one SOC1 protein and four SOC1-like proteins, which have been identified as SlTM3, SlSTM3, SlMBP18, and SlMBP23 by Zahn et al (Zahn et al., 2023). These five genes are distributed across four chromosomes (Figure 1). Their protein sequences exhibit high similarity to *Arabidopsis* SOC1, GmSOC1, ZmSOC1, and VcSOC1K. Notably, GmSOC1 and ZmSOC1 cluster with SlMBP18 and the SlSOC1-like protein XP_004252597.2, while VcSOC1 clusters with SlTM3, SlSTM3, SlMBP23, and *Arabidopsis* SOC1.

In the transcriptome reference assembled from all transcripts in the leaves of the 'Ailsa Craig' cultivar used in this study, transcripts corresponding to SlTM3, SlSTM3, SlMBP18, and the protein identical to XP_004252597.2 were detected, while SlMBP23 was absent.

The conserved nature of *SOC1* and *SOC1*-like genes suggests that their orthologs *SlTM3*, *SlSTM3*, *SlMBP18*, and *SlMBP23* in tomato may play similar roles to those in other plant species. Consequently, the constitutive expression of *GmSOC1*, *ZmSOC1*, and *VcSOC1K* may have the potential to influence fruit production.

3.2 Phenotypic changes in T_0 transgenic plants

A total of 69 hygromycin-resistant T_0 transgenic lines for GmSOC1_CX, 51 for ZmSOC1_CX, and 92 for VcSOC1K_CX were obtained across the three constructs, providing sufficient material for selecting comparable lines for phenotyping and seed harvesting (Figures 1B).

The constitutive expression of *GmSOC1* (GmSOC1_CX) resulted in non-significant changes, such as earlier flowering and increases in plant height and the number of flower clusters (Supplementary Figures S1A–C). Significant changes included increases in branch and fruit numbers, enhanced fruit production, larger fruit size, and earlier fruit maturation (Supplementary Figures S1D–H).

The constitutive expression of *ZmSOC1* (ZmSOC1_CX) led to significant changes, including earlier flowering, earlier fruit maturation, and increased fruit number and production (Supplementary Figures S1A, E–G). Non-significant changes included decreases in plant height, numbers of flower clusters and branches, and fruit size (Supplementary Figures S1B–D, H).

The constitutive expression of *VcSOC1K* (VcSOC1K_CX) induced significant changes, including earlier flowering, increased numbers of flower clusters and fruits, enhanced fruit production, and reduced fruit size (Supplementary Figures S1A, C, F–H). It also caused non-significant changes, such as earlier fruit maturation and increases in plant height and branch number (Supplementary Figures S1B, D, E).

Taken together, GmSOC1_CX, ZmSOC1_CX, and VcSOC1K_CX all contributed to increased fruit numbers and production (Supplementary Figure S1).

3.3 Phenotypic changes in T_1 transgenic plants

T_1 transgenic plants were phenotypically compared with their corresponding plants (Figures 2A). Under greenhouse conditions, among the nine groups with constitutive expression, ZmSOC1_CX17 was the only group of T_1 transgenic plants to exhibit significantly earlier flowering, while the other T_1 groups showed no difference in flowering time (Figure 2).

Consistent with observations in T_0 plants, GmSOC1_CX, ZmSOC1_CX, and VcSOC1K_CX all resulted in increased fruit numbers and overall production, while the average fruit size showed no significant decrease (Figures 2E).

3.4 Transcriptomic analysis of T_1 GmSOC1_CX plants

The T_1 GmSOC1_CX plants were selected for transcriptome analysis to confirm the presence of transgenes and identify genes responsive to *GmSOC1* expression in tomato. This selection builds on previous transcriptome analyses of ZmSOC1_CX in soybean and maize, as well as VcSOC1K_CX in blueberry and maize, which have been reported (Song and Chen, 2018; Han et al., 2021; Song and Han, 2021; Song et al., 2021).

The assembled transcriptome reference comprised 61,563 transcripts corresponding to 20,455 annotated genes. In GmSOC1_CX leaves, 565 differentially expressed transcripts (DETs) associated with 479 differentially expressed genes (DEGs) were identified. Among these DEGs, the two transgenes, *GmSOC1* and *hpt*, showed fold change (FC) of 23,170 [$Log_2^{FC(transgenic/non-transgenic)} = 14.5$] and 7,131 [$Log_2^{FC(transgenic/non-transgenic)} = 12.8$], respectively, confirming the expression of *GmSOC1* (Table 1). Additionally, RT-qPCR analysis of six selected DETs yielded results consistent with their RNA-seq data (Figure 3), supporting the reliability of the RNA-seq findings.

Analysis of the 479 DEGs using the GOSlim_Plants ontology file in BiNGO identified 27 overrepresented Gene Ontology (GO) terms. These included 11 terms under "Biological Process", seven under "Molecular Function", and nine under "Cellular Component" (Figure 4). These overrepresented GO terms suggest a broad impact of GmSOC1_CX on plant development at the transcript level, contributing to the phenotypic changes observed in GmSOC1_CX plants. For example, six overrepresented GO terms within the "Biological Process" category, namely "reproduction", "response to abiotic stimulus", "response to stress", "post-embryogenic development", "regulation of gene expression, epigenetic", and "response to endogenous stimulus", affect fruit production. These processes are linked through the overrepresented GO terms in the

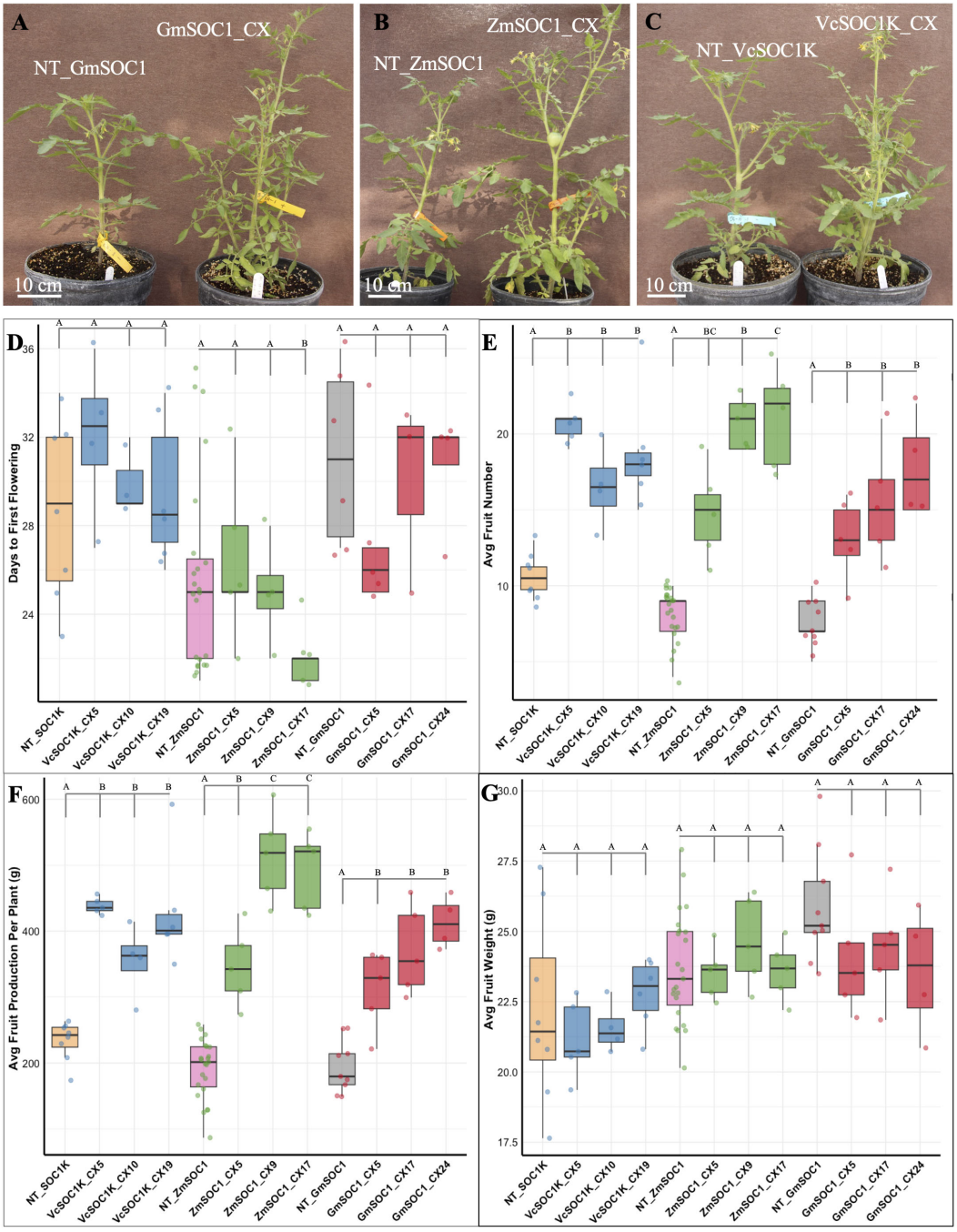

FIGURE 2
Phenotypic comparisons of three T_1 transgenic lines for GmSOC1_CX, ZmSOC1_CX, and VcSOC1K_CX, alongside their corresponding non-transgenic (NT) seedlings (NT_GmSOC1, NT_ZmSOC1, and NT_VcSOC1K), with $n > 3$ for each transgenic and NT group. **(A–C)** Representative T_1 plants. **(D)** Days to the appearance of the first flower after transplantation into a one-gallon pot. **(E)** Total number of fruits harvested. **(F)** Total weight of harvested fruits. **(G)** Average weight per fruit. The y-axis shows averages, and bars indicate standard deviations. Different letters on the bars indicate significant difference at $p \leq 0.05$ by Fisher's Protected Least Significant Difference test.

"Molecular Function" category, which regulate "transcription regulator activity" and "catalytic activity" via "binding" (Figure 4).

Further analysis of the 565 DETs for key genes identified 51 key DETs associated with flowering, phytohormones, and sucrose. These were annotated to 39 DEGs and exhibited high similarity to 28 *Arabidopsis* genes (Table 1). These DETs were annotated to 39 DEGs and showed high similarities to 28 *Arabidopsis* genes (Table 1). Among the 39 DEGs, *GmSOC1* was highly expressed ($Log_2^{FC(transgenic/non-transgenic)} = 14.5$) and suppressed the expression of two major endogenous tomato *SOC1* orthologues, *SlTM3* (TRINITY_DN7_c0_g1_i17) and *SlSTM3* (TRINITY_DN7_c0_g1_i2). In tomato, *SlTM3* and *SlSTM3* promote the floral transition but serve opposing roles in inflorescence development (Zahn et al., 2023). High expression of

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TABLE 1 Differentially expressed transcripts (DETs) of flowering pathway, hormone, MADS-box genes, and sucrose-related genes in leaves of GmSOC1-CX plants.

Transcript_ID	Log_2FC	Annotation	Pathway	Trait	Pathway gene in Arabidopsis	Arabidopsis gene name	Blast_e-value
DN6750_c0_g3_i1	-3.02	SDR3B_ARATH	Abscisic acid		AT1G52340.1	ABA2	5.88E-52
DN2346_c0_g1_i1	-1.43	ADH1_EUPLT	Abscisic acid		AT1G52340.1	ABA2	1.16E-64
DN2721_c0_g1_i1	-1.57	VICHY_VICSN	Abscisic acid		AT1G52400.1	BGL1	2.25E-143
DN1952_c0_g1_i9	0.67	ABA2_SOLLC	Abscisic acid		AT5G67030.1	ABA1	0
DN10415_c0_g1_i5	-1.25	C98A2_SOYBN	Auxin		AT4G31500.1	RNT1	4.77E-82
DN3140_c0_g1_i1	-0.65	AMI1_ARATH	Auxin		AT1G08980.1	AMI1	1.45E-154
DN6528_c0_g1_i15	-4.56	C71Z3_PASSA	Auxin		AT4G31500.1	RNT1	2.85E-158
DN7795_c0_g1_i2	-1.13	C76B6_CATRO	Auxin		AT2G30770.1	CYP71A13	2.73E-79
DN60911_c0_g1_i2	-0.49	AOC_ORYSJ	Jasmonate, auxin	Drought stress	AT3G25760.1	ERD12	5.90E-76
DN8245_c0_g1_i14	-1.30	C4H2_PETHY	Auxin		AT4G31500.1	RNT1	5.23E-58
DN8245_c0_g1_i6	-1.06	C4H2_PETHY	Auxin		AT4G31500.1	RNT1	3.40E-58
DN180_c0_g2_i3	-3.44	ST7R_ARATH	Brassinosteroid		AT1G50430.1	DWF5	0
DN180_c0_g2_i4	-5.04	ST7R_ARATH	Brassinosteroid		AT1G50430.1	DWF5	0
DN2079_c0_g1_i10	-1.96	C7A29_PANGI	Brassinosteroid		AT2G26710.1	BAS1	8.91E-134
DN31398_c0_g1_i2	-3.32	E5L3R8_SOLLC	Brassinosteroid	Biotic resistance	AT2G36800.1	DOGT1/ GAME1	1.99E-107
DN37339_c0_g1_i2	-3.24	DET2_SOLLC	Brassinosteroid		AT2G38050.1	DWF6	8.41E-92
DN5206_c0_g1_i3	-2.96	C7A15_ARATH	Brassinosteroid		AT2G26710.1	BAS1	1.81E-100
DN5206_c0_g1_i4	-2.52	72A61_PARPY	Brassinosteroid		AT1G17060.1	BAS1	2.98E-50
DN5206_c0_g1_i7	-2.73	72A61_PARPY	Brassinosteroid		AT2G26710.1	BAS1	9.83E-124
DN5206_c0_g1_i8	-2.60	C7254_GLYUR	Brassinosteroid		AT2G26710.1	BAS1	3.03E-77
DN6317_c0_g2_i2	-2.79	SC5D_TOBAC	Brassinosteroid		AT3G02580.1	DWF7	2.92E-155
DN683_c0_g1_i2	1.60	U73C4_SOLLC	Brassinosteroid		AT2G36800.1	DOGT1	0
DN8866_c0_g2_i1	-2.28	C7254_GLYUR	Brassinosteroid		AT2G26710.1	BAS1	7.98E-95
DN8866_c0_g2_i5	-2.62	72A61_PARPY	Brassinosteroid		AT2G26710.1	BAS1	1.00E-99
DN8866_c0_g2_i8	-2.66	72A61_PARPY	Brassinosteroid		AT2G26710.1	BAS1	2.97E-117
DN8866_c0_g2_i9	-3.50	72A61_PARPY	Brassinosteroid		AT2G26710.1	BAS1	4.20E-93
DN9477_c0_g1_i1	-3.13	DIM_PEA	Brassinosteroid		AT3G19820.1	DWF1	0
DN9477_c0_g1_i3	-3.05	DIM_PEA	Brassinosteroid		AT3G19820.1	DWF1	0
DN19028_c0_g3_i5	6.73	CKX7_ARATH	Cytokinin		AT5G21482.1	CKX7	3.86E-128
DN1467_c0_g1_i11	0.94	PKL_ARATH	Flowering		AT3G12810.1	PIE1	6.09E-53
DN1671_c0_g1_i17	-1.32	APRR5_ARATH	Flowering		AT5G24470.1	APRR5	2.25E-60
DN2625_c0_g1_i1	0.58	14337_SOLLC	Flowering		AT5G38480.1	GRF3	3.60E-122
DN3694_c0_g1_i2	14.50	SOC1_ARATH	Flowering		AT2G45660.1	GmSOC1	5.95E-80
DN3737_c0_g1_i55	8.74	EZA1_ARATH	Flowering		AT4G02020.1	EZA1	1.44E-177
DN5578_c0_g1_i18	7.77	CLF_ARATH	Flowering		AT2G23380.1	CLF	0
DN7_c0_g1_i17 (TM3)	-0.99	SOC1_ARATH	Flowering		AT2G45660.1	SOC1	3.42E-74
DN7_c0_g1_i2 (STM3)	-0.85	SOC1_ARATH	Flowering		AT2G45660.1	SOC1	2.74E-72

(Continued)

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TABLE 1 Continued

Transcript_ID	Log_2FC	Annotation	Pathway	Trait	Pathway gene in Arabidopsis	Arabidopsis gene name	Blast_e-value
DN12194_c0_g1_i1	-1.91	KAO2_ARATH	Gibberellin		AT1G05160.1	KAO1	2.43E-117
DN12424_c0_g1_i7	-2.15	BLH1_ARATH	Flowering, MADS-box	Abiotic stress	AT5G41410.1	BEL1	3.11E-50
DN7492_c0_g1_i1	-0.97	PGIP_PYRCO	Flowering, MADS-box		AT3G12145.1	FLOR1	4.11E-103
DN3786_c0_g1_i10	9.50	SUC4_ARATH	Sucrose	Abiotic stress	AT1G09960.1	SUC4	0
DN1108_c0_g1_i1	1.78	HSP83_IPONI	Sucrose	Drought stress	AT5G56030.2	ERD8	0
DN1108_c0_g1_i2	0.50	HSP80_SOLLC	Sucrose	Drought stress	AT5G56030.2	ERD8	3.12E-180
DN1108_c0_g1_i4	0.80	HS905_ARATH	Sucrose	Drought stress	AT5G56030.2	ERD8	3.25E-170
DN1108_c0_g1_i5	2.12	HSP83_IPONI	Sucrose	Drought stress	AT5G56030.2	ERD8	0
DN1562_c0_g5_i2	1.38	HSP7C_PETHY	Sucrose	Drought stress	AT1G56410.1	ERD2	0
DN2349_c1_g4_i1	1.73	HSP72_SOLLC	Sucrose	Drought stress	AT1G56410.1	ERD2	0
DN2349_c1_g5_i1	1.18	HSP7C_PETHY	Sucrose	Drought stress	AT1G56410.1	ERD2	1.09E-119
DN3015_c0_g2_i2	0.59	HSP7M_SOLTU	Sucrose	Drought stress	AT1G56410.1	ERD2	5.57E-170
DN500_c0_g1_i1	0.87	HS906_ARATH	Sucrose	Drought stress	AT5G56030.2	ERD8	2.37E-165
DN4220_c0_g1_i11	1.40	GST23_MAIZE	Sucrose	Biotic and abiotic resistance	AT1G10370.1	ERD9	6.22E-51

Log_2FC: Log_2(Fold change) = Log_2(transgenic/non-transgenic). The transcript DN36964_c0_g1_i2 corresponds to the transformed *GmSOC1* gene from soybean. The bold DETs were further verified by RT-qPCR.

SLSTM genes contributes to highly branched inflorescences (Wang et al., 2021).

The transcription factor BEL1-like homeodomain protein 1 (*BLH1*), which shares high similarity with the MADS-box gene *BEL1*, was downregulated. BLH family transcription factors are multifunctional, playing critical roles in plant morphogenesis, flower and fruit development, and responses to various environmental factors (Niu and Fu, 2022). Similarly, the polygalacturonase inhibitor precursor (*PGIP*), which exhibits a high similarity to the MADS-box gene *FLOR1*, known to promote flowering under long-day conditions (Torti et al., 2012), was also repressed.

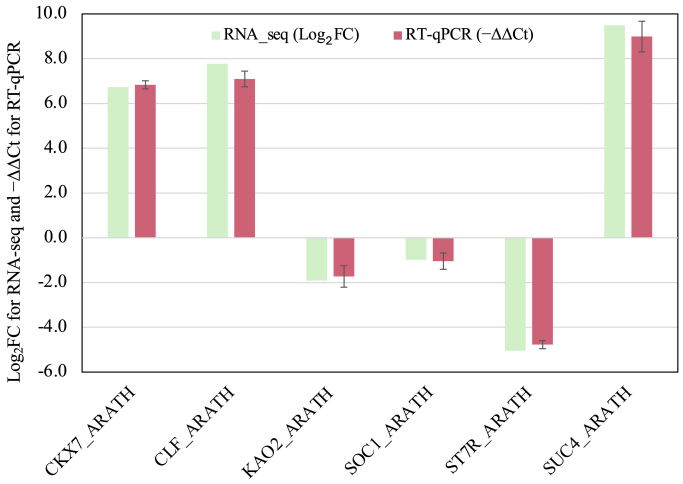

FIGURE 3
RT-qPCR analysis of the six selected DEGs listed in the Table 1. The −ΔΔCt values represent the average of three biological replicates and three technical replicates for each DEG. Tomato *ACTIN* gene SlACTIN12 (TRINITY_DN938_c0_g2_i1: ACT12_SOL) was used as the reference gene for normalization. Error bars represent standard deviation. No statistically significant differences were observed between RNA-seq and RT-qPCR data for any of the six genes analyzed ($p < 0.05$).

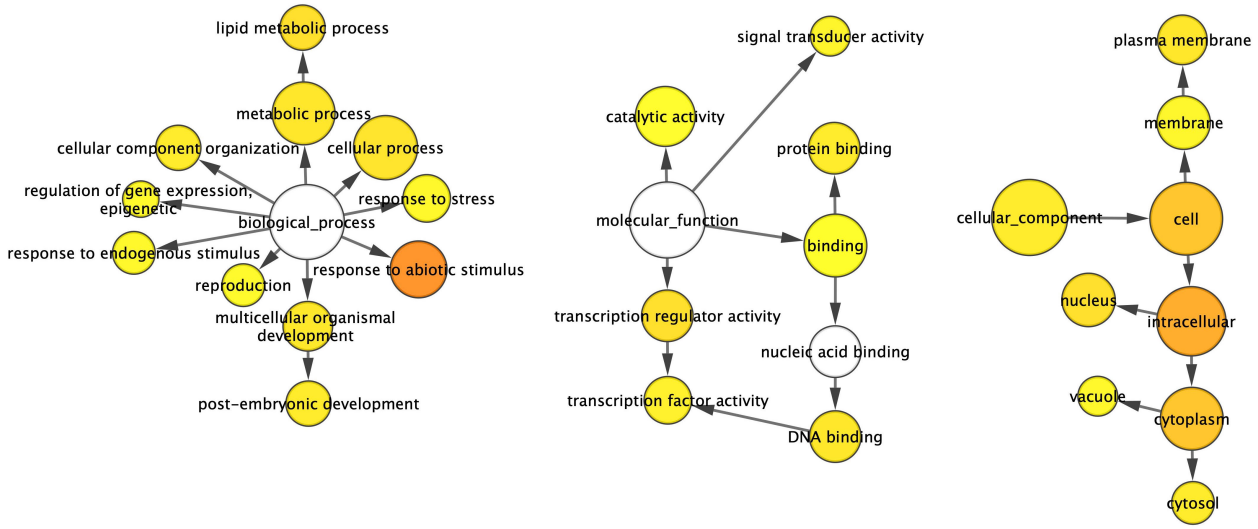

FIGURE 4
Gene networks of differentially expressed transcripts (DETs) identified from the comparison between the GmSOC1_CX and non-transgenic leaves. The ontology file of GOSlim_Plants in BiNGO was used to identify overrepresented GO terms ($p < 0.05$). Bubble size and color indicate the frequency of the GO term and the P-value, respectively.

The expression of two Histone-lysine N-methyltransferase genes *CURLY LEAF* (*CLF*) and *ENHANCER OF ZESTE 1 POLYCOMB REPRESSIVE COMPLEX 2 SUBUNIT* (*EZA1*), was enhanced. These genes encode catalytic subunits of the polycomb group (PcG) multiprotein complex. *CLF* is essential for regulating floral development by repressing the AGAMOUS homeotic gene. It achieves this by forming a nuclear complex with *EZA1* and other components, targeting ABA- and glucose-responsive elements (Saleh et al., 2007; Liu et al., 2019; Shu et al., 2020; Liu et al., 2022). Notably, a loss-of-function mutant of *Brassica rapa* exhibits early flowering (Poza-Viejo et al., 2024), suggesting that the upregulation of *CLF* and *EZA1* may contribute to either no significant impact or delaying flowering (Figure 5).

Of the genes involved in brassinosteroids (BRs) metabolism (Bajguz et al., 2020), the DEGs with repressed expressions included *BAS1* and four *DWARF* genes (*DWF1*, *DWF5*, *DWF6*, and *DWF7*) (Table 1)**;** these DEG can functionally lead to BRs-deficient changes, which are often associated with decreased BRs that can affect multiple agronomic traits (Bishop, 2003; Li et al., 2016; Zhan et al., 2022). The E5L3R8_SOLLC encodes tomato GLYCOALKALOID METABOLISM1 (GAME1) involved in the steroidal alkaloids (SAs) pathway (Itkin et al., 2011; Averello et al., 2025). The E5L3R8_SOLLC showed decreased expression (Table 1), suggesting a potential lower production of α-tomatine that has impact on plant defense system and fruit quality (Cárdenas et al., 2016; Sonawane et al., 2018; Averello et al., 2025).

For the cytokinin pathway genes, the expression of *CKX7* was enhanced, meaning a potential of a reduced cytokinin level in the GmSOC1-CX plants (Köllmer et al., 2014). Similarly, a decreased expression of the *KAO2* was found (Table 1), indicating a likely lower level of GA production (Regnault et al., 2014; Shani et al., 2024).

Among the DEGs related to sucrose, the tomato sucrose transporter *SlSUT4* (*SUC4_ARATH*) was upregulated (Table 1). While the downregulation of *SlSUT4* has been shown to promote flowering by enhancing sucrose transport to the shoot apex, its overexpression does not significantly impact flowering time or the expression of key genes in the flowering pathway (Liang et al., 2023).

Additionally, seven DEGs of HEAT SHOCK PROTEINs showed upregulation (Table 1), potentially contributing to enhanced tolerance to various abiotic stresses (Ul Haq et al., 2019). Similarly, the increased expression of the *GLUTATHIONE S-TRANSFERASE (GST) 23* is associated with both biotic and abiotic resistance (Hernández Estévez and Rodríguez Hernández, 2020). In contrast, the tomato ALCOHOL DEHYDROGENASE (ADH1_EUPLT) and its homolog YFE37, annotated as SDR3B_ARATH, were both repressed. This reduction in expression may negatively impact disease resistance (Xun et al., 2022).

4 Discussion

Tomato is commonly used as a model plant to study fruit-related traits, primarily due to its suitability for genetic transformation. In this study, we demonstrate for the first time that the ectopic expression of full-length or partial *SOC1* can enhance tomato fruit production per plant through a complex interaction of multiple genes and pathways. It is worth noting that 'Ailsa Craig' is an indeterminate tomato variety, which adds complexity to phenotyping flowering and fruiting traits in this study. When T_0 plants were examined, they were of similar size at the time of re-potting. However, for T_1 plants, which were grown

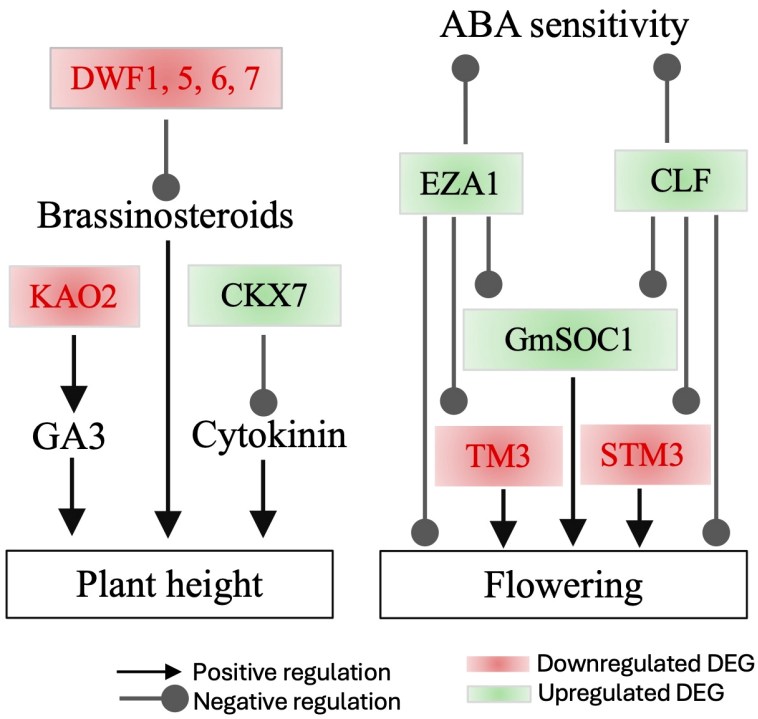

FIGURE 5
Interactions among major differentially expressed genes (DEGs) influencing tomato flowering and plant height in the leaves of GmSOC1_CX. The DEG data are presented in Table 1, and the proposed interactions are based on published information.

from seed germination, plant sizes were more variable at the time of re-potting. Importantly, phenotyping was conducted prior to genotyping by PCR, which minimized potential bias in the phenotypic data and ensured its reliability.

4.1 Flowering time and the expression of Full-length *SOC1* and partial *SOC1*

As a key integrator in the plant flowering pathway (Fornara et al., 2010; Lee and Lee, 2010; Immink et al., 2013), enhanced expression of *SOC1* or its orthologues, either though overexpression or ectopic expression, can promote flowering. This phenomenon has been reported for many plant species, including tomato (Zahn et al., 2023). Among the five *SOC1* and *SOC1-like* genes identified in tomato (Figure 1), *SlTM3* and *SlSTM3* play a more significant role in flowering initiation compared to *SlMBP23* and *SlMBP18*, at least in the indeterminate cultivar Moneyberg (Zahn et al., 2023). Additionally, the high expression of *SlSTM3* has been linked to a highly branched inflorescence phenotype (Wang et al., 2021).

In this study, phylogenetic analysis revealed that GmSOC1 and ZmSOC1 clustered closely with SlMBP18 and the fifth SlSOC1-like gene, while VcSOC1K showed closer similarity to SlTM3, SlSTM3, and SlMBP23 (Figure 1). Phenotypic observations demonstrated that both ZmSOC1-CX and VcSOC1-CX transgenic plants exhibited earlier flowering compared to non-transgenic controls, with statistically significant differences ($P < 0.01$) in the T_0 generation and no significant differences ($P < 0.01$) in the T_1 generation (Figure 2; Supplementary Figure S1A). In contrast, GmSOC1-CX plants did not show significant changes in flowering time across both T0 and T_1 generations ($P < 0.05$). The early flowering phenotype observed in ZmSOC1-CX lines suggests that the expression of *SlMBP18* and the fifth *SOC1*-like gene of tomato may play a role in promoting flowering. For the T_1 generation, it would have been valuable to investigate additional traits such as seed germination time and seedling growth, as previous studies have shown that repressed expression of DWARF genes can delay seed germination (Li et al., 2016). Notably, while GmSOC1-CX has been reported to induce early flowering in *Arabidopsis* (Zhong et al., 2012), its overexpression did not significantly promote flowering in soybean. However, *GmSOC1* knock-out mutants exhibited delayed flowering (Kou et al., 2022), highlighting the complex and species-specific regulatory roles of *GmSOC1* in flowering time control.

Interestingly, in this study, the T_0 generation of VcSOC1K-CX plants from four transgenic lines exhibited earlier flowering compared to the non-transgenic lines, whereas the T_1 generation of three transgenic lines did not show this early flowering phenotype. Previous studies have reported that overexpression of *VcSOC1K* in blueberry and *VcSOC1K-CX* in tobacco promoted flowering (Song et al., 2013; Song and Chen, 2018); however, *VcSOC1K-CX* did not significantly induce early flowering in maize (Song and Han, 2021). This variation may be attributed to many factors, including differences in expression levels, plant species, and genotype.

At the transcript levels, GmSOC1-CX did not promote flowering, at least not significantly, is likely due to the enhanced

expression of two Histone-lysine N-methyltransferase genes, *CLF* and *EZA1* (Figure 5) (Saleh et al., 2007; Liu et al., 2019; Shu et al., 2020; Liu et al., 2022; Poza-Viejo et al., 2024).

4.2 Plant architecture and the expression of full-length *SOC1* and partial *SOC1*

Plant architecture, including shoot and inflorescence structure, is an omnigenic trait that can significantly influence tomato productivity (Alonge et al., 2020; Gaarslev et al., 2021). Tomato *SOC1* genes, such as *SlTM3* and *SlSTM3*, serve as core regulators of inflorescence structure by interacting with other MADS-box genes (Alonge et al., 2020; Gaarslev et al., 2021; Wang et al., 2021; Zahn et al., 2023). Overexpression of *SlTM3* and *SlSTM3* enhances inflorescence branching (Wang et al., 2021), while their suppression reduces branching (Alonge et al., 2020; Zahn et al., 2023). In this study, transgenic lines expressing *GmSOC1*, *ZmSOC1*, and *VcSOC1K* exhibited no noticeable changes in inflorescence structure. For GmSOC1-CX lines, this was further supported at the transcript level by the unchanged expression of tomato *FRUITFULL1* (*FUL1*)(Table 1), a direct activation target of *SlSTM3* (Wang et al., 2021).

Phenotypically, the effect of *SOC1* expression on plant height and branching has been less consistent in the literature compared to its well-established role in flowering time. In soybean, *soc1* mutants have more internodes than the wild type, but the impact of *GmSOC1* overexpression on plant architecture remains unclear (Kou et al., 2022). In *Medicago truncatula*, *MtSOC1* has been shown to influence both flowering and primary stem height in both mutant and overexpression lines (Jaudal et al., 2018). Additionally, ZmSOC1-CX expression has been associated with reduced plant height in transgenic maize and soybean plants (Han et al., 2021; Song et al., 2021). In tomato, *SlTM3* and *SlSTM3* expression have not displayed any significant impact on plant height and branching (Zahn et al., 2023). In this study, GmSOC1-CX, ZmSOC1-CX, and VcSOC1-CX did not significantly change plant height but enhanced branching for at least the GmSOC1-CX and VcSOC1-CX plants (Figures 2A).

At the transcript level, it is noteworthy that the repressed expression of four DWARF genes could theoretically lead to reducing plant size in tomato due to less BRs production, as suggested by previous studies (Figure 5) (Bishop et al., 1999; Bishop, 2003; Montoya et al., 2005; Li et al., 2016; Zhan et al., 2022). The enhanced expression of *CKX7* could result in reduced cytokinin levels, which are expected to shorten plant height, increase branching, and reduce flower number (Eckardt, 2003; Bartrina et al., 2011; Köllmer et al., 2014; Waldie and Leyser, 2018). Similarly, a decreased expression of *KAO2* might reduce GA production (Regnault et al., 2014), potentially leading to delayed seed germination, stunted plant growth, and delayed flowering. In this study, although GmSOC1-CX did not exhibit phenotypic changes such as plant dwarfing, delayed flowering, or reduced flower number, it did show increased branching (Figures 1C, Figures 2A, and Supplementary Figure S1D). However, BR, cytokinin, and GA levels, as well as seed germination timing, were not investigated in this work.

4.3 Tomato fruit yield and the expression of full-length *SOC1* and partial *SOC1*

Crop yield-defining traits vary across different crops (Bailey-Serres et al., 2019). For tomatoes, yield-defining traits include both direct factors, such as fruit number, fruit size, and fruit production efficiency per unit area, as well as related traits, including tolerance to abiotic and biotic stresses (Alonge et al., 2020; Bhandari et al., 2023). Accordingly, hormone and flowering pathway genes have become the targets for genetic improvement of yield (Krieger et al., 2010; Ariizumi et al., 2013; Wang et al., 2021; Cui et al., 2022; Kang et al., 2022). In this study, two full-length *SOC1* genes from maize and soybean, along with the *K*-domain of the blueberry *SOC1* gene, were constitutively expressed in tomato. The resulting transgenic lines exhibited an increased fruit count, leading to higher total fruit production per plant, suggesting enhanced yield potential. This rise in fruit number was associated with improved branching in the transgenic plants, likely due to decreased BRs resulting from the repression of *DWARF* genes. Notably, similar effects have not been reported in tomato through the overexpression of *SlTM3* and *SlSTM3*. Interestingly, lower expression levels of *GmSOC1* have been shown to enhance soybean yield (Kou et al., 2022).

In addition to its essential role in flowering, *SOC1* also plays a role in other processes in *Arabidopsis*, such as root development and leaf senescence, both of which impact crop yield (Chen et al., 2017; Castañón-Suárez et al., 2024). However, the impact of *SOC1* overexpression on root development and leaf senescence in crops remains largely unexplored. Previously, we found that constitutive expression of three *SOC1* genes, either full-length or partial, has the potential to enhance yield in maize, soybean, and blueberry, primarily through the regulation of flowering pathway genes, as indicated by RNA-seq data (Song and Chen, 2018; Han et al., 2021; Song and Han, 2021; Song et al., 2021). In this study, unlike our previous findings, the observed increase in fruit production per transgenic plant is attributed to enhanced branching, likely due to the repressed expression of *DWARF* genes (Figure 5). Additionally, the increased expression of *CLF* and *EZA1* in the RNA-seq data of the *GmSOC1-CX* lines appears to provide evidence explaining the unchanged flowering time (Figure 5).

4.4 Tomato fruit quality, biotic and abiotic tolerance, and other traits

In this study, the biotic and abiotic tolerance of the transgenic plants were not directly assessed. However, the identification of several DEGs associated with these traits suggests that the transgenes may have influenced plant resilience to biotic and abiotic stresses (Figure 4, Table 1). Additionally, fruit quality as well as the other traits may have been impacted by the DEGs.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found in the NCBI SRA repository under accession number PRJNA1280106 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1280106).

Author contributions

GD: Investigation, Writing – review & editing, Writing – original draft. JJ: Formal analysis, Writing – review & editing, Data curation. GS: Funding acquisition, Resources, Project administration, Validation, Formal analysis, Writing – original draft, Data curation, Supervision, Writing – review & editing, Conceptualization, Methodology.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

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SUPPLEMENTARY FIGURE 1
Phenotypic comparisons of T_0 transgenic lines (GmSOC1_CX, n = 5; ZmSOC1_CX, n = 4; and VcSOC1K_CX, n = 3) and their corresponding non-transgenic (NT) lines (NT_GmSOC1, n = 4; NT_ZmSOC1, n = 3; and NT_VcSOC1K, n = 3). **(A)** Days to the appearance of the first flower after potting in a one-gallon pot. **(B)** Plant height at the time of first flowering. **(C)** Number of flower clusters counted after all fruits were harvested. **(D)** Number of branches counted after all fruits were harvested. **(E)** Days to the appearance of the first mature fruit after potting in a one-gallon pot. **(F)** Total number of fruits harvested. **(G)** Total weight of harvested fruits. **(H)** Average weight per fruit. The y-axis shows averages, and bars indicate standard deviations.

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