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ORIGINAL ARTICLE

Function of *BrSOC1b* gene in flowering regulation of Chinese cabbage and its protein interaction

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Abstract

Main conclusion **BrSOC1b may promote early flowering of Chinese cabbage by acting on BrAGL9 a, BrAGL9 b, BrAGL2 and BrAGL8 proteins.**

Abstract SOC1 is a flowering signal integrator that acts as a key regulator in controlling plant flowering time. This study focuses on the cloning of the open reading frame of *SOC1b* (*BrSOC1b*, Gene ID: *Bra000393*) gene, and analyzes its structure and phylogenetic relationships. Additionally, various techniques such as vector construction, transgenic technology, virus-induced gene silencing technology, and protein interaction technology were employed to investigate the function of the *BrSOC1b* gene and its interactions with other proteins. The results indicate that BrSOC1b consists of 642 bp and encodes 213 amino acids. It contains conserved domains such as the MADS domain, K (keratin-like) domain, and SOC1 box. The phylogenetic analysis reveals that BrSOC1b shares the closest homology with BjSOC1 from *Brassica juncea*. Tissue localization analysis demonstrates that BrSOC1b exhibits the highest expression in the stem during the seedling stage and the highest expression in flowers during the early stage of pod formation. Sub-cellular localization analysis reveals that BrSOC1b is localized in the nucleus and plasma membrane. Furthermore, through genetic transformation of the *BrSOC1b* gene, it was observed that *Arabidopsis thaliana* plants expressing *BrSOC1b* flowered earlier and bolted earlier than wild-type plants. Conversely, Chinese cabbage plants with silenced *BrSOC1b* exhibited delayed bolting and flowering compared to the control plants. These findings indicate that *BrSOC1b* promotes early flowering in Chinese cabbage. Yeast two-hybrid and quantitative real-time PCR (qRT-PCR) analyses suggest that BrSOC1b may participate in the regulation of flowering by interacting with BrAGL9a, BrAGL9b, BrAGL2, and BrAGL8 proteins. Overall, this research holds significant implications for the analysis of key genes involved in regulating bolting and flowering in Chinese cabbage, as well as for enhancing germplasm innovation in Chinese cabbage breeding.

Keywords Chinese cabbage · Flowering time · Genetic transformation · Protein interaction · SOC1

Introduction

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*), belonging to the Cruciferae *Brassica* genus and native to China, is a popular vegetable species. It is often referred to as the "head vegetable" and holds a prominent position in the "vegetable basket." Chinese cabbage has the largest cultivation area and is the most consumed vegetable in China (Liu 2016). However, during the cultivation process, Chinese cabbage is prone to early bolting and flowering due to variations in climate. Early bolting greatly affects both the yield and quality of the plants. Therefore, it is crucial to analyze the mechanisms behind the key genes regulating bolting and

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flowering in Chinese cabbage to improve its yield and germplasm innovation.

Six flowering signaling pathways have been identified, including the photoperiod pathway (Fernández et al. 2016), vernalization pathway (Zhu et al. 2015), gibberellin pathway (Galvao et al. 2015), autonomous pathway (Simpson 2004), thermosensitive pathway (Lievens et al. 2017), and age pathway (Vishal and Kumar 2018). These pathways sense and transmit flowering signals, and they converge on two key flowering integration factors, namely FLOWERING LOCUS T (FT) and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) (Yang et al. 2021). The *SOC1* gene and its homologous genes belong to the class of transcription factors that encode MADS-box and play a crucial role in regulating plant flowering.

The MADS protein is characterized by a conserved 58–60 amino acid domain called the MADS domain, located at the N-terminus, which serves as the DNA binding domain. This domain binds to the CArG box (Yanofsky et al. 1990). Phylogenetic analysis divides the plant MADS gene family into Type I and Type II. Type II *MADS* genes consist of 95 identified genes, while Type I *MADS* genes consist of 65 confirmed genes, with Type II genes being the predominant group (Bemer 2008). Type I genes are further categorized into M-α, M-β, and M-γ, while Type II genes are divided into 13 subgroups: A, B, CD, E, AGL6, AGL12, TM3-like (SOC1), AGL17, SVP, AGL18/15, BS(TT16), FLC/MAF, and MIKC. Among these subgroups, TM3-like (SOC1) has the highest number of proteins (16), whereas AGL12, AGL6, and BS (TT16) have the fewest number of proteins (only 3 each) (Zhang 2021). *MIKC* genes are further divided into two subgroups: $MIKC^{C}$ and MIKC* (Henschel et al. 2002).

SOC1 is a flowering signal integrator that participates in the photoperiod pathway, autonomous pathway, vernalization pathway, and age pathway. It acts as a key regulator in controlling the flowering time of plants (Moon et al. 2003). Extensive research on the *SOC1* gene has revealed its functions in different species. For instance, the *GhSOC1* gene in *Gerbera hybrida* is expressed in leaves, leaf buds, inflorescences, stems, and roots of wild-type *Arabidopsis thaliana* in Colombia. Its expression level increases with plant growth, particularly in mature leaves where it is the highest (Lee et al. 2000). Additionally, the *GhSOC1* gene influences flower development (Ruokolainen et al. 2011). Overexpression of *MtSOC1a* promotes flowering and main stem elongation in *Medicago truncatula*, while its mutants exhibit delayed flowering time and shortened main stem (Jaudal et al. 2018). *Petunia hybrida Fbp20*/*UNS* (FLORAL BINDING PROTEIN20/UNSHAVEN), a SOC1 homologous gene, when overexpressed, stimulates plant flowering (Ferrario et al. 2004). However, overexpression of *Fbp20*/*UNS* with missing fragments (specifically the MADS domain) results in delayed flowering of truncated plants (Ferrario et al. 2004). *ZmMADS1*, a *SOC1/TM3*-like gene, was cloned from *Zea mays* by Heuer et al. (2001). Its high expression during maize flower development suggests an important role in maize flowering. In *Dendrobium officinale*, overexpression of the *DOSOC1* gene promotes early flowering (Ding et al. 2013). These findings highlight the crucial role of the *SOC1* gene in regulating flowering.

Bolting and flowering are not only important agronomic traits in Chinese cabbage crop production but also crucial for the yield and quality of the plant organs, which directly impact human life. Based on the classification of *Arabidopsis thaliana*, the Chinese cabbage MADS family was categorized, and within a specific classification, there are 16 genes that share similarity with the *Arabidopsis thaliana SOC1* gene (Duan et al. 2015). However, it has been discovered that the *SOC1a* (*BrSOC1a*, Gene ID: *Bra000393*) gene from *Brassica rapa* promotes flowering in *Brassica oleracea* (Hong et al. 2013). Whether the homologous gene *BrSOC1b* (gene ID: *Bra004928*) is involved in flowering regulation remains unclear. Therefore, this study aims to analyze the function of *BrSOC1b* in flowering and further explore its molecular mechanism. The results of this research carry significant implications for understanding the mechanisms of key genes involved in regulating bolting and flowering in Chinese cabbage and for enhancing germplasm innovation in Chinese cabbage.

Materials and methods

Cloning of *BrSOC1b* gene

To clone the nucleotide sequence of *BrSOC1b*, total RNA was extracted from the leaves of 'HK-8' using the RNA simple total RNA kit (Tiangen, Beijing). The RNA was then reverse transcribed into cDNA using the PrimeScript-tRT reagent kit (TaKaRa, Dalian, China). Specific primers (Table S1) were designed based on the nucleotide sequence of *BrSOC1b* in the Chinese cabbage database (http://brassicadb.cn/#/), and PCR amplification was conducted using cDNA as the template. The amplified product was ligated to the pEASY-Blunt Cloning vector and transformed into *E. coli* (*DH5α*). Monoclonal, shaking, bacterial PCR detection, and sequencing were performed to confirm the correct cloning recombinant vector, which was named pEASY-Blunt-*BrSOC1b*.

Analysis of physical and chemical characteristics of *BrSOC1b*

The number of amino acids, theoretical isoelectric point (pI), relative molecular mass, protein stability, and hydrophobicity of BrSOC1b were analyzed using the ExPASy

online software (https://web.expasy.org/cgi-bin/protparam/protparam). The secondary structure and transmembrane structure of the BraSOC1 protein were analyzed using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) and DeepTMHMM Server v.2.0 (https://dtu.biolib.com/DeepTMHMM) (Hallgren et al. 2022), respectively. DNAMAN software (http://www.lynnon.com/) was utilized to align the cloned DNA fragment with the BrSOC1b fragment in the database. The conserved domain and motif of BrSOC1b were analyzed using SMART software (http://smart.embl-heidelberg.de/) (Letunic et al. 2012) and MEME online software (http://meme.nbcr.net/meme/cgi-bin/meme.cgi) (Bailey et al. 2006), respectively. To determine the subcellular localization of BrSOC1b, we employed Plant-mPLoc (Chou and Shen 2010) for protein prediction.

Phylogenetic analysis of BrSOC1b

The phylogenetic relationship between BrSOC1b and SOC1 in other species was analyzed using the Maximum Likelihood method with MEGA5.0 software (Tamura et al. 2011). The protein sequences of OsSOC1, TaSOC1, and GmSOC1 were obtained from previous reports (Lee et al. 2004; Shitsukawa et al. 2007; Zhong et al. 2012). The sequences of AtSOC1, BjSOC1, and BnSOC1 were retrieved from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/). The abbreviations and accession numbers of the protein sequences used are provided in Table S2.

Analysis of tissue expression pattern of *BrSOC1b*

To analyze the tissue expression pattern of *BrSOC1b,* the root, stem and leaf of Chinese cabbage 'HK-8' cultured normally were taken as samples. In the early stage of podding, the root, stem, stem old leaves, stem young leaves, flowers, pods and buds were taken as samples to analyze the tissue expression pattern of *BrSOC1b*. Each tissue selected three biological replicates.

Construction of *BrSOC1b*-heterologous expression vector and its genetic transformation in *Arabidopsis thaliana*

To construct the *BrSOC1b*-heterologous expression vector, specific primers containing *Kpn* I and *Spe* I restriction sites were designed (Table S1). Firstly, the cDNA fragment containing *Kpn* I and *Spe* I restriction sites was cloned from pEASY-Blunt-*BrSOC1b*. Secondly, the amplified fragment was ligated to the PTCK303 vector digested by *Kpn* I-*Spe* I using T4 ligase and then transformed into *E. coli* (*DH5α*). Monoclonal shaking, bacterial PCR detection, and sequencing were performed. The correct recombinant vector was named pTCK303-*BrSOC1b* (Fig. S4).

The vector pTCK303-*BrSCO1b* was transformed into *Agrobacterium GV3101* using the freeze–thaw method (Yu et al. 2003). It was then plated on LB plates containing Kanamycin and incubated in an inverted position in a 28℃ dark incubator for 2–3 days. Randomly selected single colonies were subjected to PCR, and the colonies with correct bands were selected for further use. *Agrobacterium* containing pTCK303-*BrSCO1b* was inoculated into LB liquid medium containing Kanamycin and Rifampicin antibiotics, and cultured at 28 °C and 250 rpm until the OD_{600} reached approximately 1.0. After centrifugation at 6,000 rpm for 5 min at 20 °C, the cells were collected. The cells were then evenly resuspended in a solution containing 5% sucrose (with 0.02% silwet L-77) to achieve an OD_{600} of approximately 1.0. The well-grown and flowering *Arabidopsis thaliana* plants were watered one day in advance. The small pots containing *Arabidopsis thaliana* plants were inverted the next day, ensuring that all inflorescences were immersed in the suspension buffer for approximately 50 s. After 7 days, the transformation process was repeated using the same method. After 2–3 weeks, watering with nutrient solution was minimized, and the plants were subjected to accelerated aging until mature seeds were obtained. Subsequently, the T3 homozygous lines were obtained through multi-generation selfing and single plant seed collection.

Positive identification of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants

Considering that the pTCK303 vector carries hygromycin resistance genes, we performed DNA detection of the hygromycin resistance genes in *BrSOC1b*-infected *Arabidopsis thaliana* plants. Furthermore, since the pTCK303 vector also contains a GUS reporter gene, GUS histochemical staining was employed to identify *BrSOC1b*-heterologous expression Arabidopsis thaliana plants. Additionally, RNA was extracted from the leaves of transgenic and wild-type *Arabidopsis thaliana* plants, and the relative expression of *BrSOC1b* was determined using reverse transcription and quantitative real-time PCR (qRT-PCR) technology.

Construction of *Brsoc1b*-silenced vector and its genetic transformation in Chinese cabbage

For the virus-induced gene silencing (VIGS) experiments, a suitable 40 bp sequence and its reverse complementary sequence were selected from *BrSOC1b*: 5'-ATAGGATCATGTTCGATAGAGGAGCTGCAGCAAATTGAGCGCTCAATTTGCTGCAGCTCCTCTATCGAACATGATCCTAT-3'. These sequences were synthesized, and the resulting 80 bp sequence was phosphorylated. The vector pTY was digested

with the *SnaB* I restriction enzyme and subsequently dephosphorylated. The phosphorylated DNA fragment and the dephosphorylated pTY vector were ligated using T4 ligase. The ligated construct was then transformed into *DH5α*, followed by screening and sequencing, leading to the identification of positive clones. Following the principles of virus-induced gene silencing, *Brsoc1b*-silenced Chinese cabbage plants were obtained through friction inoculation (Yu et al. 2018) and designated as pTY-*Brsoc1b* (Fig. S5).

Statistics of flowering related indexes of transgenic plants

According to the method of Chen et al. (2012), the days required for bolting and initial flowering of transgenic plants and control plants were counted.

Construction and transient transformation of *BrSOC1b*-subcellular localization vector

To investigate the subcellular localization of the BrSOC1b protein, an open reading frame fragment of BrSOC1b (without the stop codon) containing the *SalI-KpnI* restriction site was cloned using pEASY-Blunt-*BrSOC1b* as a template. The amplified fragment was then ligated to the PRI101-GFP vector, which had been digested with *SalI-KpnI*, using T4 ligase. The resulting construct was transformed into *E. coli* (*DH5α*) for monoclonal selection, followed by bacterial PCR detection and sequencing. The correctly recombined vector was designated as PRI101-GFP-*BrSOC1b*.

The generated recombinant vector PRI101-GFP-*BrSOC1b* (Fig. S6) and the empty PRI101-GFP vector were separately transformed into Agrobacterium GV3101 using the freeze–thaw method (Dupadahalli 2007). Subsequently, the Agrobacterium strains were infiltrated into tobacco leaves using the injection infiltration method (Li and Zhang 2010). The PRI101-GFP vector was used as a control in the experiment. After 40 h of dark incubation, the subcellular localization of the BrSOC1b protein was analyzed using laser confocal microscopy.

Verification of the interaction between BrSOC1b and the target proteins

To clarify the network relationship of BrSOC1b in flowering regulation, we utilized the STRING online tool (https://www.string-db.org) to predict the protein interaction network of BrSOC1b. To validate the interaction between BrSOC1b and the target proteins, we constructed the pGBKT7-*BrSOC1b* bait vector and the pGADT7-target protein vectors. Firstly, we verified the self-activation of the BrSOC1b protein, and subsequently confirmed the interaction between BrSOC1b and the target proteins.

a. Construction of yeast two-hybrid vector

   To construct pGBKT7-*BrSOC1b* bait vector, the cDNA fragmenof of *BrSOC1b* containing *Nco* I / *BamH* I restriction sites (without the stop codon) was cloned using pEASY-Blunt-*BrSOC1b* as template and specific primers containing *Nco* I / *BamH* I restriction sites. The amplified fragment was ligated to the pGBKT7 vector digested by *Nco* I / *BamH* I using T4 ligase. The target protein gene was inserted into the *EcoR* I / *Xho* I restriction site of pGADT7 using the same method, and the vector was named pGADT7-target protein vector. The information of proteins interacting with BrSOC1b in this experiment is list in Table S3.

b. Co-transformation and interaction verification of bait vector and target protein

   The pGBKT7-*BrSOC1b* and pGADT7-target proteins were co-transformed into yeast strain Y2HGold by Yeastmaker yeast transformation system 2 (Cat. No.630439), and then cultured on SD / -Trp / -Leu medium. Monoclonal cells normally grown on SD / -Trp / -Leu solid medium were inoculated into 5 mL SD / -Trp / -Leu liquid medium and cultured at 30 ℃ until OD_{600} was 1.0. The growth of yeast was observed after 3–5 days on SD / -Trp / -Leu / -His / -Ade / + X-*α*-Gal solid medium.

RNA extraction, reverse transcription and qRT-PCR analysis

Total RNA was extracted from leaf samples using RNA simple Total RNA Kit (Tiangen, Beijing), and RNA was reverse transcribed into cDNA using Prime Script RT reagent kit (TaKaRa, Dalian, China). Finally, qRT-PCR was performed using the SYBR Premix ExTaq kit (TaKaRa, Dalian, Beijing). The total PCR system was 20 μL, including 10 μL 2 × SYBR® Premix Ex Taq™ II (TaKaRa), 6.8 μL ddH_2O, 0.4 μL ROX II, 0.8 μL primers and 2.0 μL cDNA template. PCR conditions were as follows: stage 1, 95 °C 20 s; stage 2, 95 °C 3 s; 60 °C 30 s (40 cycles); stage 3, 95 °C 15 s; 60 °C 1 min; 95 °C 15 s. Stage 3 was used to create a melting curve. The results were calculated according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) and plotted using Origin.

Statistical analysis

Data were collected from a minimum of three biological replicates and analyzed using Student's *t*-test or one-way ANOVA followed by post hoc Duncan's test using SPSS software (version 20, Chicago, IL, USA). The presented data represent the mean of three replicates, and the error bars indicate the standard deviation. Different letters above the

columns indicate significant differences between different tissues or lines ($P < 0.05$).

Results and analysis

Cloning and bioinformatics analysis of the *BrSOC1b* genes

By cloning the nucleotide sequence of *BrSOC1b*, we found that *BrSOC1b* contains 642 bp (Fig. S1). The nucleotide sequence of the cloned *BrSOC1b* was aligned with the nucleotide sequence of the gene in the Chinese cabbage database, and the sequence identity was 100.00%. *BrSOC1b* was analyzed by ExPASy online software (https://web.expasy.org/cgi-bin/protparam/protparam). The results showed that *BrSOC1b* encoded 213 amino acids (Fig. 1a), the theoretical isoelectric point (*pI*) was 9.16, and the relative molecular mass was 24 399.92. The protein belongs to unstable protein (instability index is 59.72) and hydrophobic protein (hydrophilic average index is −0.814). SOPMA analysis showed that BrSOC1b protein was composed of 56.34% α-helix, 26.76% random coil, 11.27% extended strand and 5.63% β-turn (Fig. 1b). In addition, the transmembrane structure of BrSOC1b protein was predicted by DeepTMHMM Server v.2.0, and it was found that the protein belonged to the non-trans-membrane proteins protein (Fig. 1c). The conserved domain of BrSOC1b was analyzed by SMART online software. The results showed that BrSOC1b protein contained conserved MADS domain and K domain (Fig. 1a). Finally, the subcellular localization of BrSOC1b protein was predicted by Plant-mPLoc, and it was speculated that BrSOC1b may be localized in the nucleus.

Amino acid sequence alignment and phylogenetic tree analysis of BrSOC1b and SOC1 of other species

By comparing the amino acid sequence of BrSOC1b with SOC1 protein sequences from other species, it was observed that BrSOC1b and SOC1 proteins of other species shared the same domains, namely the MADS domain, K (keratin-like) domain, and SOC1 box at the C-terminus (Fig. 2a). To further investigate the evolutionary relationship between BrSOC1b and SOC1 proteins from *Oryza sativa, Triticum aestivum, Hordeum vulgare, Arabidopsis thaliana, Brassica juncea*, and *Brassica napus*, phylogenetic analysis was performed using MEGA5.0 software. The results revealed that BrSOC1b exhibited the

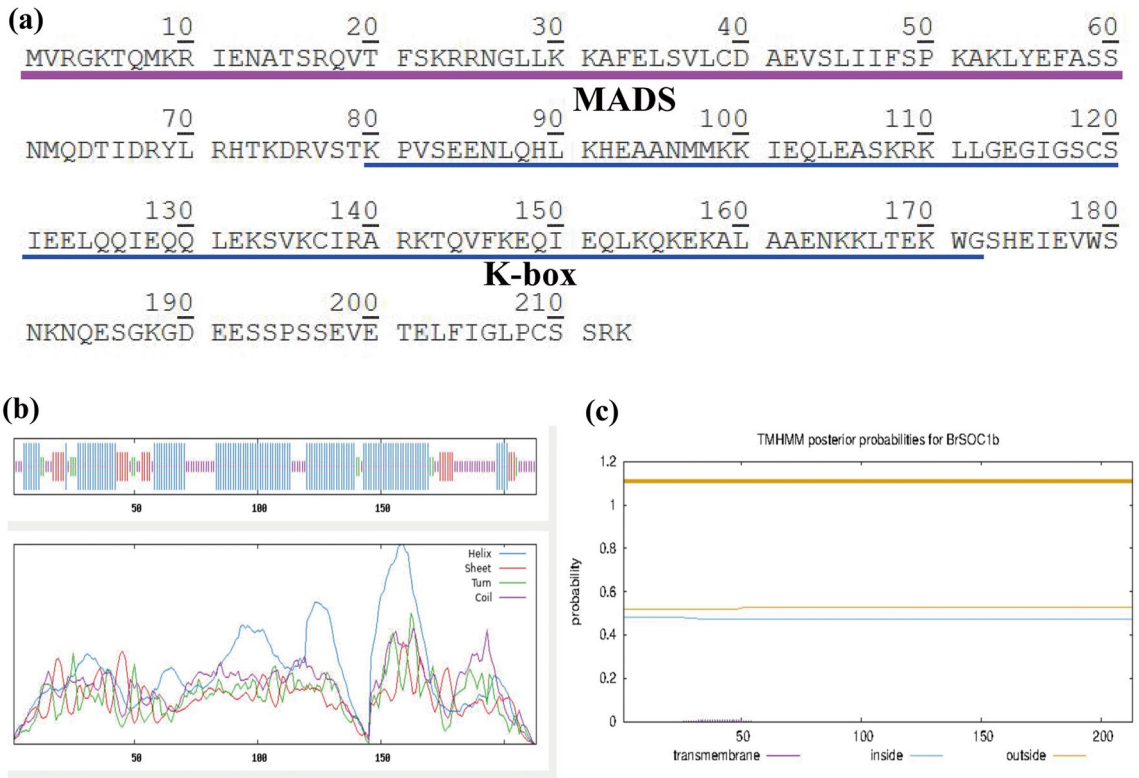

Fig. 1 Bioinformatics analysis of *BrSOC1b*. **a** The predicted amino acid sequence and conserved domain prediction of BrSOC1b. MADS, MADS domain, K-box, K domain. **b** The secondary structure of the BrSOC1b. Helix, represents α-helix; Sheet, represents extended strand; Turn, represents β-turn; Coil, represents random coil. **c** The transmembrane structure prediction of BrSOC1b

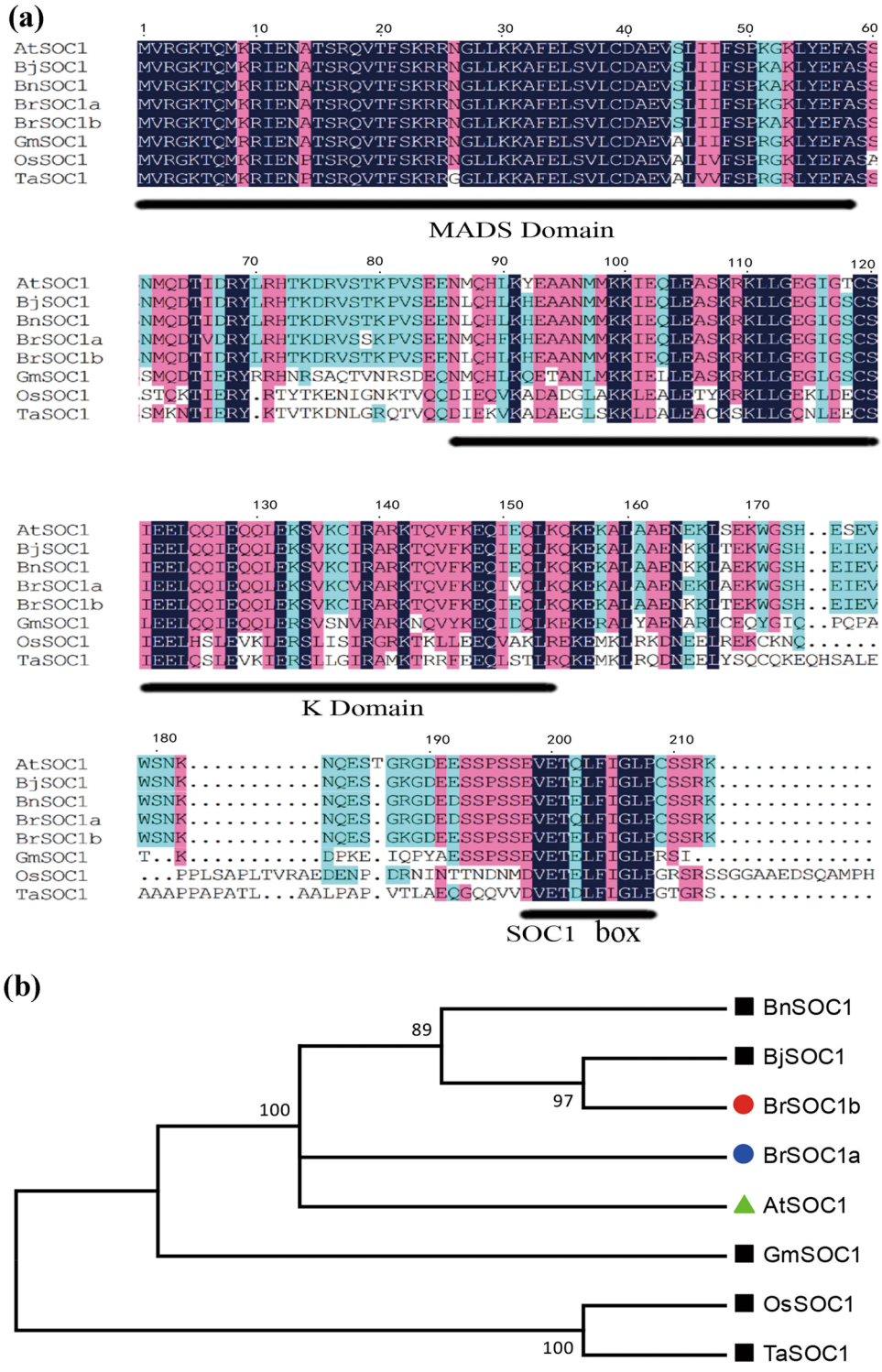

Fig. 2 Amino acid sequence alignment and phylogenetic relationship between BrSOC1b and SOC1 proteins of other species. **a** Amino acid sequence and conserved domain analysis of BrSOC1b protein. Identical and conserved residues are highlighted with the same color background color. The corresponding conserved domains are marked by thick black lines. **b** A phylogenetic tree of BrSOC1b and SOC1 proteins from other species. The phylogenetic tree was constructed by MEGA5.0 software with the method of Maximum Likelihood. Bootstrap values higher than 89% are shown on the nodes

closest homology with BjSOC1b from *Brassica juncea*, followed by BnSOC1b from *Brassica napus*. Additionally, BrSOC1b displayed relatively close homology with BrSOC1a (Fig. 2b). The evolutionary branch indicates that BrSOC1b is relatively distant from OsSOC1, TaSOC1, and GmSOC1.

Tissue expression pattern analysis of *BrSOC1b* gene

To explore the expression of *BrSOC1b* gene in various tissues, we explored it at seedling stage and early podding stage. Firstly, the relative expression of *BrSOC1b* in roots, stems and leaves was determined at seedling stage. The

results showed that *BrSOC1b* had the highest expression in stem, which was 77.12 times higher than that in root, followed by the expression in leaf, which was 45.21 times higher than that in root (Fig. 3a).

Furthermore, we studied the relative expression of *BrSOC1b* in roots, stems, old leaves, young leaves, flowers, pods and buds at the early stage of pod formation. The results showed that *BrSOC1b* had the highest expression level in flowers, which was 11.56 times that of roots (Fig. 3b). Secondly, it is also expressed in stems, stem born old leaves and stem born young leaves. The lowest expression level was in the bud, which was 0.27 times that of the root (Fig. 3b). Based on the above experimental results, we speculate that *BrSOC1b* may be related to the growth and development of stems and leaves at the seedling stage, and is mainly expressed in flowers at the early stage of pod formation. Therefore, we speculate that *BrSOC1b* may play a key role in flower development.

Sub-cellular localization analysis of BrSOC1b

To explore the subcellular localization of BrSOC1b protein, we transiently expressed *BrSOC1b* gene in tobacco. The results showed that PRI101-GFP, as a control, emitted green light in the whole cell of tobacco, while GFP, which formed a fusion protein with BrSOC1b, emitted light in the nucleus and cell membrane, and the green fluorescence on the membrane coincided with the red fluorescence of the membrane tag mCherry (Fig. 4). Therefore, we speculated that BrSOC1b was localized in the nucleus and plasma membrane.

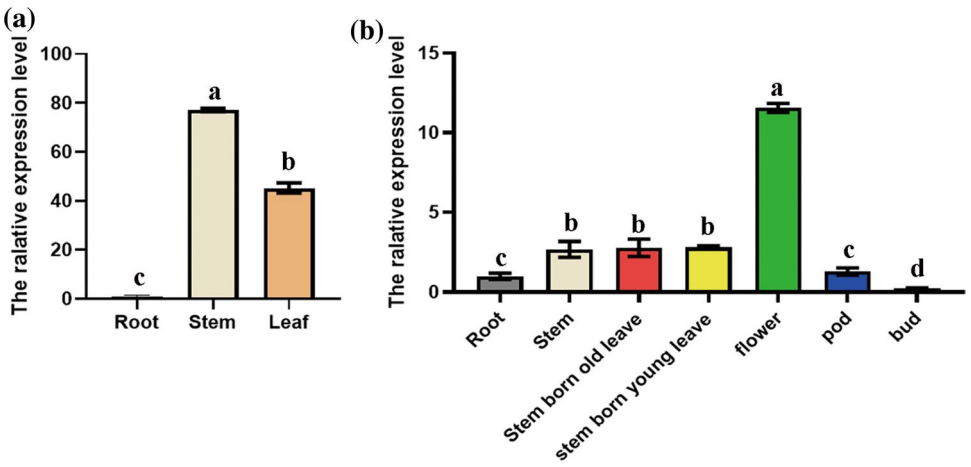

Fig. 3 Expression profiles of *BrSOC1b* in various tissues. **a** Expression profile of *BrSOC1b* in root, stem and leaf at seedling stage. **b** Expression profiles of *BrSOC1b* in the root, stem, stem born old leave, stem born young leave, flower, pod, and bud. The data represent the mean of three biological replicates and three technical replicates, and the error line represents the standard deviation of the mean. The gene expression was carried out by $2^{-\Delta\Delta Ct}$ method, and then the expression of *BrSOC1b* in the root was corrected to 1. *BrActin* (*Bra028615*) was as reference gene. Different letters above the column indicated significant differences between different tissues ($P < 0.05$)

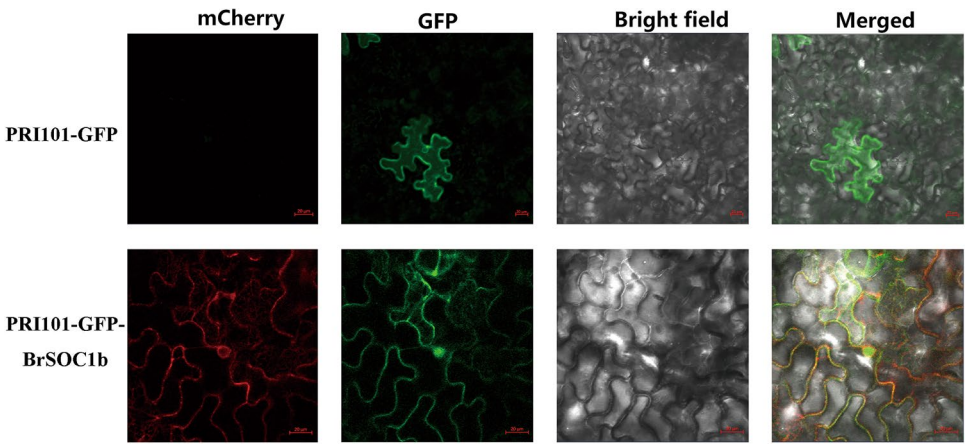

Fig. 4 Sub-cellular localization analysis of *BrSOC1b* in *Nicotiana benthamiana* leaf cells. mCherry, membrane marker; pm-rbCD3-1008; GFP, dark field image of tobacco leaf cells; Bright field, bright field image of tobacco leaf cells; Merged, the superposition of red fluorescence image, green fluorescence image and bright field; Scale: 20 μm

Positive identification of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants

To investigate the function of *BrSOC1b*, we conducted heterologous expression of *BrSOC1b* to generate transgenic *Arabidopsis thaliana* plants. To identify positive seedlings of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants, we initially performed hygromycin resistance gene detection using DNA extracted from transgenic *Arabidopsis thaliana* leaves. The results indicated the presence of hygromycin resistance genes in 11 T1 plants (Fig. 5a). Through successive selfing and individual plant selection, we obtained T3 generation plants.

In the T3 generation, we employed the GUS (β-D-glucuronidase) histochemical staining method to verify partial staining in the leaves of transgenic *Arabidopsis thaliana* under a stereomicroscope, whereas no staining was observed in the leaves of wild-type Arabidopsis thaliana (Fig. 5b). Subsequently, we randomly examined the relative expression levels of *BrSOC1b* in two transgenic lines (OE1 and OE4) as well as wild-type plants using qRT-PCR. The results revealed that the relative expression level of *BrSOC1b* in wild-type plants was very low, while in transgenic plants, it was 10.09–12.57 times higher than that in wild-type plants (Fig. 5c). Based on this analysis, we selected the transgenic lines OE1 and OE4 for further phenotypic observation and statistical analysis.

Phenotypic observation and flowering time statistics of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants

To investigate the impact of *BrSOC1b* on plant flowering, we utilized two lines of transgenic plants (OE1 and OE4) along with wild-type plants as the experimental materials. We observed the phenotypes of these plants and recorded the bolting time and flowering time. The results revealed that the bolting time and flowering time of the transgenic plants were significantly earlier compared to those of the wild-type plants (Fig. 6a, b). Specifically, the bolting time of *BrSOC1b*-overexpressing *Arabidopsis thaliana* plants occurred 7 to 11 days earlier than that of the wild type (Fig. 6c). Moreover, the flowering time of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants was 6 to 7 days earlier than that of the wild type (Fig. 6d).

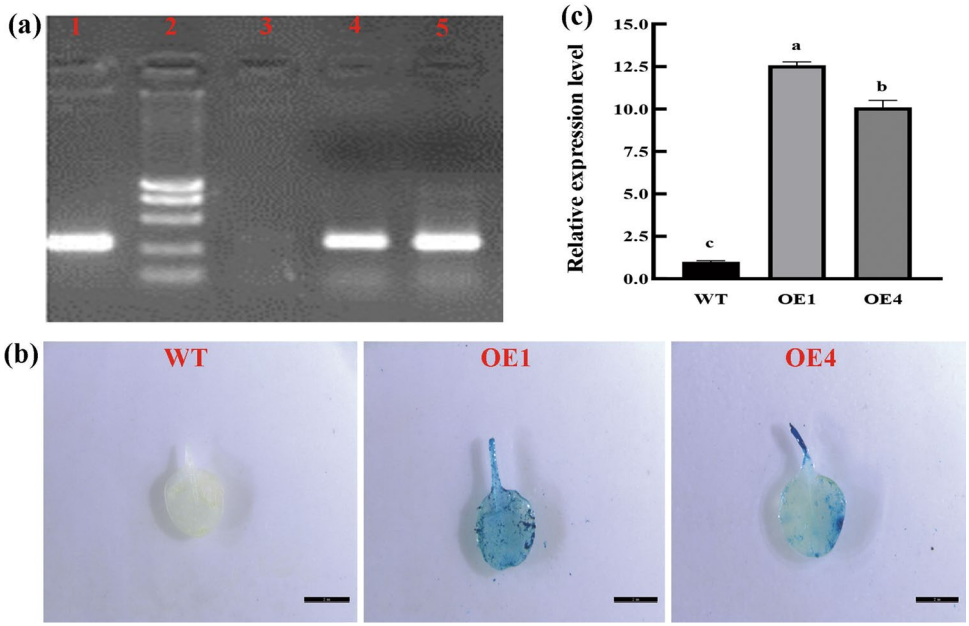

Fig. 5 Positive identification of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants. **a** DNA analysis of hygromycin resistance genes in *BrSOC1b*-heterologous expression *Arabidopsis thaliana.* Note: Column 1 is shown as a positive control. Column 2 is 1000 marker, 100, 250, 500, 750 and 1000 bp from bottom to top. Column 3 is negative control. Column 4 and 5 are transgenic seedling detection samples. **b** β-glucuronidase (GUS) histochemical staining of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* lines. **c** The qRT-PCR analysis of *BrSOC1b* in the leaves of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* lines. The gene expression was carried out by $2^{-\Delta\Delta Ct}$ method, and then the expression of *BrSOC1b* in the root was corrected to 1. The *eIF4Actin* gene was used as an internal control. The data represents the average of three replicates, and the error line represents the standard deviation of the average. Different letters above the column indicated significant differences between different tissues ($P < 0.05$)

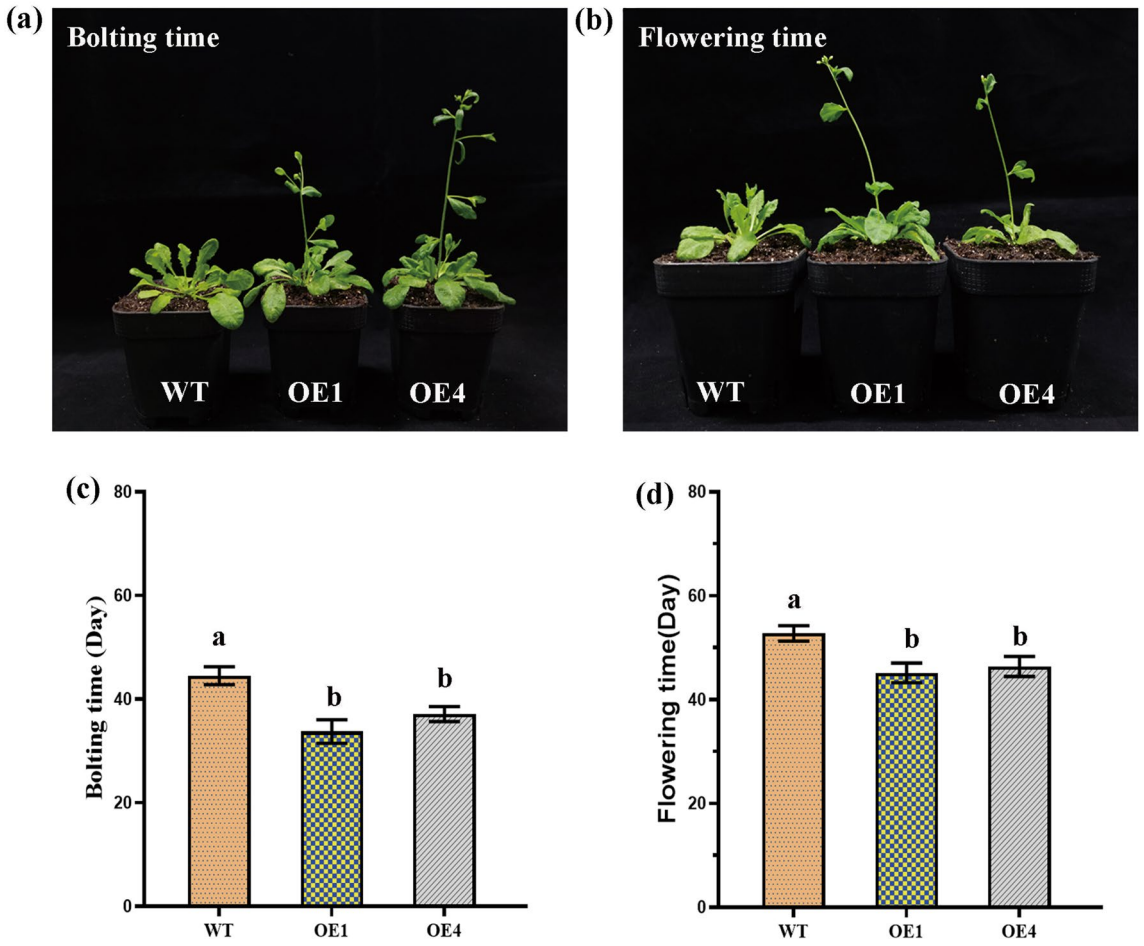

Fig. 6 Phenotypic observation and related data statistics of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants and wild-type plants. **a** The phenotype of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants and wild-type plants at bolting stage. **b** Phenotypes of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants and wild-type plants at flowering stage. **c** Bolting time statistics of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants and wild-type plants. **d** Flowering time statistics of *BrSOC1b*-heterologous expression *Arabidopsis thaliana* plants and wild-type plants. We take the initial flowering period as the flowering time. Each line and wild-type plants were counted 8 plants. The data represents the average, and the error line represents the standard deviation of the average. Different letters above the column indicated that there were significant differences between different lines ($P < 0.05$)

BrSOC1b protein interaction network prediction and the self-activation detection of BrSOC1b

To clarify the interaction relationship of BrSOC1b protein, we used STRING online tool to predict the interaction network diagram of BrSOC1b protein (Fig. S2). The interaction diagram showed that BrSOC1b interacted with BrAP1 (Bra038326), BrAGL9a / SEP3 (Bra010955), BrAGL9b / SEP3 (Bra030032), BrAGL2 / SEP1 (Bra008674), FUL / BrAGL8 (Bra029347) (Fig. S2). Therefore, I speculate that BrSOC1b may interact with the above proteins.

To verify the interaction between BrSOC1b and the above proteins, the self-activation of BrSOC1b protein was first verified. The pGBKT7-*BrSOC1b* and pGADT7 were co-transformed into the yeast strain Y2HGold as the experimental group. The negative control was the co-transformation of pGBKT7 and pGADT7 in the yeast strain, and the positive control was the co-transformation of pGBKT7-53 and pGADT7-T in the yeast strain. The positive control, negative control and experimental groups were cultured on SD / -Trp / -Leu and SD / -Trp / -Leu / -His / -Ade / + X-α-Gal solid medium, respectively. As shown in Fig. S3, these three groups can grow normally on SD / -Trp / -Leu, and the positive control can grow normally and show blue on SD / -Trp / -Leu / -His / -Ade / + X-α-Gal solid medium. However, negative control and experimental groups in SD / -Trp / -Leu / -His / -Ade / + X-α-Gal could not grow normally (Fig. S3). Based on the above analysis, we speculate that there is no self-activation of BrSOC1b protein in yeast.

Verification of the interaction between BrSOC1b and predicted interacting proteins

To explore the interaction between BrSOC1b and BrAP1, BrAGL9a, BrAGL9b, BrAGL2, BrAGL8 proteins, we used BrSOC1b as a bait protein and verified by yeast two-hybrid technology. PGBKT7-BrSOC1b + pGADT7-BrAP1, pGBKT7-BrSOC1b + pGADT7-BrAGL9a, pGBKT7-BrSOC1b + pGADT7-BrAGL9b, pGBKT7-BrSOC1b + pGADT7-BrAGL8, pGBKT7-BrSOC1b + pGADT7-BrAGL2 were coated on SD / -Trp / -Leu and SD / -Trp / -Leu / -His / -Ade / + X-α-Gal plates, respectively. After 3 days of culture, it was found that all combinations could grow on SD / -Trp / -Leu medium (Fig. 7a). In addition, the experimental groups pGBKT7-BrSOC1b + pGADT7-BrAGL9a, pGBKT7-BrSOC1b + pGADT7-BrAGL9b, pGBKT7-BrSOC1b + pGADT7-BrAGL8 and pGBKT7-BrSOC1b + pGADT7-BrAGL2 could grow and turn blue on SD / -Trp / -Leu / -His / -Ade / + X-α-Gal medium. However, the experimental group pGBKT7-BrSOC1b + pGADT7-BrAP1 could not grow normally. Based on the above experimental results, we concluded that BrSOC1b interacts with BrAGL9a, BrAGL9b, BrAGL2, BrAGL8 proteins, but not with BrAP1 (Fig. 7b). Considering that BrAGL9a, BrAGL9b, BrAGL2 and BrAGL8 proteins all belong to MADS transcription factors, this family plays a key role in regulating plant flowering pathways, so we speculate that BrSOC1b protein may interact with the above proteins to regulate flowering.

Phenotypic observation of *Brsoc1b*-silenced Chinese cabbage plants

The leaves of *Brsoc1b*-silenced Chinese cabbage plants exhibited significant chlorosis compared to the wild-type (WT) plants, 20 days after virus inoculation. They displayed typical symptoms of turnip yellow mosaic virus (Fig. 8a). A total of 20 pTY-*Brsoc1b* plants and 10 pTY control plants were obtained. The Brsoc1b-silenced Chinese cabbage plants, as well as the control (pTY) plants, were further cultivated, and it was observed that the Brsoc1b-silenced Chinese cabbage plants exhibited delayed bolting and flowering compared to the control plants (Fig. 8b, c). The expression of the *BrSOC1b* gene was assessed by qRT-PCR when Chinese cabbage plants were close to bolting. The results demonstrated that the relative expression of *BrSOC1b* in pTY-*Brsoc1b* plants was approximately 0.65–0.78 times that of pTY plants (Fig. 9a). Consequently, we hypothesized that *BrSOC1b* was partially silenced in Chinese cabbage, and this gene played a positive role in regulating the bolting time and flowering time of Chinese cabbage.

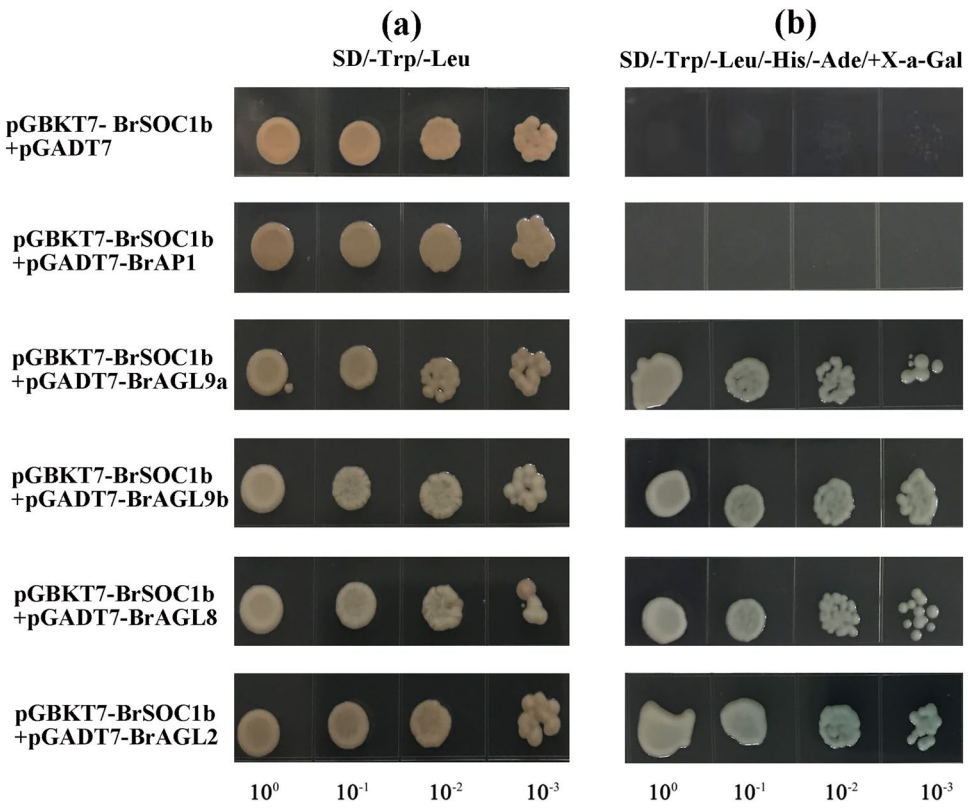

Fig. 7 Verification of the interaction between BrSOC1b and BrAP1, BrAGL9a, BrAGL9b, BrAGL2, BrAGL8 proteins. The pGBKT7-BrSOC1b bait vector and the target vector were co-transformed into the yeast strain Y2HGold, and then detected on SD / -Trp / -Leu (**a**) and SD / -Trp / -Leu / -His / -Ade / + X-α-Gal (**b**) plates

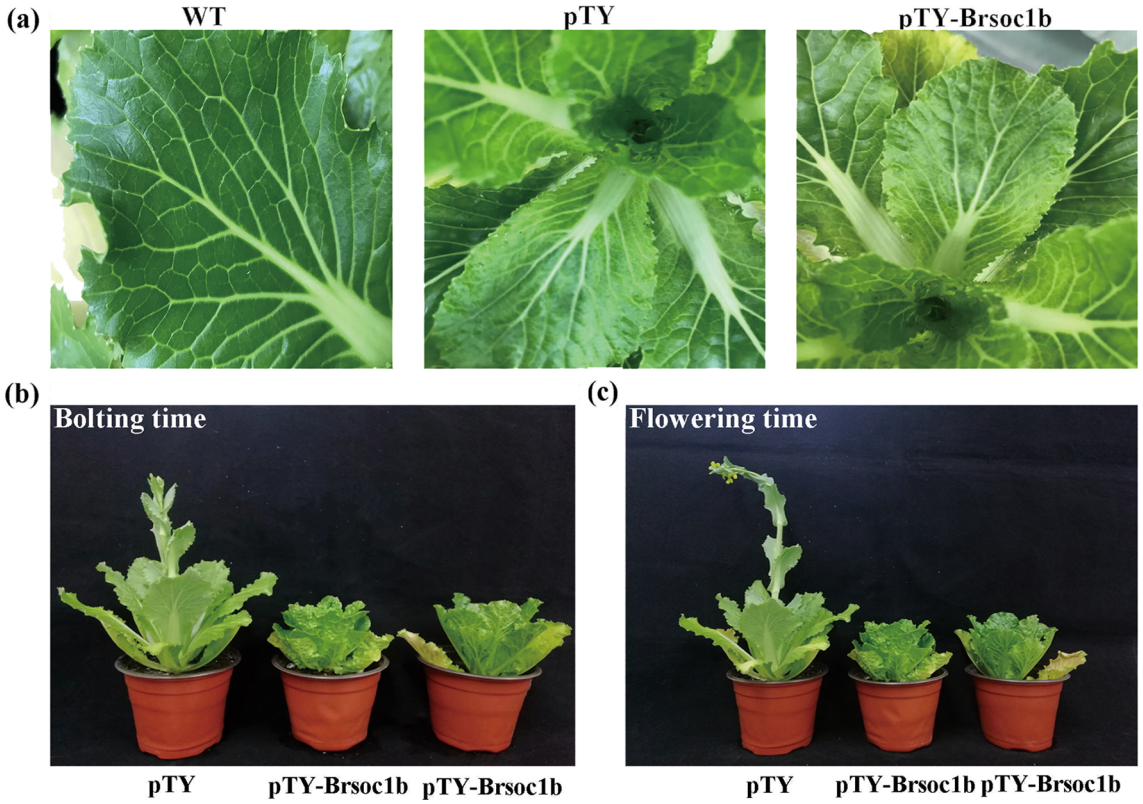

Fig. 8 Phenotypic analysis of *Brsoc1b*-silenced Chinese cabbage plants and control plants. **a** Local phenotypes of *Brsoc1b*-silenced Chinese cabbage pTY-*Brsoc1b* and control pTY leaves. **b** Phenotype of *Brsoc1b*-silenced Chinese cabbage plants and control plants at bolting stage. **c** The phenotype of *Brsoc1b*-silenced Chinese cabbage plants and control plants at the initial flowering stage

Expression analysis of genes interacting with BrSOC1b in pTY-*Brsoc1b* plants and pTY plants

Three pTY-*Brsoc1b* plants and three pTY control plants were selected for further analysis. The relative expression profiles of *BrAGL9a, BrAGL9b, BrAGL2, BrAGL8*, and *BrAP1* genes were measured in the pTY-*Brsoc1b* plants and control plants. It was observed that when the *BrSOC1b* gene was partially silenced, the expression of *BrAGL9a, BrAGL9b, BrAGL2*, and *BrAGL8* genes was significantly down-regulated, except for the *BrAP1* gene (Fig. 9). For instance, in pTY-*Brsoc1b* plants, the expression of *BrAGL9a* was approximately 0.38–0.45 times that of pTY plants (Fig. 9c), and the expression of *BrAGL2* was around 0.65–0.76 times that of pTY plants (Fig. 9f). Taking into account the results of the protein interaction experiments, it is speculated that *BrAGL9a*, *BrAGL9b*, *BrAGL2*, and *BrAGL8* genes are down-regulated genes of the *BrSOC1b* gene, which may play a pivotal role in flowering regulation.

Discussion

Flowering time is a critical period for plant reproduction, and the regulation of flowering holds great significance for plant production and breeding. The *SOC1* gene acts as an integrator that combines multiple flowering signals. Through the analysis of the structure of *BrSOC1b* in Chinese cabbage, it was observed that the BrSOC1b protein contains a SOC1 box, which is unique to the TM3-like branch of the MADS family gene. This suggests that *BrSOC1b* belongs to the TM3-like branch gene (Fig. 2a). Furthermore, the transmembrane structure prediction of BrSOC1b protein revealed that it is a non-transmembrane protein (Fig. 1c). Additionally, the subcellular localization prediction of BrSOC1b protein indicated that it localizes to the nucleus. However, when *BrSOC1b* was transiently expressed in tobacco cells, it was observed to localize to both the nucleus and plasma membrane (Fig. 4). Based on these findings, it can be speculated that *BrSOC1b* may exhibit spatiotemporal differential expression characteristics.

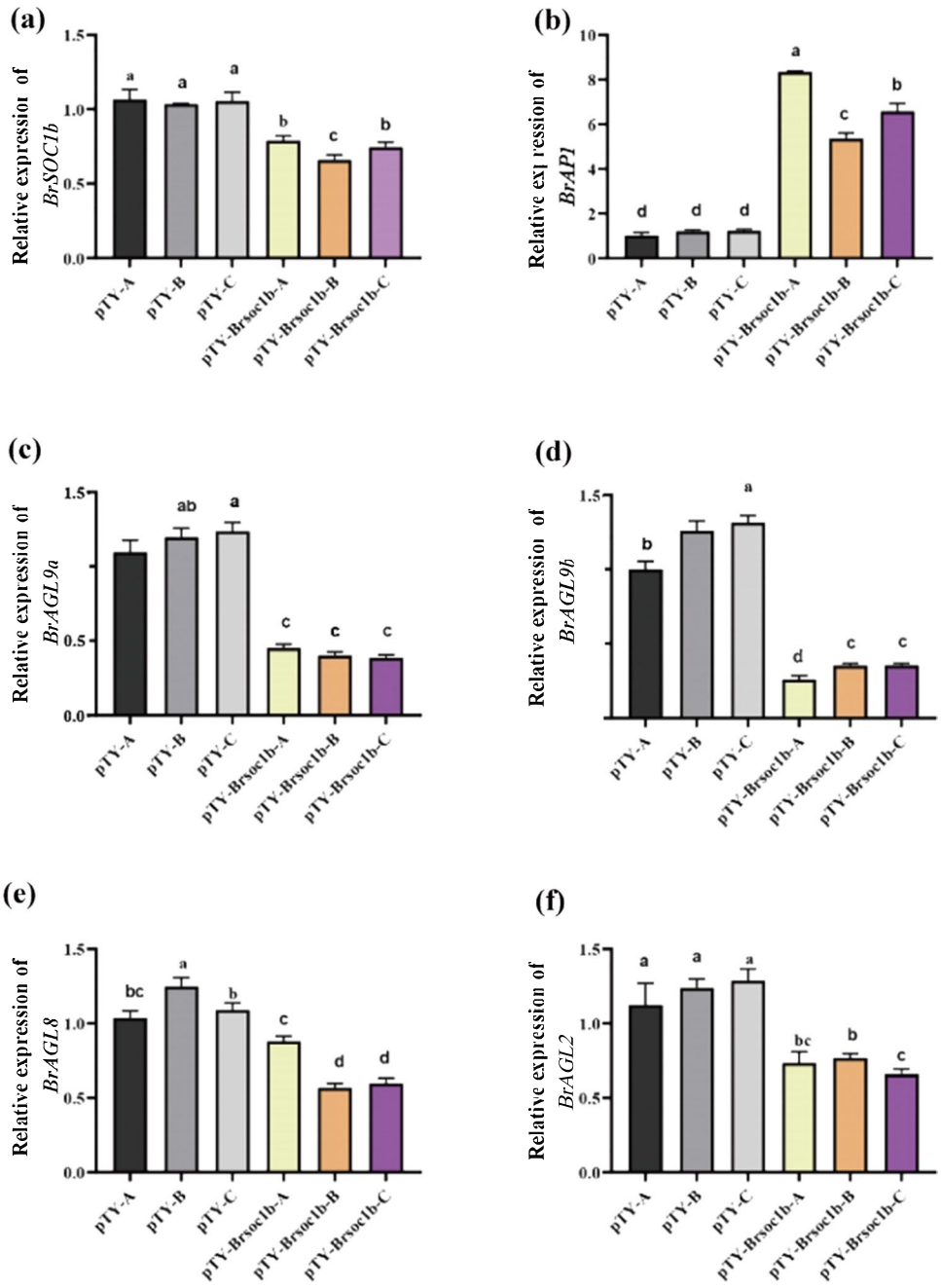

Fig. 9 Expression analysis of genes interacting with BrSOC1b in pTY-BrSOC1b plants and pTY plants. *BrActin* (Bra028615) gene was used as an internal control. The gene expression was carried out by $2^{-\Delta\Delta Ct}$ method. The data represents the average of three replicates, and the error line represents the standard deviation of the average. Different letters above the column indicated significant differences between different lines ($P < 0.05$)

BrSOC1b promotes early flowering in *Arabidopsis thaliana*, and the phylogenetic relationship suggests that *BrSOC1b* has the closest homology with *BjSOC1b*. This indicates that BjSOC1b may also be involved in flowering regulation in mustard. However, further experiments are required to confirm this speculation. Moreover, considering that *Oryza sativa*, *Triticum aestivum*, and *Hordeum vulgare* are monocots, while *Arabidopsis thaliana*, *Brassica juncea*, and *Brassica napus* are dicots, it can be observed that the homologous relationship between BrSOC1b and SOC1 proteins in dicots is closer than that in monocots. This finding is consistent with the evolutionary characteristics of organisms.

Currently, researchers have conducted extensive investigations into the function of the *SOC1* gene. The *Arabidopsis thaliana SOC1* (*AtSOC1*) gene has been found to regulate flowering time and floral tube development (Zhao et al. 2014). Overexpression of the *SOC1* gene promotes early flowering in alfalfa (Mauren et al. 2018). In *Bambusa oldhamii*, *SOC1* exhibits the highest expression in

flower buds and the lowest expression in stems (Onouchi et al. 2000). Additionally, *CsSOC1a* was found to accumulate the most in mature leaves and also promoted flowering induction in Gannan early navel orange trees (He et al. 2022). *BrSOC1b* displays high expression levels in stems and leaves during the seedling stage. Furthermore, *BrSOC1b* is highly expressed in flowers during the early podding stage (Fig. 3), suggesting a potential key role for *BrSOC1b* in flower development.

Through phenotypic analysis of *BrSOC1b*-heterologous expression in *Arabidopsis thaliana* plants and wild-type *Arabidopsis thaliana* plants, it was discovered that heterologous expression of *BrSOC1b* promotes flowering in *Arabidopsis thaliana*. Conversely, partial silencing of *BrSOC1b* results in delayed flowering in Chinese cabbage. These results indicate that *BrSOC1b* plays a crucial role in the regulation of flowering in Chinese cabbage. While this study reveals that *BrSOC1b* regulates the flowering time of plants, the molecular mechanism underlying *SOC1's* regulation of plant flowering remains unclear (Xian et al. 2013). Current research has mainly focused on the regulation of *SOC1* in the shoot apical meristem to control the process of flowering induction in plants. However, it is still necessary to verify whether *SOC1* in leaves can translocate to the shoot apex, warranting further exploration of the molecular mechanism of *SOC1*.

Proteins serve as the executors of function, and protein interactions are the primary means through which functions are performed. The yeast two-hybrid experiment revealed interactions between BrSOC1b and BrAGL9a, BrAGL9b, BrAGL8b, and BrAGL2b. The *AtAGL9* gene was initially cloned in *Arabidopsis thaliana*, and its close association with flower development was observed (Mandel and Yanofsky 1998; Yang et al. 2019). Based on this, we speculate that *BrSOC1b* may regulate plant flower development by interacting with BrAGL9a and BrAGL9b, potentially affecting flowering time regulation in plants. Additionally, several studies have reported on the *AP1* gene. It was discovered that the apple *AP1* gene (*MdAP1*) and petunia *AP1* gene promote early flowering in transgenic *Arabidopsis thaliana* plants (Kotoda et al. 2002; An et al. 2001). Heterologous expression of *Arabidopsis thaliana AP1* in tomato shortens the vegetative cycle without affecting plant production (Ellul et al. 2004). In this study, yeast two-hybrid assays did not indicate an interaction between BrSOC1b and BrAP1. However, qRT-PCR analysis demonstrated an increase in BrAP1 expression after partial silencing of BrSOC1b (Fig. 9b), suggesting that the *BrSOC1b* gene may indirectly influence the expression of BrAP1. Further verification is necessary to determine if BrAP1 is involved in flowering regulation. These results provide a foundation for further investigation into the mechanisms underlying *BrSOC1b's* involvement in flowering regulation.

Conclusion

Based on the aforementioned studies, *BrSOC1b* consists of 642 base pairs, encoding 213 amino acids. It contains a conserved MADS domain, K (keratin-like) domain, and SOC1 box. The BrSOC1b protein is localized in the nucleus and plasma membrane, with specific expression primarily observed in Chinese cabbage flowers. *BrSOC1b* promotes early flowering in plants and may regulate the flowering time of Chinese cabbage by interacting with BrAGL9a, BrAGL9b, BrAGL2, and BrAGL8 proteins. This study lays the groundwork for the comprehensive analysis of the molecular mechanisms underlying the regulation of Chinese cabbage flowering by the *BrSOC1b* gene. Furthermore, it holds significant implications for the genetic enhancement of Chinese cabbage germplasm resources in future breeding efforts.

Author contribution statement JY (Jingping Yuan) and CS conceived and designed the experiments, BS, RC and DL performed the experiments. XG, CW, NK and BC performed the qRT-PCR. JY, CS and XL wrote the manuscript. All authors read and approved the final manuscript.

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Data availability No data was used for the research described in the article.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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