The Crop Journal 10 (2022) 75–87

Contents lists available at ScienceDirect

The Crop Journal

journal homepage: www.elsevier.com/locate/cj

# Cotton *GhBRC1* regulates branching, flowering, and growth by integrating multiple hormone pathways

Quan Sun [a,b,1], Yuanhui Xie [a,1], Huimin Li [a], Jinlei Liu [a], Rui Geng [a], Ping Wang [a], Zongyan Chu [d], Ying Chang [a], Guanjun Li [a], Xiao Zhang [a], Youlu Yuan [c,*], Yingfan Cai [a,*]

[a] State Key Laboratory of Cotton Biology, Henan Key Laboratory of Plant Stress Biology, School of Life Sciences, Henan University, Kaifeng 475000, Henan, China
[b] Chongqing Key Laboratory of Big Data for Bio Intelligence, College of Bioinformation, Chongqing University of Posts and Telecommunications, Chongqing 400065, China
[c] State Key Laboratory of Cotton Biology, Key Laboratory of Cotton Genetic Improvement, Ministry of Agriculture, Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang 455000, Henan, China
[d] Kaifeng Academy of Agriculture and Forestry, Kaifeng 475000, Henan, China

ARTICLE INFO

*Article history:*
Received 24 September 2020
Revised 24 December 2020
Accepted 29 January 2021
Available online 14 March 2021

*Keywords:*
Cotton
*GhBRC1*
Branching
Flowering
Growth

ABSTRACT

Cotton architecture is partly determined by shoot branching and flowering patterns. *GhBRC1* was previously identified by RNA-seq analysis of nulliplex-branching and normal-branching cotton. However, the roles of *GhBRC1* in cotton remain unclear. In the present study, investigations of nuclear localization and transcriptional activity indicated that GhBRC1 has characteristics typical of transcription factors. Gene expression analysis showed that *GhBRC1* was highly expressed in axillary buds but displayed different expression patterns between the two branching types. Overexpression of *GhBRC1* in *Arabidopsis* significantly inhibited the number of branches and promoted flowering. In contrast, silencing *GhBRC1* in cotton significantly promoted seedling growth. *GhBRC1* was induced by multiple hormones, including strigolactones, which promoted seedling growth and seed germination of *Arabidopsis* plants overexpressing *GhBRC1*. Consistent with these findings, RNA-seq analysis of virus-induced gene silencing treated cotton revealed that a large number of genes were differentially expressed between *GhBRC1*-silenced and control plants, and these genes were significantly enriched in plant hormone signalling pathways. Together, our data indicates that *GhBRC1* regulates plant branching and flowering through multiple regulatory pathways, especially those regulating plant hormones, with functions partly differing from those of *Arabidopsis BRC1*. These results provide insights into the molecular mechanisms controlling plant architecture, which is important for breeding cotton with ideal plant architecture and high yield.



## 1. Introduction

Both adaptation to the environment and growth shape plant architecture. For example, plants often absorb more light by fine-tuning growth characteristics. Regulation of plant branching is a major strategy for plant survival and space occupation, and is accomplished by a complex regulatory network [1]. Shoot branching establishes leaf area and distribution and determines the amount of light interception and photosynthesis, which in turn influence the number of flowers and fruits, fruit filling and yield. The mechanisms regulating plant architecture have been extensively studied over the past several decades [1–3]. It has been reported that DNA methylation and transcriptional regulators controlling the signalling pathways of hormones, sugar, light and water are involved in shoot branching [2,4–10]. Among these regulators, *BRANCHED 1* (*BRC1*) is an important hub controlling bud outgrowth in many species [11–13].

BRC1 is a member of the TCP family, a plant-specific transcription factor (TF) family. Examples of TCP TFs include TEOSINTE BRANCHED1 (TB1) from *Zea mays* (maize), CYCLOIDEA (CYC) from *Antirrhinum majus*, and PROLIFERATING CELL FACTORS (PCF) from *Oryza sativa* (rice). TCP TFs are predominantly involved in plant development, regulating processes such as branching [11,14], leaf development [15], and seed germination [16]. TCP proteins are characterized by the presence of a TCP domain, which is a non-canonical beta helix-loop-helix domain. TCPs are widespread in plants, but the number of TCP family genes largely varies among species. There are only six *TCP* genes in bryophyte species [17],

* Corresponding authors.
*E-mail addresses:* yingfancai@outlook.com (Y. Cai), yylcri@126.com (Y. Yuan).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.cj.2021.01.007

whereas there are more than 60 *TCP* genes in tobacco (*Nicotiana tabacum*) [18], cotton (*Gossypium hirsutum* and *Gossypium barbadense*) [19,20], *Brassica juncea* var. *tumida* [21], and oilseed rape (*Brassica napus*) [22].

TCP proteins are divided into two classes, I and II, with the main difference being a four amino acid deletion in the basic region of the class I TCPs. Class II further comprises two distinct subclasses, namely CINCINNATA (CIN) and CYCLOIDEA/TB1 (CYC/TB1). BRC1 is a member of the CYC/TB1 clade of class II, and is mainly involved in regulating shoot branching [11], the floral transition [23] and other aspects of plant development [24]. In *Arabidopsis thaliana*, *BRC1* and *BRC2* are closely related to *TB1* in maize and *FINE CULM 1* (*FC1*) in rice. These genes are conserved in many plant species.

To date, studies of *BRC1* have mainly focused on its roles in the molecular mechanisms and networks regulating plant development. Multiple studies have implicated auxin/indole-3-acetic acid (IAA), cytokinin (CK), strigolactone (SL) and others in the regulation of *BRC1* gene expression [2,25–27]. Auxin indirectly promotes *BRC1* expression through CK and SL in the bud [27,28]. High CK levels can downregulate *BRC1* expression in axillary buds [29,30], and the promotion of cross-stem PIN-mediated auxin transport and branching by CK may be partly caused by direct repression of *BRC1* in the bud [31]. Regulation of *BRC1* expression by SL was first reported in *Arabidopsis* and pea (*Pisum sativum*) [11,32]. DWARF53 (D53), an important molecule in the SL signalling pathway, can interact with IPA1 (IDEAL PLANT ARCHITECTURE1), which directly binds to the *TB1* promoter in rice and activates TB1 transcriptional activity [33–37]. In addition, gibberellin (GA), light and sugar might also be involved in the regulation of *BRC1* expression, but the mechanism is still unclear [38–42].

Cotton (*Gossypium* spp.) is an economically important fibre and oil crop, and the main source of renewable textile fibres. Cotton architecture is determined by shoot branching and flowering patterns, which directly influence light exploitation, yield, and planting area, as well as the efficiency of harvesting and the cost of planting. Our previous study demonstrated that *GbDWARF14* (rice *D14* homolog) is receptor in the SL signalling pathway that may regulate *BRC1* expression [35,37,43,44]. As mentioned above, BRC1 is an integrator involved in shoot branching and response to many endogenous and exogenous inputs. Genome sequencing has been completed for five cotton species, including *G. australe* [45], *G. hirsutum* [46,47], *G. barbadense* [48,49], *G. arboreum* [50], *G. raimondii* [51], and the most noteworthy are tetraploids; their genome sequences have revealed 75 *TCP* genes in *G. barbadense* and 73 or 74 *TCP* genes in *G. hirsutum* [19,20,52]. However, whether the cotton BRC1 homolog is also involved in shoot branching is unknown. In addition, the detailed mechanism regulating *BRC1* expression in cotton, including the stimuli involved, has yet to be determined.

In this study, we cloned a cotton *BRC1* homolog (named *GhBRC1*), and performed a preliminary analysis of the function of this gene. The results provide valuable information for future studies of the role of GhBRC1 during floral transition and lay a foundation for exploring the molecular mechanism of shoot branching in cotton.

## 2. Materials and methods

### 2.1. Plant materials and growth conditions

Cotton lines Huazhong-94, 3798, and Jinxianduanguozhi (JX or C5) were cultivated in a constant-temperature incubator under long-day conditions (16 h light/8h dark) with approximately 80% relative humidity and a temperature of 25 °C. *N. benthamiana* was grown in an incubator maintained at 25 °C. *Arabidopsis thaliana* (*Arabidopsis*) ecotype Columbia-0 and other relative mutant plants were grown under a 16 h light/8 h dark photoperiod at 21 °C.

### 2.2. RNA extraction and real-time quantitative PCR (qRT-PCR) analysis

Total RNA was extracted from different plant materials using RNA Plant Plus Reagent (Tiangen Biotech Co., Ltd., Beijing, China). The total RNA samples were treated with DNase I (Takara, Qingdao, China) to remove contaminating genomic DNA. First-strand cDNA was synthesized from the total RNA using a HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, Jiangsu, China). Analysis of qRT-PCR was performed using TB Green *Premix Ex Ta* II (TliRNaseH Plus) (Takara, Qingdao, China). *ACTIN3* was used as the internal reference gene for qRT-PCR, and the gene-specific primers are listed in Table S1.

### 2.3. Subcellular localization

The full-length coding sequence of *GhBRC1* was cloned into the plasmid pK7FWGF2.0 in frame with GFP. The recombinant vector and the control vector were transformed into *Agrobacterium tumefaciens* (GV3101). Subcellular localization was analysed by injecting the *Agrobacterium* into the abaxial side of tobacco leaves (4–6 weeks old) as described previously [43]. The leaves were then incubated in the dark for 2–3 days. A drop of 4′,6-Diamidino-2-Phenylindole (DAPI) solution (Solarbio, Beijing, China) was added to the tissues, and after 5 min the solution was removed, and the tissue was washed two to three times with phosphate buffer saline (PBS). The tissue was covered with a cover slip, then examined under a microscope.

### 2.4. Transactivation activity analysis in yeast

The selected full-length and truncated *GhBRC1* open reading frames were PCR amplified and cloned into the pGBKT7 vector (Clontech, Mountain View, CA, USA) using the gene-specific primers listed in Table S1. Then the pGBKT7-GhBRC1 recombinant vector, the positive control vector pGBKT7-53 + pGADT7-T, and the negative control pGBKT7 empty plasmid were transformed into the yeast strain Y2H using the lithium acetate method. The transformed strains were further serially cultured on various SD selective media, including SD/-Trp and SD/-Trp/-His/-Ade/x-α-Gal, and incubated at 30 °C for 3–5 days.

### 2.5. Yeast one- and two-hybrid assays

The regions of the *GhBRC1* and cotton *FLOWERING LOCUS T* (*GhFT*) promoters containing *cis* elements were ligated into the vector pLacZi. The cotton *FLOWERING LOCUS C1* (*GhFLC1*), *GhFLC2*, and *GhBRC1* coding regions were cloned into the vector pJG4-5 for yeast one-hybrid assays. The coding sequence of *GhBRC1* was cloned into the bait vector pGBKT7 and/or the prey vector pGADT7 for the yeast two-hybrid assay. The two vectors were then co-transformed into the Ym4271 (one-hybrid) or Y2H Gold (two-hybrid) yeast strain (Clontech). After being incubated on double dropout (DDO) medium at 30 °C for 2–3 days, yeast cells were selected on DDO medium lacking Leu (Ura) and Trp. Colonies that grew were further selected on quadruple dropout (QDO) media lacking Leu (Ura), Trp, His and Ade, and x-gal/x-α-gal was used for the colour reaction. Empty vectors were used as controls [23].

### 2.6. Overexpression of GhBRC1 in Arabidopsis and transgenic seed germination under GR24 treatment

The open reading frame of *GhBRC1* was cloned into the vector pK7WG2.0 and then transformed into *A. tumefaciens* GV3101.

Transformation of *Arabidopsis* plants was performed using the floral dip method. For selection, seeds were planted in aseptic conditions on MS agar containing 25 mg $L^{-1}$ hygromycin. Selection was performed for three generations. T3 lines displaying 100% hygromycin resistance were considered homozygous and were used for further experiments.

The *Arabidopsis* seeds were sown on MS medium with 0.5, 1, or 3 μmol $L^{-1}$ GR24. Seeds were incubated for 2–3 days at 4 °C in darkness to break dormancy, and then were transferred to the culture room (21 °C). Germination was recorded daily for 6 days. Seeds were scored as germinated when the radicle emerged.

### 2.7. Phytohormone treatments

Cotton seedlings of the fifth true leaves (3798 line) were sprayed with 50 μmol $L^{-1}$ IAA, 50 mg $L^{-1}$ GA3, 50 mg $L^{-1}$ 6-BA, 100 μmol $L^{-1}$ ABA, 3 μmol $L^{-1}$ SL, 50 mg $L^{-1}$ BR or 50 mg $L^{-1}$ JA. Control plants were treated similarly with the solvent that the hormones were dissolved in. Then the third and fourth true leaves of seedlings were harvested at 0 min, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, 24 h, 36 h and 48 h. The treated tissues were quickly frozen in liquid nitrogen and stored at −80 °C until use.

### 2.8. VIGS of GhBRC1 in cotton

VIGS vectors were constructed and *A. tumefaciens*-mediated gene silencing in cotton was performed as previously described [53]. Cotton *Cloroplastos alterados1* (*GhCLA1*) and *GhBRC1* cDNA were PCR-amplified using the primers listed in Table S1. The amplified fragments were cloned into the Tobacco Rattle Virus (TRV) cDNA clones of RNA2 (pTRV2) vectors to construct pTRV2: GhCLA1 and pTRV2:GhBRC1, respectively. These vectors were transformed into *A. tumefaciens* GV3101. The cultures and cells were handled as described earlier. Cultures of bacteria harbouring the TRV cDNA clones of RNA1 (pTRV1) and pTRV2 vectors were mixed in a 1:1 ratio, and the cotyledons of 2-week-old cotton seedlings were infiltrated with a needleless syringe. Small holes were punched on the underside of the cotyledon to facilitate infiltration [54]. The assays were performed with at least six plants for each construct, and the experiments were repeated at least three times.

### 2.9. RNA-seq and differential gene expression analysis

The expression of the *GhBRC1* gene in JX and 3798 was silenced by VIGS, then the stem tips of seedlings (true leaves at day 45) were used to extract total RNA, which was sent to BioMarker (Beijing, China) for library construction and sequencing. The clean data were mapped to the cotton reference genome (*G. hirsutum* TM-1 v2.1) [55] using HISAT2 [56] and assembled using StringTie [57]. RPKM was used to obtain the relative levels of expression [58]. Differential expression analysis was performed using DEseq for comparisons between the experimental group and control group with four biological replicates each [59]. GO and KEGG enrichment analyses were performed in BioMarker iCloud (https://international.biocloud.net/).

## 3. Results

### 3.1. The expression profile of GhBRC1

We first identified the cotton *GhBRC1* gene from RNA-seq analysis of nulliplex-branched (3798) and normal branching (Huazhong-94) Upland cottons (Fig. 1A, B) [60]. Quantitative reverse-transcription PCR (qRT-PCR, primers in Table S1) analysis showed that the expression levels of *GhBRC1* at the seedling, squaring, flowering and wadding stages differed between two lines of upland cotton, 3798 and Huazhong-94. In the results, the expression pattern was similar in both cotton lines, except for the ovule tissues. In the 3798 line, high expression of the *GhBRC1* was detected in axillary buds and the stem apex at the squaring stage, almost no expression was detected at the flowering stage and weak expression was detected at the wadding stage. During seedling development, the expression of *GhBRC1* reached the highest level and then began to decrease (Fig. 1C). In the Huazhong-94 line, *GhBRC1* expression was detected in axillary buds, the stem apex, and ovule tissues at the flowering stage; the expression levels in other tissues were relatively low (Fig. 1C). The ratios of axillary bud/stem apex expression levels of *GhBRC1* were 1.099 and 7.00 in the 3798 and Huazhong-94 lines, respectively.

### 3.2. The cotton GhBRC1 gene inhibits Arabidopsis branching

The cotton *GhBRC1* gene is a member of *TCP* gene family. We analysed the TCP TF family in cotton (*G. hirsutum*) and found 73 TCP family proteins. GhBRC1 has high similarity with AtTCP18 (also known as BRC1). In a phylogenetic tree, GhBRC1 (GhTCP62) and BRC1 clustered on the same branch, indicating that they may have similar functions (Fig. S1A, B). GhBRC1 proteins also had the typical TCP domain (Fig. S1C, D). To determine whether the *GhBRC1* gene has a function similar to that of the *AtBRC1* gene, *GhBRC1* was introduced into *Arabidopsis* wild-type (WT) plants; the *GhBRC1* overexpression (OE) plants had fewer branches than WT and *Arabidopsis brc1* (*Atbrc1*) plants (Fig. 2A, B). A high level of *GhBRC1* expression was also detected in *Arabidopsis* OE plants by semi-quantitative RT-PCR and qRT-PCR (Fig. 2C, D). These results showed that GhBRC1 has the same function as *Arabidopsis* BRC1 in repressing axillary bud development [11].

### 3.3. Overexpression of GhBRC1 in Arabidopsis results in early flowering

We compared the phenotypes of the OE, WT and *brc1* mutant plants, and found that the *GhBRC1* OE plants obviously flowered earlier than the WT and *brc1* plants; the OE plants flowered after an average of 28 days, but WT flowered after an average of 33 days (Fig. 3A, B). Statistical analysis of *Arabidopsis* rosette leaf number showed that the OE lines had significantly fewer rosette leaves compared with the WT, and the number of rosette leaves of the mutants was significantly higher than WT (Fig. 3C–F). Transgenic *GhBRC1* plants were further obtained with *Atbrc1* complemented plants, and results showed that *GhBRC1* rescued the phenotype to be similar to that of the WT (Fig. S2)

### 3.4. Silencing GhBRC1 promotes cotton seedling growth and development

To study the function of the *GhBRC1* gene in cotton, we knocked down expression of *GhBRC1* in cotton using the virus-induced gene silencing (VIGS) method. The results showed that the growth rate and height of silenced plants were significantly higher than those of the control plants (Fig. 4A, B); the expression level of the *GhBRC1* gene was obviously lower than that in the control (Fig. 4C). In *Arabidopsis*, BRC1 interacts with FT to repress the floral transition [23]. Therefore, we also silenced the cotton *GhFT* gene. *GhFT*-silenced plants showed a phenotype opposite to that of *GhBRC1*-silenced plants; they were significantly shorter than control plants (Fig. 4D–F). This result implied that GhBRC1 and GhFT may be involved in growth.

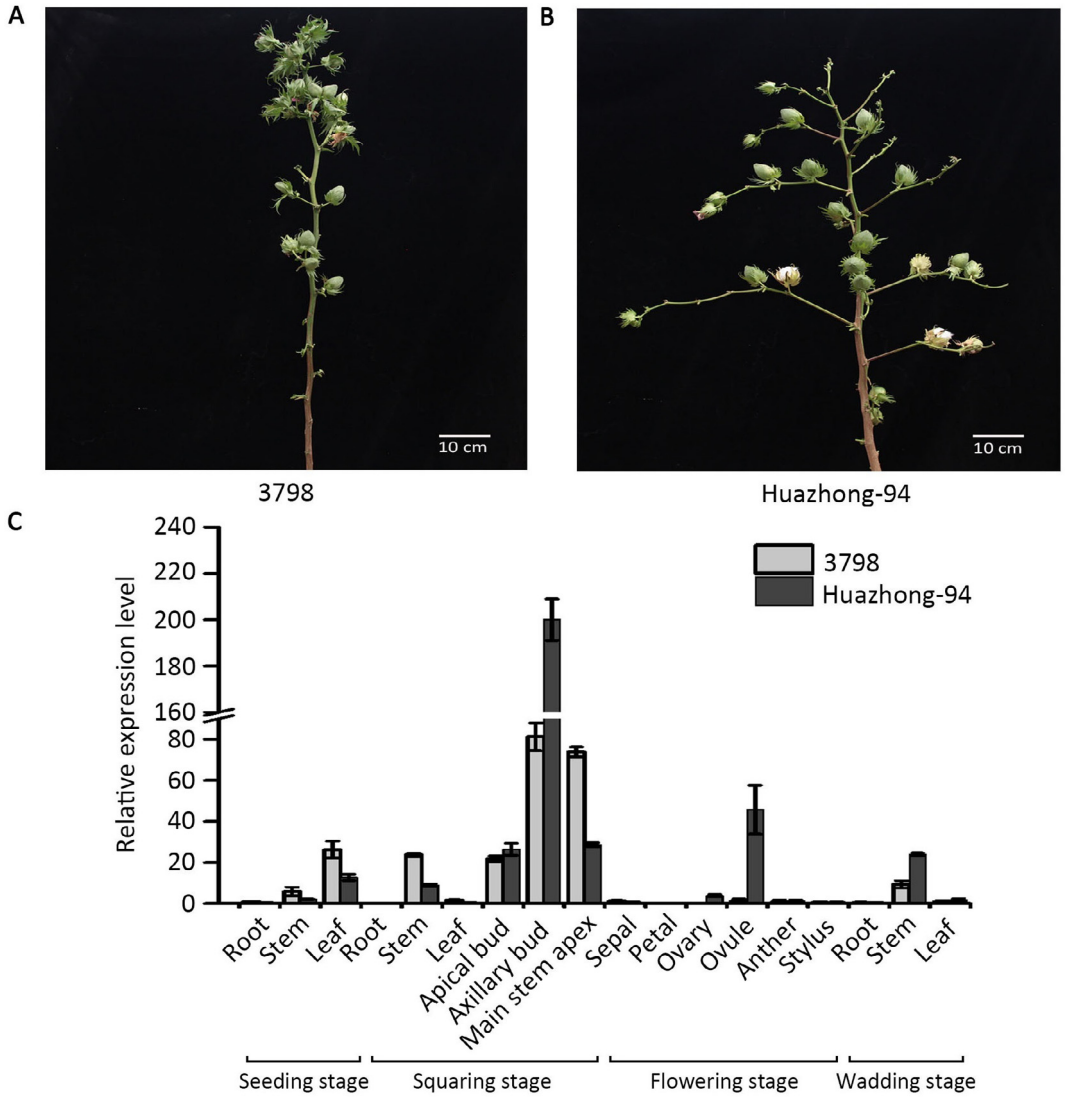


**Fig. 1.** Plant appearance and the expression profile of *GhBRC1* determined by qRT-PCR. (A) Nulliplex-branch 3798 and (B) normal-branching Huazhong-94. (C) Expression patterns of the *GhBRC1* gene in 3798 and Huazhong-94 determined by qRT-PCR analysis. Tissues were collected at the seedling, squaring, flowering, and wadding stages. The *GhUBQ7* gene was used as the endogenous reference gene. Values are means ± SD of three technical replicates.

### 3.5. GhBRC1 has characteristics typical of TCP family transcription factors

To determine whether GhBRC1 has characteristics typical of TFs, the GhBRC1-GFP fusion protein was transiently expressed in tobacco leaves. Analysis of fluorescence showed that GhBRC1-GFP was only localized in the nucleus, consistent with its putative function as a TF (Fig. 5A, B). Further analysis revealed that the transcriptional activation domain of the GhBRC1 protein was mainly located in protein region 1–162; this region was sufficient to activate hydrolysis of x-α-gal and production of blue plaques in a transcriptional activation assay (Fig. 5C, D).

### 3.6. GhBRC1 may be regulated by FLC proteins

According to the above results, GhBRC1 may function in promoting flowering in *Arabidopsis*, so we further analysed the interaction between GhBRC1 and multiple flowering-related proteins. The results of yeast two-hybrid assays showed that GhBRC1 cannot interact with the cotton homologs of the SHORT VEGETATIVE PHASE (SVP), SQUAMOSA promoter binding protein-like 9a (SPL9a), SPL9b, DWARF53 (D53), FLOWERING LOCUS C1 (FLC1), FT, HOMEOBOX protein 12 (HB12), MOTHER OF FT AND TFL-like1 (MFT-L1), MFT-L2, Nulliplex-branch (NB), TERMINAL FLOWER-like 1 (TFL-L1), TFL-L2, and Brother of FT and TFL1 (BFT) proteins (Fig. S3). Further analysis of the promoter regions of the *GhFT* and *GhBRC1* genes revealed that these promoters contained multiple *cis*-acting elements recognized by FLC, and there were two TCP binding sites in the promoter of the *FT* gene (Fig. 6A). The yeast one-hybrid experiment was used to determine if *GhBRC1* is regulated by cotton FLC proteins. The results showed that the two cotton homologs of FLC, GhFLC1 and GhFLC2, can interact with several predicted FLC binding sites (Fig. 6B). The two FLC proteins can also bind to the *GhFT* promoter, but they bind to slightly different binding sites. FLC1 can bind to the predicted motifs 1, 2, and 5, while FLC2 can bind to motifs 1, 4, and 5. The yeast one-hybrid experiment also showed that GhBRC1 can bind to motifs 6 and 7 of the *GhFT* promoter (Fig. 6C). These results indicated that GhBRC1 cannot directly interact with the above-mentioned flowering-related proteins to regulate flowering, but that FLC may regulate the

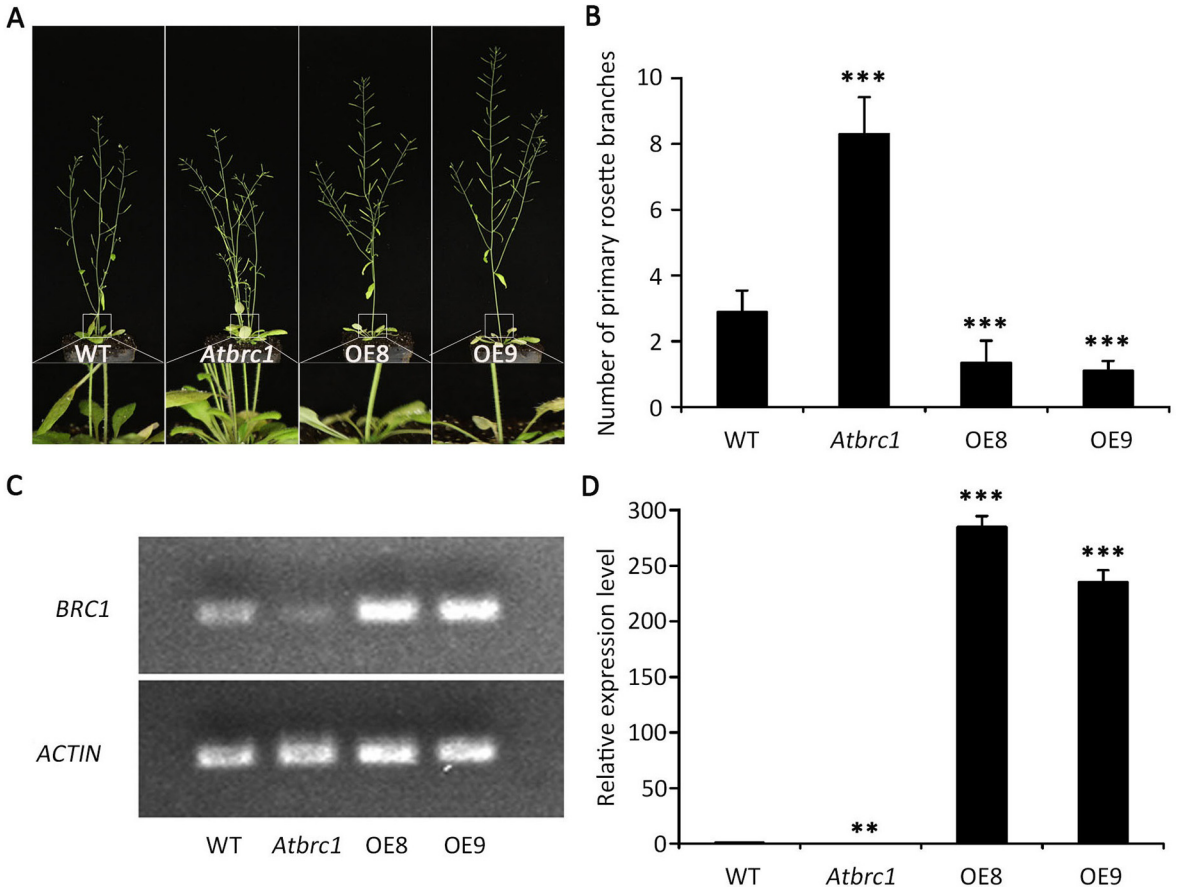


**Fig. 2.** The cotton *GhBRC1* gene inhibits *Arabidopsis* branch development. (A) Representative images showing primary rosette branching phenotypes and (B) analysis of the number of primary rosette branches of wild-type Col-0 (WT), *Atbrc1* and two independent transgenic p35S::GhBRC1 overexpression lines (OE8 and OE9). Scale bars, 1 cm. Values are means ± SD ($n$ = 3 biological replicates of 20 plants per genotype); Student's $t$-test: **, $P < 0.01$, ***, $P < 0.001$. (C) RT-PCR and (D) qRT-PCR analysis of *GhBRC1* expression in WT, *Atbrc1*, OE8, and OE9. *ACTIN2* was used as the reference gene. Values are means ± SD of three technical replicates.

expression of *GhBRC1*, and GhBRC1 may participate in the regulation of the flowering pathway by regulating the expression of the *GhFT* gene.

### 3.7. GR24 involved in GhBRC1 regulation of plant development

Multiple studies have shown that the effects of BRC1 on plant growth, flowering, and branching may be closely related to SL [29,61]. In this study, we found that the germination rates of WT, *brc1* mutant and *GhBRC1* transgenic *Arabidopsis* plants were not significantly different at lower concentrations of GR24 (a synthetic SL analogue) (Fig. 7A, B). When the GR24 concentration was 1 μmol $L^{-1}$, the germination of *Arabidopsis* seeds was obviously delayed by about one day (Fig. 7A, B). With increased SL concentrations *Arabidopsis* seeds seldom germinated until day 6 (Fig. 7A, B).

By observing the early stage of germination, we found that the development of hypocotyls and roots was inhibited by GR24, and the inhibitory effect on hypocotyls and roots was more obvious with higher concentrations of GR24. However, regardless of GR24 application, overexpression of *GhBRC1* promoted hypocotyl growth and inhibited root elongation (Fig. 7C–F).

### 3.8. GhBRC1 responds to multiple hormones

When hormones, namely GR24, abscisic acid (ABA), brassinolide (BR), IAA, 6-benzylamino-purine (6-BA), Gibberellin A3 (GA3) and jasmonic acid (JA), were sprayed on cotton seedlings, we found that all except ABA significantly induced the expression of *GhBRC1* between 2 and 8 h after spraying; expression levels then gradually decreased to normal levels (Fig. 8A–G). The *GhBRC1* expression level in BR-, IAA-, 6BA-, GA3- and JA-treated plants sharply decreased at 30 min (Fig. 8B–D, F and G), and at 5 min the *GhBRC1* expression level in GR24-, IAA-, ABA- and GA3-treated plants obviously increased (Fig. 8A, B, D, and E). These results indicate that GhBRC1 is responsive to multiple hormones (Fig. 8A–F).

### 3.9. Silencing of GhBRC1 affects genes involved in plant hormone signal transduction pathways

To further identify the downstream genes regulated by GhBRC1, we performed high-throughput sequencing of the shoot apexes of the 3798 (nulliplex-branch) and JX (has shorter fruiting branches than cultivars with normal fruiting branches) lines 45 days after silencing of *GhBRC1* by VIGS. The results revealed 3519 differentially expressed genes (DEGs) between the 3798 treatment group and the 3798 control group (2091 up-regulated genes and 1428 down-regulated genes) (Table S2), while only 17 differentially expressed genes (9 up-regulated and 8 down-regulated) were identified between the JX treatment group and the JX control group (Fig. 9A; Table S3).

Functional enrichment analysis of the DEGs in *GhBRC1*-silenced 3798 revealed that genes involved in DNA replication, homologous recombination, mismatch repair, and plant hormone signal transduction pathways were highly enriched (Fig. 9C; Table S4). In particular, 66 genes were annotated to 8 plant hormone signal transduction pathways (Table S5), mainly the signalling pathways

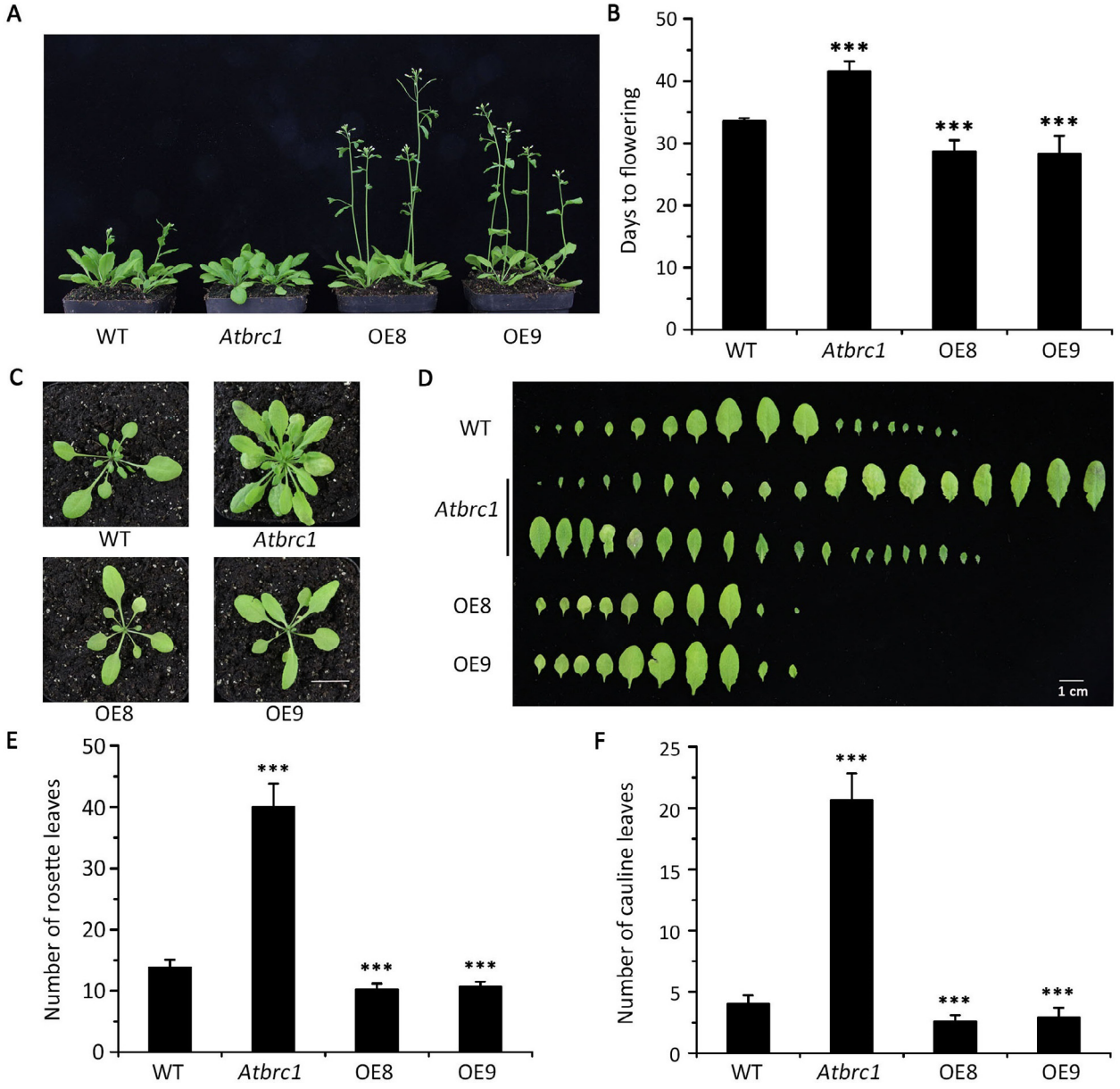


**Fig. 3.** Ectopic expression of *GhBRC1* affects phase change in *Arabidopsis*. (A) Flowering time and number of cauline and rosette leaves of wild-type Col-0 (WT), *Atbrc1*, and two independent transgenic 35S::GhBRC1 overexpression lines (OE8 and OE9) under long-day conditions (16 h light/8 h dark). (B) Quantitative analysis of days to flowering (based on the opening of the first flower) of WT, *Atbrc1*, OE8, and OE9. (C) Representative images of the flowering phenotype. (D) Representative rosette leaf phenotypes. (E) Quantitative analysis of the number of rosette leaves present at flowering, and (F) the number of cauline leaves present in WT, *Atbrc1*, OE8, and OE9. Values are means ± SD ($n$ = 3 biological replicates of 20 plants per genotype); Student's $t$-test, ***, $P < 0.001$.

of auxin, ABA, GA, and BR (Fig. 9D; Table S5). In addition, two SL esterase D14-like genes, Gh_A05G016800 and Gh_D05G022800, were down-regulated in *GhBRC1*-silenced 3798 [43]; this is consistent with the close association between the signal transduction pathways of SL and other plant hormones.

There are only five genes in the intersection between the 3798 (control: experiment, c:e) comparison group and the JX (c:e) comparison group, namely Gh_D01G158300 (Non-specific lipid-transfer protein 1), Gh_D08G057000 (unknown), Gh_A04G054200 (Jade1), Gh_A13G172900 (9-*cis*-epoxycarotenoid dioxygenase NCED3), and Gh_A11G065800 (seed biotin-containing protein SBP65) (Fig. 9B). However, there have been no reports of these genes being associated with BRC1 and branch development. The huge difference in the number of differentially expressed genes between the 3798 (c:e) and JX (c:e) comparison groups may be related to the traits of these two lines; the molecular mechanism underlying this difference needs to be further studied.

## 4. Discussion

Cotton architecture is closely associated with cotton yield and harvesting efficiency. In *Arabidopsis*, rice, wheat and maize, BRC1 (TB1) regulates branch development, and because branch morphology determines yield, this trait is closely related to economic value [7,14,62,63]. Cotton *GhBRC1* is homologous to *BRC1*, but studies of its function have not been reported.

In this study, we compared the phenotypes of transgenic *Arabidopsis GhBRC1* OE plants with those of *Atbrc1* mutant and WT, and found that OE plants produced significantly fewer branches than WT plants (Fig. 1), indicating that GhBRC1 can inhibit

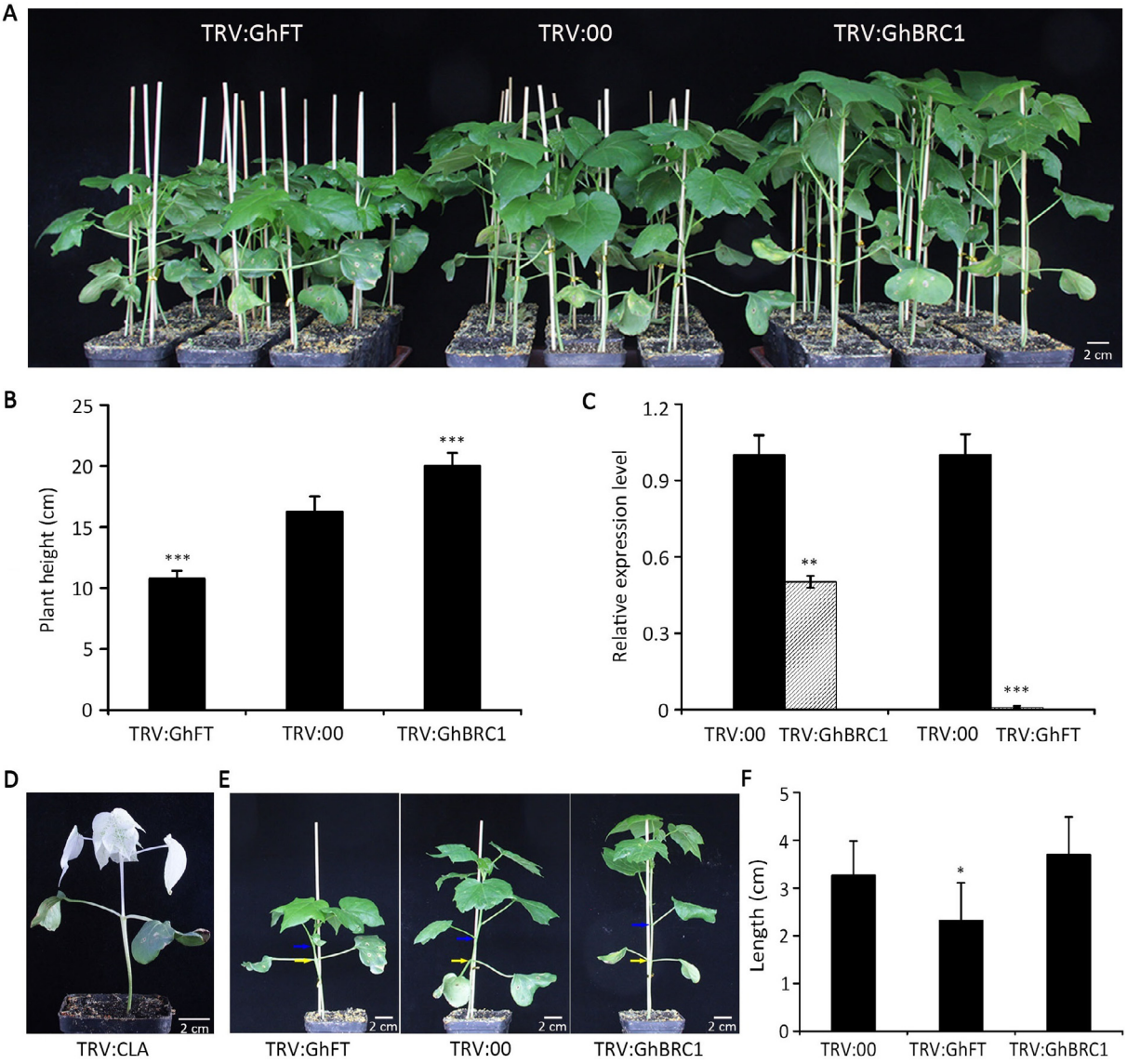


**Fig. 4.** Silencing of *GhBRC1* by VIGS can promote cotton seedling growth and development. (A) The phenotype of upland cotton line 3798 at the seedling stage after silencing of *GhBRC1* using VIGS technology. (B) Quantitative analysis of the heights of the indicated plants. (C) Transcript levels of *GhBRC1* and *GhFT* in WT 3798 and *GhBRC1*-silenced leaves. (D) The phenotype after silencing of the *CLA* control using VIGS technology. (E) Images and (F) quantitative analysis of node-internode length in WT and *GhBRC1*-silenced plants. Values are means ± SD ($n$ = 3 biological replicates of 20 plants per genotype); Student's $t$-test, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

*Arabidopsis* branch production and that its function is similar to that of *Arabidopsis* BRC1 and rice TB1 [23,64].

Our study indicated that GhBRC1 is a nuclear localized protein (Fig. 3A), similar to the BRC1 (*Arabidopsis*) and TB1 (maize) proteins, indicating that GhBRC1 is a TF and may play a conserved role in branching regulation. Yeast two-hybrid assays further demonstrated that GhBRC1 has a self-activation domain, which is a typical characteristic of TFs (Fig. 5D). In *Arabidopsis*, BRC1 interacts with FT to repress the floral transition [23]. However, *Arabidopsis* plants overexpressing *GhBRC1* exhibited early flowering, which is different from the effects of *Arabidopsis* BRC1 overexpression [23]. At the same time, overexpression and the rescued plant of *GhBRC1* obviously inhibited the production of rosette leaves. These results show that cotton GhBRC1 can inhibit *Arabidopsis* vegetative development and promote the transition from the vegetative to the flowering phase.

In *Arabidopsis*, *BRC1* is mainly expressed in leaves, leaf bases, inflorescences and flowers, and especially in the shoot apical meristem [11]. In our study, *GhBRC1* was mainly expressed in leaves and meristems (such as apical buds, axillary buds, and the main stem apex). The expression profile of *GhBRC1* is highly similar to that of *Arabidopsis BRC1*, which further indicates that GhBRC1 may share a conserved function with *Arabidopsis* BRC1. As previously mentioned, GhBRC1 may interact with GhFT and regulate flowering development and transition [23]. In this study, a nulliplex-branch line, 3798, and a line with normal fruiting branches, Huazhong-94, were used to determine the expression levels of *GhBRC1*. The nulliplex-branch phenotype may be regulated by ATC/GhSP (*A. thaliana* CENTRORADIALIS homolog), which is a major factor involved in inhibiting flowering [65–68]. In addition, the expression pattern of *GhBRC1* in axillary buds and stem apices in the 3798 and Huazhong-94 lines are obviously different, indicating that *GhBRC1* may be an upstream regulatory factor in the ATC/GhSP pathway.

In *GhBRC1*-silenced cotton lines, the seedlings were significantly taller than those of the control group, indicating that GhBRC1 inhibits plant growth. In wheat, TB1 has been shown to regulate height; *TB1* is expressed at a low level in the nodes of the main

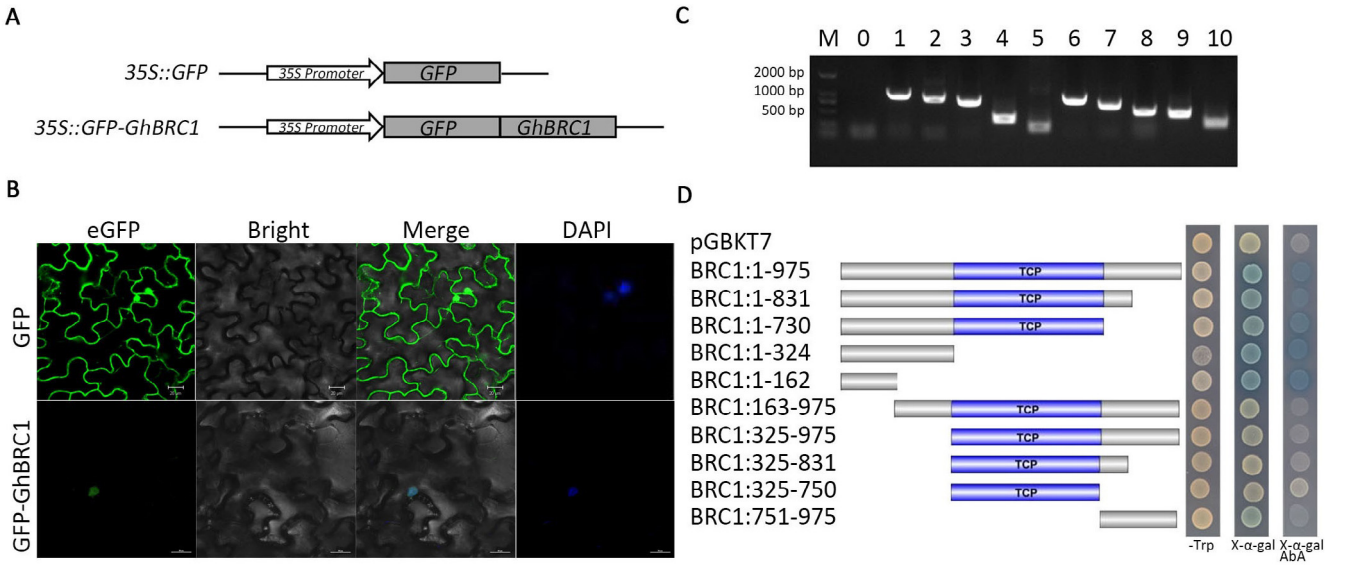


**Fig. 5.** Analysis of the subcellular localization and transcriptional activation activity of the GhBRC1 protein. (A) Schematic diagram of p35S-GFP::GhBRC1. Drawing is not to scale. (B) Subcellular localization of the GhBRC1-GFP protein in tobacco leaf epidermal cells. (C) PCR electrophoresis assay of each truncated fragment of the *GBRC1* gene. (D) Schematic diagram of each fragment of GhBRC1 tested and the results of yeast x-α-gal and Aureobasidin A (AbA) staining, which indicate transcriptional activation activity of the GhBRC1 protein.

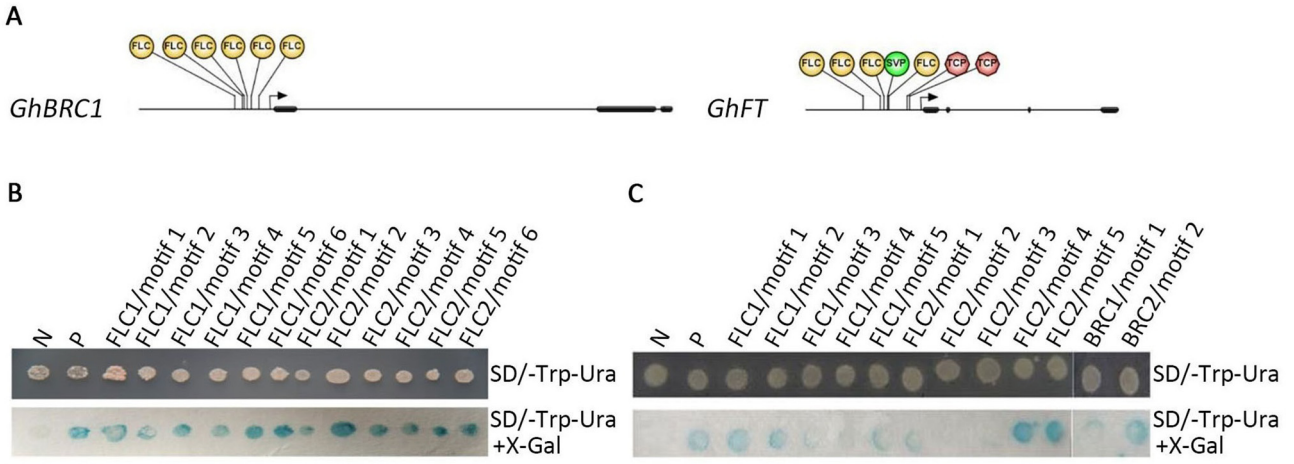


**Fig. 6.** Yeast one-hybrid and yeast two-hybrid assays. (A) Gene structure diagram of *GhBRC1* and *GhFT*. (B) The interaction of GhFLC1 and GhFLC2 with the *cis*-elements in the promoter of *GhBRC1*. (C) The interaction of GhFLC1, GhFLC2 and GhBRC1 with *cis*-elements in the promoter of *GhFT*. Yeast cells were cultured on SD/-Trp -Leu medium and SD/-Trp -Leu -His -Ade -x-gal medium.

culm prior to elongation, and increased levels of TB1 restrict the elongation of stem internodes [62]. Our results are consistent with the function of TB1 in wheat. However, *GhFT*-silenced cotton lines had significantly shorter seedlings than the control group, indicating that GhFT has the opposite function of GhBRC1: promoting plant growth. The opposite phenotypes of the *GhFT*- and *GhBRC1*-silenced lines also demonstrate that GhBRC1 and GhFT may inhibit each other in cotton just as BRC1 and FT may neutralize each other by interacting in *Arabidopsis* [23,27]. Many studies have shown that the regulation of *BRC1* and *FT* expression is closely associated with plant hormones, which may also be the reason for the significant change in plant growth after these two genes are silenced [2,7,27,32,69,70]. In this study, we also analysed the interaction of GhBRC1 with cotton homologs of multiple flowering-related proteins, but no interactions were observed in yeast two-hybrid assays. However, the results of yeast one-hybrid assays show that *GhBRC1* may be regulated by GhFLC proteins, and that GhBRC1 may also regulate *GhFT* expression. These results may explain why GhBRC1 can decrease plant height in cotton and promote flowering in *Arabidopsis*.

Transcriptome sequencing of *GhBRC1*-silenced lines of 3798 and JX revealed that the number of DEGs between the silenced lines and the controls was significantly different between the two lines. There were significantly more differentially expressed genes in 3798 than in JX. As mentioned above, 3798 is a nulliplex-branch line, in which most of the flowers arise directly from the leaf axils on the main shoot, and these individuals typically do not have a fruiting branch. However, JX has shorter fruiting branches than cultivars with normal fruiting branches. The nulliplex-branch phenotype of 3798 may be caused by the inhibition of axillary bud growth [60,65]. According to previous reports, BRC1 functions in inhibiting the growth of axillary buds [69]; silencing of *GhBRC1* in 3798 may have a stronger inhibitory effect on the growth of fruiting branches or axillary buds than in JX [5,23,69,71].

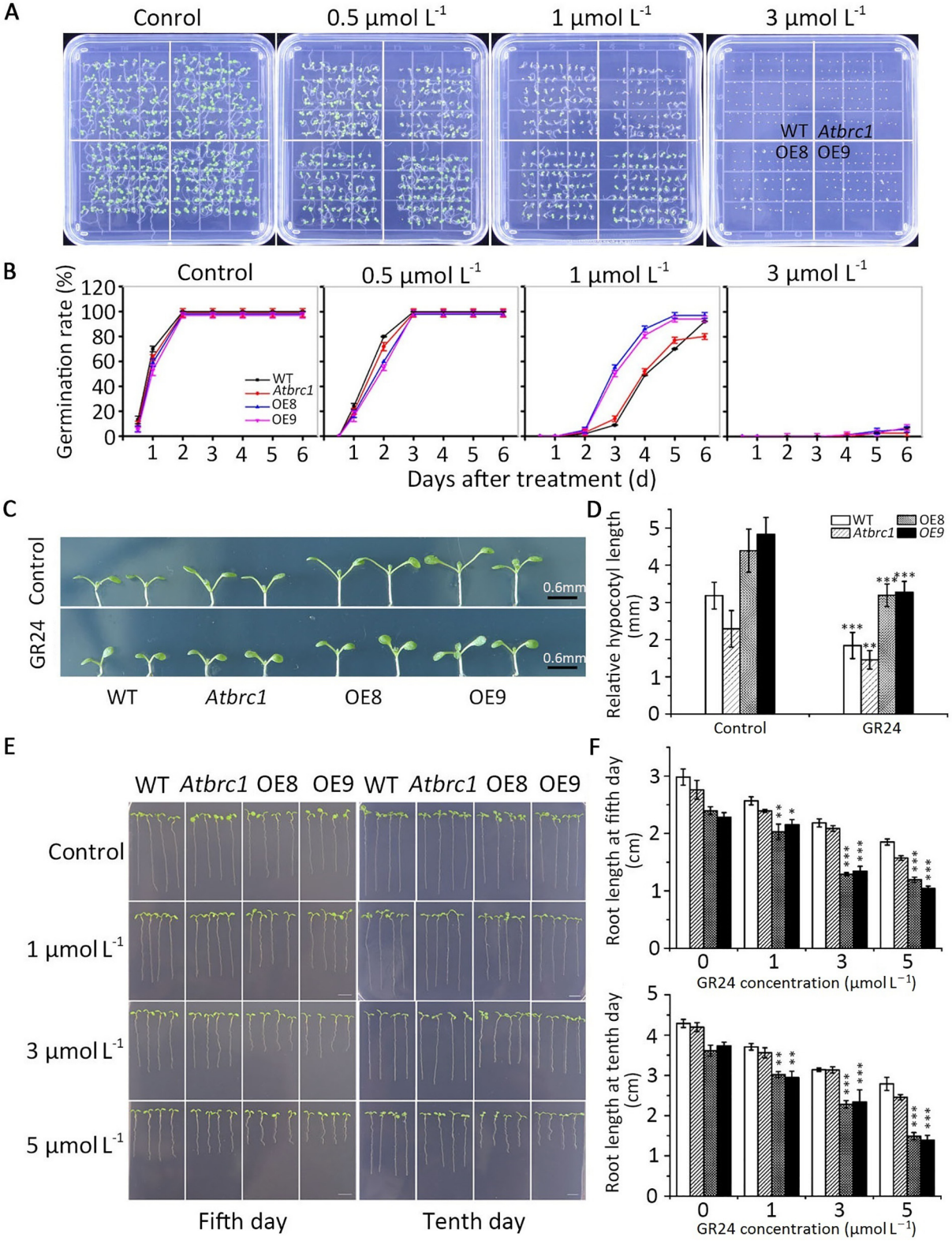


**Fig. 7.** Regulation of SL-mediated plant development by GhBRC1. (A) Representative images of seed germination at different concentrations of GR24 and (B) quantitative analysis of germination rate. (C) Representative images of hypocotyls of seedlings treated with 1 μmol $L^{-1}$ GR24 and (D) relative hypocotyl lengths of the indicated seedlings. Scale bars, 5 mm. (E) Representative images of roots of seedlings treated with different concentrations of GR24 and (F) quantitative analysis of root length. Images were taken 5 days and 10 days after growth on vertical plates. Bar, 6 mm. Root growth was monitored and analysed using Image J software. Values are means ± SD ($n$ = 3 biological replicates of 20 plants per genotype); Student's $t$-test, *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

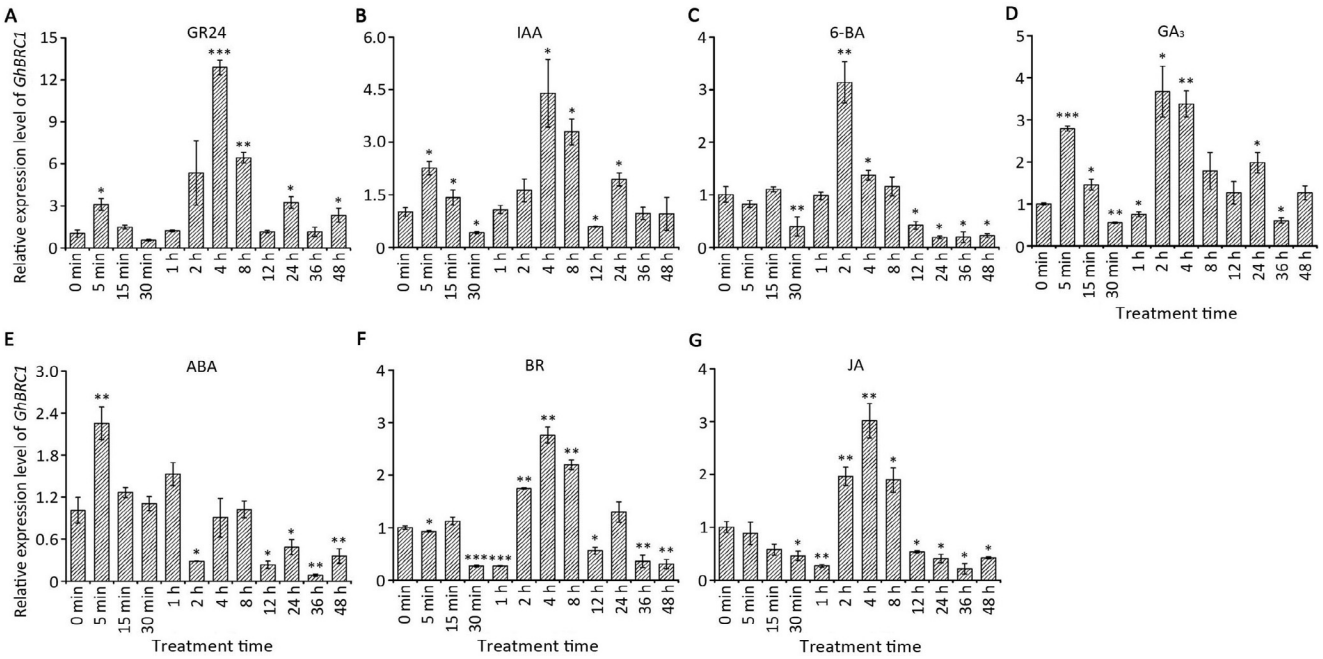


**Fig. 8.** *GhBRC1* responds to multiple hormones. Expression levels of *GhBRC1* gene in response to plant hormones after treated the fifth true leaf seedings. *ACTIN2* was used as the reference gene. Values are means ± SD of three technical replicates. Student's *t*-test: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Unfortunately, the duration of VIGS was not long enough, and we did not observe significant phenotypic changes at the seedling stage. Silencing of *GhBRC1* in 3798 and JX resulted in a decrease in the expression level of the BRC1 downstream gene NCED3, which may lead to a decrease in ABA and trigger a hormone response, thus promoting bud development [5].

KEGG enrichment analysis of differentially expressed genes in *GhBRC1*-silenced 3798 seedlings revealed enrichment in the plant hormone pathway, which is consistent with the notion that the effects of *GhBRC1* on axillary bud growth and plant development are mainly related to the regulation of plant hormones; these results are consistent with the results of a previous experiment in which VIGS of *GhBRC1* resulted in longer seedlings [2,5,7,11,24,27,32,69,71–75].

Based on the above results, we speculate that *GhBRC1* may be regulated by multiple plant hormones in cotton. In *Arabidopsis* axillary buds, *BRC1* regulates NCED3 expression and ABA levels; increased expression of NCED3 leads to ABA accumulation and triggers a hormone response [5]. Indeed, ABA accumulation is required for suppression of bud outgrowth in plants, and ABA functions downstream of BRC1 to control axillary bud dormancy [5,71]. This may explain why *BRC1* expression did not increase significantly after ABA treatment, but gradually decreased after 12 h of treatment. The up-regulation of *BRC1* at 5 min may be due to feedback regulation.

Plant hormones auxin and SL can indirectly promote *BRC1* expression [11,29,32,36,40]. However, auxin regulates *BRC1* by controlling two antagonistic factors, CK and SL [27]. After treatment with GR24 and IAA, the expression level of *GhBRC1* significantly increased after 4 to 8 h. We found that applying 1 μmol $L^{-1}$ GR24 to GhBRC1 transgenic *Arabidopsis* seeds significantly promoted germination; GR24 also promoted the growth of GhBRC1 OE seedlings. These results are consistent with the finding that *FC1* (a *BRC1* homolog in rice) works downstream of SLs in rice [28]. High CK levels in axillary buds result in downregulation of *BRC1* expression in pea [29]. In our study, *GhBRC1* was rapidly downregulated at 30 min and at 8 h, and was only upregulated at 2 h after being treated with 6BA. CK acts as a repressor, with downregulated expression according to a previous report [2], but the mechanism of upregulation at 2 h after treatment is still unexplained.

After treatment with ABA, the expression levels were not obviously changed after 8 h excepting a transitory upregulation at 5 min. This result was consistent with Gonzalez-Grandio and Cubas's study supporting a model in which ABA acts downstream of BRC1 [69]. In addition, the change in *GhBRC1* expression in cotton treated with BR or JA is similar to that in plants treated with 6BA or GA3. Ni et al. [76] found that GA negatively influences BRC1 expression in *Jatropha curcas*, but the expression was not obviously downregulated except from 30 min to 1 h in this study. Although there are no reports that BR and JA regulate the expression of the *BRC1* gene, we speculate that BR and JA may participate in regulating the development of plant branches. According to Ferguson and Beveridge [77], this kind of regulation could be involved in various metabolism pathways, such as feedback regulation, long-distance hormone transport and the interplay of plant hormone metabolism and signalling.

## 5. Conclusions

This is the first report of the function of BRC1 in cotton. This work describes detailed expression and functional studies of *GhBRC1*. The results demonstrate that GhBRC1 may have a pivotal role in branch development, flowering, growth, and germination by integrating multiple hormone pathways, including SL and other hormone pathways. The study further showed that the functions of GhBRC1 partly differ from those of *Arabidopsis* BRC1. Further research is needed to elucidate the gene network of *GhBRC1* in cotton and to clarify its roles in the control of branching and hormone response in this economically important crop plant.

## Data availability

All the RNA-seq and small RNA raw data and processed data used in this study have been deposited at NCBI Gene Expression Omnibus under accession number GSE142638.

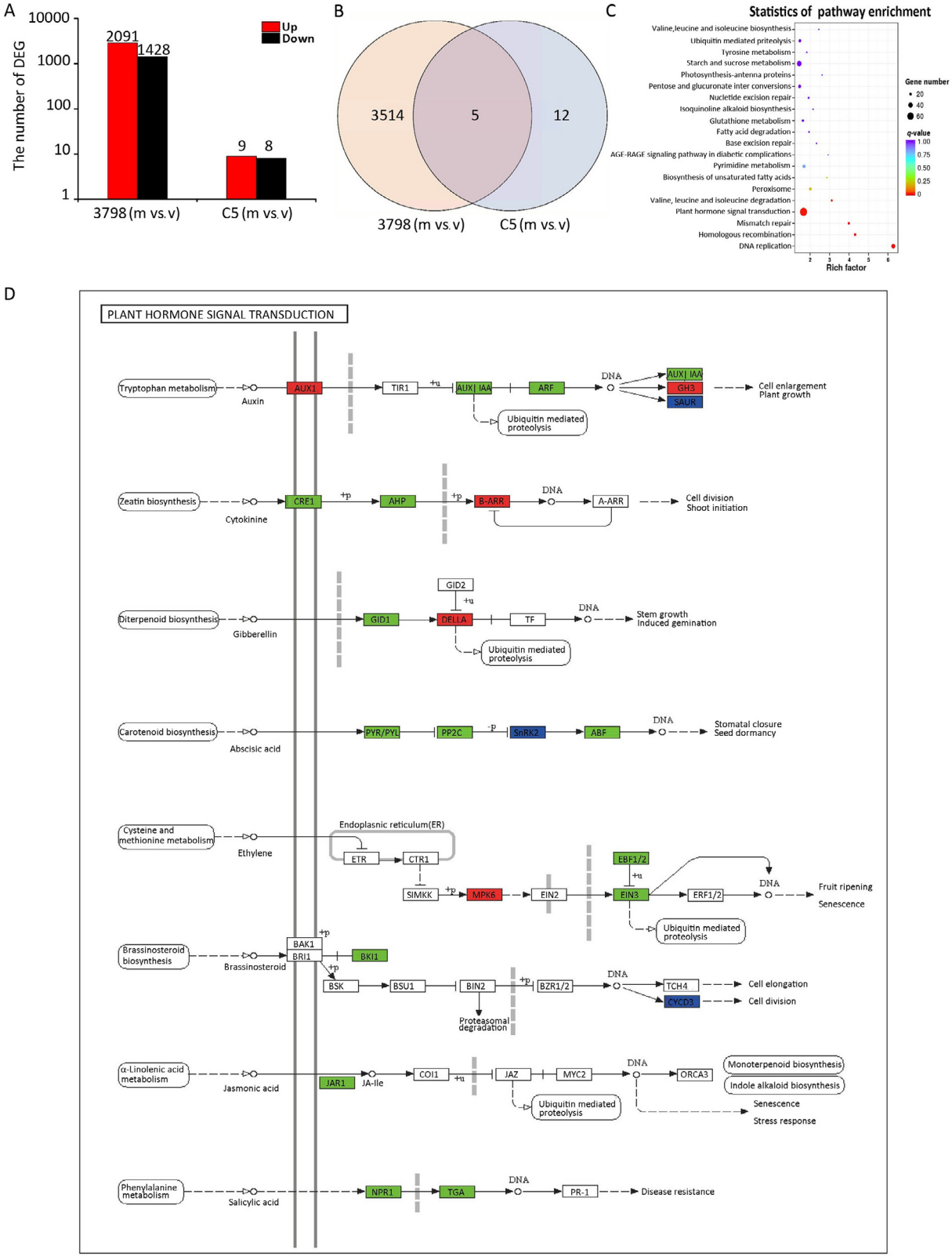


**Fig. 9.** Functional enrichment analysis of genes differentially expressed in *GhBRC1*-silenced lines. (A) The number of differentially expressed genes identified between *GhBRC1*-silenced and control of 3798 and JX lines. (B) Venn diagram showing the overlap in differentially expressed genes for the two comparison groups. 3798 (c:e) indicates the differentially expressed genes between the control (c) and experimental (e) groups for variety 3798; JX (c:e) indicates the differentially expressed genes between the control and experimental groups for variety JX. The number represents the number of differentially expressed genes. (C) Kyoto Encyclopedia of Genes and Genomes (KEGG) functional enrichment analysis of genes differentially expressed between *GhBRC1*-silenced and control 3789 lines. (D) Enrichment of the differentially expressed genes in *GhBRC1*-silenced 3798 in plant hormone signalling pathways.

## CRediT authorship contribution statement

**Quan Sun:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Yuanhui Xie:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization. **Huiming Li:** Investigation, Methodology. **Jinlei Liu:** Investigation, Methodology. **Rui Geng:** Investigation, Methodology. **Ping Wang:** Investigation, Methodology. **Zongyan Chu:** Investigation, Methodology. **Ying Chang:** Investigation, Methodology. **Guanjun Li:** Investigation, Methodology. **Xiao Zhang:** Conceptualization. **Yingfan Cai:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing - original draft, Writing - review & editing. **Youlu Yuan:** Conceptualization, Resources.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (U1704104) and the National Key Research and Development Program of China (2018YFD0100304, 2016YFD0101902).

## Appendix A. Supplementary data

Supplementary data for this article can be found online at https://doi.org/10.1016/j.cj.2021.01.007.

## References

[1] B. Wang, S.M. Smith, J. Li, Genetic regulation of shoot architecture, Annu. Rev. Plant Biol. 69 (2018) 437–468.
[2] T. Teichmann, M. Muhr, Shaping plant architecture, Front Plant Sci. 6 (2015) 233.
[3] Z.W.N. Teo, S. Song, Y.Q. Wang, J. Liu, H. Yu, New insights into the regulation of inflorescence architecture, Trends Plant Sci. 19 (2014) 158–165.
[4] P. McSteen, O. Leyser, Shoot branching, Annu. Rev. Plant Biol. 56 (2005) 353–374.
[5] E. González-Grandío, A. Pajoro, J.M. Franco-Zorrilla, C. Tarancón, R.G.H. Immink, P. Cubas, Abscisic acid signaling is controlled by a *BRANCHED1/HD-ZIP I* cascade in *Arabidopsis* axillary buds, Proc. Natl. Acad. Sci. U. S. A. 114 (2017) E245–E254.
[6] A. Corot, H. Roman, O. Douillet, H. Autret, M.D. Perez-Garcia, S. Citerne, J. Bertheloot, S. Sakr, N. Leduc, S. Demotes-Mainard, Cytokinins and abscisic acid act antagonistically in the regulation of the bud outgrowth pattern by light intensity, Front Plant Sci. 8 (2017) 1724.
[7] M. Wang, M.A. Le Moigne, J. Bertheloot, L. Crespel, M.D. Perez-Garcia, L. Oge, S. Demotes-Mainard, L. Hamama, J.M. Daviere, S. Sakr, BRANCHED1: a key hub of shoot branching, Front Plant Sci. 10 (2019) 76.
[8] F.F. Barbier, E.A. Dun, S.C. Kerr, T.G. Chabikwa, C.A. Beveridge, An update on the signals controlling shoot branching, Trends Plant Sci. 24 (2019) 220–236.
[9] A. Vayssières, P. Mishra, A. Roggen, U. Neumann, K. Ljung, M.C. Albani, Vernalization shapes shoot architecture and ensures the maintenance of dormant buds in the perennial *Arabis alpina*, New Phytol. 227 (2020) 99–115.
[10] Q. Sun, J. Qiao, S. Zhang, S. He, Y. Shi, Y. Yuan, X. Zhang, Y. Cai, Changes in DNA methylation assessed by genomic bisulfite sequencing suggest a role for DNA methylation in cotton fruiting branch development, PeerJ. 6 (2018).
[11] J.A. Aguilar-Martínez, C. Poza-Carrión, P. Cubas, *Arabidopsis BRANCHED1* acts as an integrator of branching signals within axillary buds, Plant Cell 19 (2007) 458–472.
[12] E.A. Dun, J. Hanan, C.A. Beveridge, Computational modeling and molecular physiology experiments reveal new insights into shoot branching in pea, Plant Cell 21 (2009) 3459–3472.
[13] C.A. Beveridge, J. Kyozuka, New genes in the strigolactone-related shoot branching pathway, Curr. Opin. Plant Biol. 13 (2010) 34–39.
[14] L.E. Dixon, J.R. Greenwood, S. Bencivenga, P. Zhang, J. Cockram, G. Mellers, K. Ramm, C. Cavanagh, S.M. Swain, S.A. Boden, *TEOSINTE BRANCHED1* regulates inflorescence architecture and development in bread wheat (*Triticum aestivum*), Plant Cell 30 (2018) 563–581.
[15] M. Kieffer, V. Master, R. Waites, B. Davies, TCP14 and TCP15 affect internode length and leaf shape in Arabidopsis, Plant J. 68 (2011) 147–158.
[16] S. Danisman, TCP transcription factors at the interface between environmental challenges and the plant's growth responses, Front Plant Sci. 7 (2016) 1930.
[17] O. Navaud, P. Dabos, E. Carnus, D. Tremousaygue, C. Hervé, TCP transcription factors predate the emergence of land plants, J. Mol. Evol. 65 (2007) 23–33.
[18] L. Chen, Y.Q. Chen, A.M. Ding, H. Chen, F. Xia, W.F. Wang, Y.H. Sun, Genome-wide analysis of TCP family in tobacco, Genet. Mol. Res. 15 (2016) gmr7728.
[19] W. Li, D.D. Li, L.H. Han, M. Tao, Q.Q. Hu, W.Y. Wu, J.B. Zhang, X.B. Li, G.Q. Huang, Genome-wide identification and characterization of TCP transcription factor genes in upland cotton (*Gossypium hirsutum*), Sci. Rep. 7 (2017) 10118.
[20] K. Zheng, Z. Ni, Y. Qu, Y. Cai, Z. Yang, G. Sun, Q. Chen, Genome-wide identification and expression analyses of TCP transcription factor genes in *Gossypium barbadense*, Sci. Rep. 8 (2018) 14526.
[21] J. He, X. He, P. Chang, H. Jiang, D. Gong, Q. Sun, Genome-wide identification and characterization of TCP family genes in *Brassica juncea* var. *tumida*, PeerJ 8 (2020) e9130.
[22] M.M. Liu, M.M. Wang, J. Yang, J. Wen, P.C. Guo, Y.W. Wu, Y.Z. Ke, P.F. Li, J.N. Li, H. Du, Evolutionary and comparative expression analyses of tcp transcription factor gene family in land plants, Int. J. Mol. Sci. 20 (2019) 3591.
[23] M. Niwa, Y. Daimon, K. Kurotani, A. Higo, J.L. Pruneda-Paz, G. Breton, N. Mitsuda, S.A. Kay, M. Ohme-Takagi, M. Endo, T. Araki, BRANCHED1 interacts with *FLOWERING LOCUS T* to repress the floral transition of the axillary meristems in *Arabidopsis*, Plant Cell 25 (2013) 1228–1242.
[24] M. Muhr, N. Prüfer, M. Paulat, T. Teichmann, Knockdown of strigolactone biosynthesis genes in *Populus* affects *BRANCHED1* expression and shoot architecture, New Phytol. 212 (2016) 613–626.
[25] E. Stes, S. Depuydt, A. De Keyser, C. Matthys, K. Audenaert, K. Yoneyama, S. Werbrouck, S. Goormachtig, D. Vereecke, Strigolactones as an auxiliary hormonal defence mechanism against leafy gall syndrome in *Arabidopsis thaliana*, J. Exp. Bot. 66 (2015) 5123–5134.
[26] T. Bennett, Y. Liang, M. Seale, S. Ward, D. Müller, O. Leyser, Strigolactone regulates shoot development through a core signalling pathway, Biol. Open 5 (2016) 1806–1820.
[27] C. Rameau, J. Bertheloot, N. Leduc, B. Andrieu, F. Foucher, S. Sakr, Multiple pathways regulate shoot branching, Front. Plant Sci. 5 (2014) 741.
[28] K. Minakuchi, H. Kameoka, N. Yasuno, M. Umehara, L. Luo, K. Kobayashi, A. Hanada, K. Ueno, T. Asami, S. Yamaguchi, J. Kyozuka, FINE CULM1 (FC1) works downstream of strigolactones to inhibit the outgrowth of axillary buds in rice, Plant Cell Physiol. 51 (2010) 1127–1135.
[29] N. Braun, A. de Saint Germain, J.P. Pillot, S. Boutet-Mercey, M. Dalmais, I. Antoniadi, X. Li, A. Maia-Grondard, C. Le Signor, N. Bouteiller, D.a. Luo, A. Bendahmane, C. Turnbull, C. Rameau, The pea TCP transcription factor PsBRC1 acts downstream of strigolactones to control shoot branching, Plant Physiol. 158 (2012) 225–238.
[30] C.A. Helliwell, A.N. Chin-Atkins, I.W. Wilson, R. Chapple, E.S. Dennis, A. Chaudhury, The Arabidopsis *AMP1* gene encodes a putative glutamate carboxypeptidase, Plant Cell 13 (2001) 2115–2125.
[31] T. Waldie, O. Leyser, Cytokinin targets auxin transport to promote shoot branching, Plant Physiol. 177 (2018) 803–818.
[32] E.A. Dun, A. de Saint Germain, C. Rameau, C.A. Beveridge, Antagonistic action of strigolactone and cytokinin in bud outgrowth control, Plant Physiol. 158 (2012) 487–498.
[33] F. Zhou, Q. Lin, L. Zhu, Y. Ren, K. Zhou, N. Shabek, F. Wu, H. Mao, W. Dong, L. Gan, W. Ma, H. Gao, J. Chen, C. Yang, D. Wang, J. Tan, X. Zhang, X. Guo, J. Wang, L. Jiang, X. Liu, W. Chen, J. Chu, C. Yan, K. Ueno, S. Ito, T. Asami, Z. Cheng, J. Wang, C. Lei, H. Zhai, C. Wu, H. Wang, N. Zheng, J. Wan, D14-SCF$^{D3}$-dependent degradation of D53 regulates strigolactone signalling, Nature 504 (2013) 406–410.
[34] L. Jiang, X. Liu, G. Xiong, H. Liu, F. Chen, L. Wang, X. Meng, G. Liu, H. Yu, Y. Yuan, W. Yi, L. Zhao, H. Ma, Y. He, Z. Wu, K. Melcher, Q. Qian, H.E. Xu, Y. Wang, J. Li, DWARF 53 acts as a repressor of strigolactone signalling in rice, Nature 504 (2013) 401–405.
[35] X. Song, Z. Lu, H. Yu, G. Shao, J. Xiong, X. Meng, Y. Jing, G. Liu, G. Xiong, J. Duan, X.F. Yao, C.M. Liu, H. Li, Y. Wang, J. Li, IPA1 functions as a downstream transcription factor repressed by D53 in strigolactone signaling in rice, Cell Res. 27 (2017) 1128–1141.
[36] J. Liu, X. Cheng, P. Liu, J. Sun, miR156-targeted SBP-Box transcription factors interact with DWARF53 to regulate *TEOSINTE BRANCHED1* and *BARREN STALK1* expression in bread wheat, Plant Physiol. 174 (2017) 1931–1948.
[37] J. Wang, H. Yu, G. Xiong, Z. Lu, Y. Jiao, X. Meng, G. Liu, X. Chen, Y. Wang, J. Li, Tissue-specific ubiquitination by IPA1 INTERACTING PROTEIN1 modulates IPA1 protein levels to regulate plant architecture in rice, Plant Cell 29 (2017) 697–707.
[38] T.H. Kebrom, B.L. Burson, S.A. Finlayson, Phytochrome B represses *Teosinte Branched1* expression and induces sorghum axillary bud outgrowth in response to light signals, Plant Physiol. 140 (2006) 1109–1117.
[39] S.A. Finlayson, S.R. Krishnareddy, T.H. Kebrom, J.J. Casal, Phytochrome regulation of branching in *Arabidopsis*, Plant Physiol. 152 (2010) 1914–1927.
[40] O. Lantzouni, C. Klermund, C. Schwechheimer, Largely additive effects of gibberellin and strigolactone on gene expression in *Arabidopsis thaliana* seedlings, Plant J. 92 (2017) 924–938.
[41] N. Leduc, H. Roman, F. Barbier, T. Péron, L. Huché-Thélier, J. Lothier, S. Demotes-Mainard, S. Sakr, Light signaling in bud outgrowth and branching in plants, Plants 3 (2014) 223–250.

[42] T. Girault, F. Abidi, M. Sigogne, S. Pelleschi-Travier, R. Boumaza, S. Sakr, N. Leduc, Sugars are under light control during bud burst in *Rosa* sp., Plant Cell Environ. 33 (2010) 1339–1350.
[43] P. Wang, S. Zhang, J. Qiao, Q. Sun, Q. Shi, C. Cai, J. Mo, Z. Chu, Y. Yuan, X. Du, Y. Miao, X. Zhang, Y. Cai, Functional analysis of the *GbDWARF14* gene associated with branching development in cotton, PeerJ. 7 (2019) e6901.
[44] L. Zhang, H. Yu, B. Ma, G. Liu, J. Wang, J. Wang, R. Gao, J. Li, J. Liu, J. Xu, Y. Zhang, Q. Li, X. Huang, J. Xu, J. Li, Q. Qian, B. Han, Z. He, J. Li, A natural tandem array alleviates epigenetic repression of *IPA1* and leads to superior yielding rice, Nat. Commun. 8 (2017) 14789.
[45] Y. Cai, X. Cai, Q. Wang, P. Wang, Y. Zhang, C. Cai, Y. Xu, K. Wang, Z. Zhou, C. Wang, S. Geng, B. Li, Q. Dong, Y. Hou, H. Wang, P. Ai, Z. Liu, F. Yi, M. Sun, G. An, J. Cheng, Y. Zhang, Q. Shi, Y. Xie, X. Shi, Y. Chang, F. Huang, Y. Chen, S. Hong, L. Mi, Q. Sun, L. Zhang, B. Zhou, R. Peng, X. Zhang, F. Liu, Genome sequencing of the Australian wild diploid species *Gossypium australe* highlights disease resistance and delayed gland morphogenesis, Plant Biotechnol. J. 18 (2020) 814–828.
[46] T. Zhang, Y. Hu, W. Jiang, L. Fang, X. Guan, J. Chen, J. Zhang, C.A. Saski, B.E. Scheffler, D.M. Stelly, A.M. Hulse-Kemp, Q. Wan, B. Liu, C. Liu, S. Wang, M. Pan, Y. Wang, D. Wang, W. Ye, L. Chang, W. Zhang, Q. Song, R.C. Kirkbride, X. Chen, E. Dennis, D.J. Llewellyn, D.G. Peterson, P. Thaxton, D.C. Jones, Q. Wang, X. Xu, H. Zhang, H. Wu, L. Zhou, G. Mei, S. Chen, Y. Tian, D. Xiang, X. Li, J. Ding, Q. Zuo, L. Tao, Y. Liu, J. Li, Y. Lin, Y. Hui, Z. Cao, C. Cai, X. Zhu, Z. Jiang, B. Zhou, W. Guo, R. Li, Z.J. Chen, Sequencing of allotetraploid cotton (*Gossypium hirsutum* L. acc. TM-1) provides a resource for fiber improvement, Nat. Biotechnol. 33 (2015) 531–537.
[47] F. Li, G. Fan, C. Lu, G. Xiao, C. Zou, R.J. Kohel, Z. Ma, H. Shang, X. Ma, J. Wu, X. Liang, G. Huang, R.G. Percy, K. Liu, W. Yang, W. Chen, X. Du, C. Shi, Y. Yuan, W. Ye, X. Liu, X. Zhang, W. Liu, H. Wei, S. Wei, G. Huang, X. Zhang, S. Zhu, H. Zhang, F. Sun, X. Wang, J. Liang, J. Wang, Q. He, L. Huang, J. Wang, J. Cui, G. Song, K. Wang, X. Xu, J.Z. Yu, Y. Zhu, S. Yu, Genome sequence of cultivated Upland cotton (*Gossypium hirsutum* TM-1) provides insights into genome evolution, Nat. Biotechnol. 33 (2015) 524–530.
[48] D. Yuan, Z. Tang, M. Wang, W. Gao, L. Tu, X. Jin, L. Chen, Y. He, L. Zhang, L. Zhu, Y. Li, Q. Liang, Z. Lin, X. Yang, N. Liu, S. Jin, Y. Lei, Y. Ding, G. Li, X. Ruan, Y. Ruan, X. Zhang, The genome sequence of Sea-Island cotton (*Gossypium barbadense*) provides insights into the allopolyploidization and development of superior spinnable fibres, Sci. Rep. 5 (2015) 17662.
[49] X. Liu, B. Zhao, H.J. Zheng, Y. Hu, G. Lu, C.Q. Yang, J.D. Chen, J.J. Chen, D.Y. Chen, L. Zhang, Y. Zhou, L.J. Wang, W.Z. Guo, Y.L. Bai, J.X. Ruan, X.X. Shangguan, Y.B. Mao, C.M. Shan, J.P. Jiang, Y.Q. Zhu, L. Jin, H. Kang, S.T. Chen, X.L. He, R. Wang, Y. Z. Wang, J. Chen, L.J. Wang, S.T. Yu, B.Y. Wang, J. Wei, S.C. Song, X.Y. Lu, Z.C. Gao, W.Y. Gu, X. Deng, D. Ma, S. Wang, W.H. Liang, L. Fang, C.P. Cai, X.F. Zhu, B. L. Zhou, Z. Jeffrey Chen, S.H. Xu, Y.G. Zhang, S.Y. Wang, T.Z. Zhang, G.P. Zhao, X. Y. Chen, *Gossypium barbadense* genome sequence provides insight into the evolution of extra-long staple fiber and specialized metabolites, Sci. Rep. 5 (2015) 14139.
[50] F. Li, G. Fan, K. Wang, F. Sun, Y. Yuan, G. Song, Q. Li, Z. Ma, C. Lu, C. Zou, W. Chen, X. Liang, H. Shang, W. Liu, C. Shi, G. Xiao, C. Gou, W. Ye, X. Xu, X. Zhang, H. Wei, Z. Li, G. Zhang, J. Wang, K. Liu, R.J. Kohel, R.G. Percy, J.Z. Yu, Y.X. Zhu, J. Wang, S. Yu, Genome sequence of the cultivated cotton *Gossypium arboreum*, Nat. Genet. 46 (2014) 567–572.
[51] K. Wang, Z. Wang, F. Li, W. Ye, J. Wang, G. Song, Z. Yue, L. Cong, H. Shang, S. Zhu, C. Zou, Q. Li, Y. Yuan, C. Lu, H. Wei, C. Gou, Z. Zheng, Y. Yin, X. Zhang, K. Liu, B. Wang, C. Song, N. Shi, R.J. Kohel, R.G. Percy, J.Z. Yu, Y.X. Zhu, J. Wang, S. Yu, The draft genome of a diploid cotton *Gossypium raimondii*, Nat. Genet. 44 (2012) 1098–1103.
[52] Z. Yin, Y. Li, W. Zhu, X. Fu, X. Han, J. Wang, H. Lin, W. Ye, Identification, characterization, and expression patterns of TCP genes and microRNA319 in cotton, Int. J. Mol. Sci. 19 (2018) 3655.
[53] W. Gao, L. Long, L.F. Zhu, L. Xu, W.H. Gao, L.Q. Sun, L.L. Liu, X.L. Zhang, Proteomic and virus-induced gene silencing (VIGS) analyses reveal that gossypol, brassinosteroids, and jasmonic acid contribute to the resistance of cotton to *Verticillium dahliae*, Mol. Cell Proteomics 12 (2013) 3690–3703.
[54] S. Bachan, S.P. Dinesh-Kumar, Tobacco rattle virus (TRV)-based virus-induced gene silencing, Methods Mol. Biol. 894 (2012) 83–92.
[55] Y. Hu, J. Chen, L. Fang, Z. Zhang, W. Ma, Y. Niu, L. Ju, J. Deng, T. Zhao, J. Lian, K. Baruch, D. Fang, X. Liu, Y.L. Ruan, M. Rahman, J. Han, K. Wang, Q. Wang, H. Wu, G. Mei, Y. Zang, Z. Han, C. Xu, W. Shen, D. Yang, Z. Si, F. Dai, L. Zou, F. Huang, Y. Bai, Y. Zhang, A. Brodt, H. Ben-Hamo, X. Zhu, B. Zhou, X. Guan, S. Zhu, X. Chen, T. Zhang, *Gossypium barbadense* and *Gossypium hirsutum* genomes provide insights into the origin and evolution of allotetraploid cotton, Nat. Genet. 51 (2019) 739–748.
[56] D. Kim, B. Langmead, S.L. Salzberg, HISAT: a fast spliced aligner with low memory requirements, Nat. Methods 12 (2015) 357–360.
[57] M. Pertea, G.M. Pertea, C.M. Antonescu, T.C. Chang, J.T. Mendell, S.L. Salzberg, StringTie enables improved reconstruction of a transcriptome from RNA-seq reads, Nat. Biotechnol. 33 (2015) 290–295.
[58] S. Audic, J.M. Claverie, The significance of digital gene expression profiles, Genome Res. 7 (1997) 986–995.
[59] S. Anders, W. Huber, Differential expression analysis for sequence count data, Genome Biol. 11 (2010) R106.
[60] Q. Sun, X. Du, C. Cai, L. Long, S. Zhang, P. Qiao, W. Wang, K. Zhou, G. Wang, X. Liu, H. Zhang, S. Geng, C. Yang, W. Gao, J. Mo, C. Miao, C. Song, Y. Cai, To be a flower or fruiting branch: insights revealed by mRNA and small RNA transcriptomes from different cotton developmental stages, Sci. Rep. 6 (2016) 23212.
[61] M. Seale, T. Bennett, O. Leyser, *BRC1* expression regulates bud activation potential but is not necessary or sufficient for bud growth inhibition in *Arabidopsis*, Development 144 (2017) 1661–1673.
[62] L.E. Dixon, M. Pasquariello, S.A. Boden, *TEOSINTE BRANCHED1* regulates height and stem internode length in bread wheat (*Triticum aestivum*), J. Exp. Bot. (2020) eraa252.
[63] T. Takeda, Y. Suwa, M. Suzuki, H. Kitano, M. Ueguchi-Tanaka, M. Ashikari, M. Matsuoka, C. Ueguchi, The *OsTB1* gene negatively regulates lateral branching in rice, Plant J. 33 (2003) 513–520.
[64] J.M. Lewis, C.A. Mackintosh, S. Shin, E. Gilding, S. Kravchenko, G. Baldridge, R. Zeyen, G.J. Muehlbauer, Overexpression of the maize *Teosinte Branched1* gene in wheat suppresses tiller development, Plant Cell Rep. 27 (2008) 1217–1225.
[65] W. Chen, J. Yao, L. Chu, Z. Yuan, Y. Li, Y. Zhang, Genetic mapping of the nulliplex-branch gene (*gb_nb1*) in cotton using next-generation sequencing, Theor. Appl. Genet. 128 (2015) 539–547.
[66] R.C. McGarry, S.F. Prewitt, S. Culpepper, Y. Eshed, E. Lifschitz, B.G. Ayre, Monopodial and sympodial branching architecture in cotton is differentially regulated by the *Gossypium hirsutum SINGLE FLOWER TRUSS* and *SELF-PRUNING* orthologs, New Phytol. 212 (2016) 244–258.
[67] N.C. Huang, W.N. Jane, J. Chen, T.S. Yu, *Arabidopsis thaliana CENTRORADIALIS homologue (ATC)* acts systemically to inhibit floral initiation in *Arabidopsis*, Plant J. 72 (2012) 175–184.
[68] Z. Si, H. Liu, J. Zhu, J. Chen, Q. Wang, L. Fang, F. Gao, Y. Tian, Y. Chen, L. Chang, B. Liu, Z. Han, B. Zhou, Y. Hu, X. Huang, T. Zhang, Mutation of SELF-PRUNING homologs in cotton promotes short-branching plant architecture, J. Exp. Bot. 69 (2018) 2543–2553.
[69] E. González-Grandío, C. Poza-Carrión, C.O.S. Sorzano, P. Cubas, *BRANCHED1* promotes axillary bud dormancy in response to shade in *Arabidopsis*, Plant Cell 25 (2013) 834–850.
[70] M. Martin-Trillo, E.G. Grandio, F. Serra, F. Marcel, M.L. Rodriguez-Buey, G. Schmitz, K. Theres, A. Bendahmane, H. Dopazo, P. Cubas, Role of tomato BRANCHED1-like genes in the control of shoot branching, Plant J. 67 (2011) 701–714.
[71] C. Yao, S.A. Finlayson, Abscisic acid is a general negative regulator of *Arabidopsis* axillary bud growth, Plant Physiol. 169 (2015) 611–626.
[72] P. Prusinkiewicz, S. Crawford, R.S. Smith, K. Ljung, T. Bennett, V. Ongaro, O. Leyser, Control of bud activation by an auxin transport switch, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 17431–17436.
[73] C. Schommer, J.F. Palatnik, P. Aggarwal, A. Chételat, P. Cubas, E.E. Farmer, U. Nath, D. Weigel, J.C. Carrington, Control of jasmonate biosynthesis and senescence by miR319 targets, PLoS Biol. 6 (2008) e230.
[74] B. Jiang, H. Miao, S. Chen, S. Zhang, F. Chen, W. Fang, The *lateral suppressor*-like gene, *DgLsL*, alternated the axillary branching in transgenic chrysanthemum (*Chrysanthemum* × *morifolium*) by modulating IAA and GA content, Plant Mol. Biol. Rep. 28 (2010) 144–151.
[75] S.F. Lo, S.Y. Yang, K.T. Chen, Y.I. Hsing, J.A.D. Zeevaart, L.J. Chen, S.M. Yu, A novel class of gibberellin 2-oxidases control semidwarfism, tillering, and root development in rice, Plant Cell 20 (2008) 2603–2618.
[76] J. Ni, C. Gao, M.S. Chen, B.Z. Pan, K. Ye, Z.F. Xu, Gibberellin promotes shoot branching in the perennial woody plant *Jatropha curcas*, Plant Cell Physiol. 56 (2015) 1655–1666.
[77] B.J. Ferguson, C.A. Beveridge, Roles for auxin, cytokinin, and strigolactone in regulating shoot branching, Plant Physiol. 149 (2009) 1929–1944.