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Identification of bromodomain family genes across the cotton genome and the enhancement of plant growth and development by GhBRD34

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Abstract

Background

Bromodomain (BRD)-containing proteins are important regulators of plant growth, development, and responses to environmental stress. However, the understanding of their specific biological functions and molecular mechanisms in cotton is limited.

Results

Here, we performed a genome-wide survey and identified 69 GhBRD family members, which are located on 24 chromosomes of the upland cotton genome. Analysis of the promoter regions revealed several conserved cis-acting elements that play roles in light signaling, abiotic stress responses, and phytohormone signaling. Available transcriptome datasets revealed that four of these genes (*GhBRD1*, *GhBRD33*, *GhBRD34*, and *GhBRD68*) exhibited consistently high expression across different tissues, suggesting that these genes participate in various aspects of plant growth and development. When *GhBRD34* was silenced through virus-induced gene silencing in upland cotton, plant growth and development were markedly suppressed, resulting in reduced plant height and a relatively small leaf area. Conversely, *GhBRD34* overexpression in *Arabidopsis* not only promoted growth and development but also rendered the plants more sensitive to drought conditions.

Conclusions

Our findings lay a solid foundation for future studies aimed at dissecting the molecular pathways through which *GhBRD34* influences growth, development, and drought responsiveness in upland cotton.

1. Introduction

The dynamic regulation of protein structure and function is accomplished primarily via post-translational modifications. Among these, lysine acetylation stands out as a ubiquitous mark, occurring not only on histones but also on nonhistone proteins. It is involved in a diverse array of biological processes, ranging from transcriptional control and chromatin compaction to protein-protein interactions, cell cycle progression, and metabolic pathways **[1]**. Bromodomain (BRD) serves as a crucial epigenetic reader module on chromatin-associated proteins. Its main function is to specifically identify and attach to acetylated lysine residues found on both histones and nonhistones **[2]**. Genes containing BRDs were first identified in *Drosophila melanogaster* **[3]**, and subsequent work has shown that these genes are widely distributed across diverse eukaryotic lineages. Structurally, BRD proteins harbor a conserved region of approximately 110 amino acids, folded into four α-helices (αZ, αA, αB, αC) that are connected by ZA and BC loops **[3]**. The ability of BRDs to engage acetylated lysines and form specific protein-protein and

protein-nucleic acid complexes at acetylated lysine-marked functional sites is fundamental to epigenetic regulation **[4]**.

In *Arabidopsis*, a total of 28 AtBRD proteins have been identified and assigned to four functional groups: proteins associated with chromatin-remodeling complexes; BRD and extraterminal domain (BET) family transcriptional regulators; BRD proteins with intrinsic histone acetyltransferase (HAT) activity; and other miscellaneous BRD proteins **[5]**. Chromatin-remodeling complexes modulate gene transcription by altering chromatin architecture; the well-studied SWI/SNF complex, for example, promotes nucleosome sliding and disrupts histone-DNA contacts. Within this complex, AtBRD1, AtBRD2, and AtBRD13 are integral components **[6]**, and together with the BRM protein, they regulate plant growth and development **[7]**. Moreover, brdx3 and brm-defective mutants display aberrant phenotypes in response to abscisic acid (ABA) and gibberellin (GA), along with altered expression of ABA/GA-responsive genes, suggesting that BRD1, BRD2, and BRD13 play essential roles in SWI/SNF-mediated ABA and GA signaling. *Arabidopsis* also possesses 12 genes encoding BET proteins, known as general transcription factor group E (GTE) 1 through 12 **[8]**. Among these transcriptional regulators, AtGTE1 influences the transcript levels of genes involved in cell wall metabolism, photosynthesis, and lipid metabolism **[9]**. AtGTE4, on the other hand, participates in mitotic cell cycle maintenance **[10].** It can directly bind to and inhibit 12 oxophytodienoic acid reductase 3 (OPR3), leading to the accumulation of jasmonic acid and heightened expression of jasmonate responsive genes, thereby acting as a negative regulator of plant immune responses **[11]**. BRD proteins with HAT activity can catalyze the acetylation of adjacent histones, resulting in the formation of a positive feedback loop that amplifies epigenetic signals and ultimately regulates chromatin accessibility and gene transcription efficiency. The HAT family in *Arabidopsis* precisely regulates histone acetylation levels and is widely involved in plant growth, development, stress responses, and epigenetic regulation. As core members of the family, HAG1 and HAT1/HAT2 can achieve spatiotemporal dynamic regulation of gene transcription through domain specific interactions and complex assembly, offering a remarkable epigenetic basis for plant adaptation to environmental changes and growth processes **[12, 13]**.

Cotton is a major economic crop and the world's leading source of natural fiber for the textile industry **[14]**. Upland cotton is the most extensively planted and commercially significant species globally. It is an allotetraploid species derived from the hybridization of two diploid progenitors carrying the At and Dt subgenomes, followed by chromosome doubling **[15]**. This unique evolutionary history has endowed upland cotton with genes related to fiber develoment from the At genome and genes related to environmental adaptation from the Dt genome, laying a crucial genetic foundation for its worldwide cultivation **[16]**. The biological functions of the BRD family in cotton are largely unexplored. To address this gap, we conducted a comprehensive genome-wide survey of *BRD* genes across four cotton species, *Gossypium hirsutum*, *G. barbadense*, *G. arboreum*, and *G. raimondii*. We methodically examined the tissue-specific expression profiles, chromosomal distribution, structural diversity, and phylogenetic relationships of the *GhBRD* gene in upland cotton. Furthermore, by using virus-induced gene silencing (VIGS) in upland cotton and heterologous overexpression in *Arabidopsis*, we obtained preliminary insights into the biological function of *GhBRD34*. Taken together, the results of this study offer a

theoretical basis for further exploration of the molecular mechanisms through which *GhBRD34* modulates cotton growth, development, and drought tolerance.

2. Materials and methods

2.1. Genome-wide identification of *BRD* genes in cotton

The genomic sequences and corresponding protein datasets for *G. hirsutum, G. barbadense, G. arboreum*, and *G. raimondii* were retrieved from the CottonMD database, whereas those for *Arabidopsis thaliana* were obtained from the TAIR repository. To identify candidate *BRD* genes, we employed HMMER 3.2.1 with the hidden Markov model profile of the BRD (PF00439) as a search query against the aforementioned proteomes **[17]**. Following the initial screening, redundant entries were eliminated, and the remaining candidate sequences were subjected to validation using the NCBI Conserved Domain Database (NCBI-CDD) to confirm their membership in the cotton BRD protein family. The physicochemical parameters of the GhBRD proteins were calculated using the ExPASy portal **[18]**, while their subcellular localizations were predicted using the Plant-mPLoc tool **[19]**.

2.2. Phylogenetic reconstruction of the BRD family across cotton species

With respect to phylogenetic analysis, multiple sequence alignments were carried out on BRD protein sequences generated from *A. thaliana*, *G. hirsutum*, *G. barbadense*, *G. arboreum*, and *G. raimondii* by using the ClustalW module implemented in MEGA X under default alignment parameters **[20]**. A neighbor-joining phylogenetic tree based on this alignment was then constructed, with bootstrap support for each node determined by 1000 replicates. The iTOL web-based platform was subsequently used to visualize and annotate the resulting tree.

2.3. Chromosomal distribution, exon-intron organization, and conserved motif composition of the GhBRD family in upland cotton

Chromosomal position data for each *BRD* gene were extracted from the genome annotation files of upland cotton, and a physical distribution map across the 26 chromosomes was generated using MapChart software. A phylogenetic tree was constructed independently using MEGA X and saved in Newick (nwk) format **[20]**. To detect conserved sequence features, the MEME suite **[21]** was run with the maximum number of motifs set to 10. The resulting XML output from MEME, the nwk tree file, and the GFF-based gene structure records were integrated and rendered using the TBtools package **[22]**.

2.4. Prediction of promoter cis-regulatory elements

DNA sequences located 1500 bp upstream of the start codon of the *GhBRD* gene were retrieved. Putative cis-acting elements in these locations were predicted using the PlantCare database **[23]**, and the results

were visualized using TBtools software.

2.5. Transcriptome profiling across different tissues

RNA-seq datasets corresponding to eight distinct organs of upland cotton, namely roots, stems, leaves, pistils, stamens, sepals, petals, and receptacles were downloaded from the CottonMD resource **[24]**. The expression abundance of each *GhBRD* gene was quantified as fragments per kilobase of transcript per million mapped reads (FPKM). Heatmaps illustrating the expression patterns throughout various tissues were constructed using TBtools software.

2.6. Virus-induced gene silencing assay

The recombinant plasmids pCLCrVA::*GhBRD34* and pCLCrVA::*GhPDS*, the empty vector pCLCrVA, and the helper plasmid pCLCrVB were each transformed into *Agrobacterium tumefaciens*. To achieve an OD_{600} of 1.2–1.5, the modified strains were cultivated in LB broth supplemented with the correct antibiotics at 28°C and 180 rpm. The cells were centrifuged at 4500 rpm for 10 min to form a pellet and then resuspended in MMA buffer to achieve an OD_{600} of approximately 1.8. They were mixed with the helper plasmid pCLCrVB at a 1:1 ratio and incubated in the dark for 2 h. The resulting mixtures were infiltrated into the abaxial leaf surfaces using a needleless syringe. The infiltrated plants were placed in darkness for 24 h before being returned to standard light conditions. Silencing efficiency was assessed based on the albino phenotype of positive control plants 10–14 days post infiltration and further confirmed by qRT-PCR using GhActin as the internal reference. Total RNA was extracted from leaves and reverse-transcribed into cDNA at a concentration of 100 ng/μL **[25]**.

2.7. Generation of transgenic Arabidopsis lines overexpressing GhBRD34

The coding region of GhBRD34 was amplified from CRI24 using KOD DNA polymerase (Toyobo, Shanghai, China). The amplified fragment was cut with Kpn I and BamH I and then ligated into the pCAMBIA2300 plant overexpression vector to generate the 35S::*GhBRD34* construct. The *A. thaliana* ecotype Col-0 served as the recipient material, and transgenic plants were generated using the floral dip method **[26]**. T_0 transgenic seeds were sterilized and planted on 1/2 MS medium enriched with 35 mg/L kanamycin. Seedlings with green leaves and normal roots were chosen and transplanted into soil. The T_2 transgenic lines showing a 3:1 segregation ratio were further screened on 1/2 MS medium supplemented with 35 mg/L kanamycin to obtain homozygous T_3 seeds for subsequent experiments.

2.8. Evaluation of drought stress tolerance

To assess drought tolerance, seeds from wild-type (Col-0) and *GhBRD34*-overexpressing *Arabidopsis* lines were surface-sterilized and seeded on MS media supplemented with 400 mM mannitol to simulate osmotic stress. Following sowing, the plates were subjected to stratification at 4°C for 48 h to induce germination, after which they were moved to a growth chamber maintained at 22°C with a 16-h light/8-h dark cycle. After the seedlings reached the three-true-leaf stage, both the transgenic and control plants

were subject to water deficit stress by completely withholding irrigation for approximately 2 weeks, and then, watering was resumed. The phenotypic response and overall growth status of the plants were monitored throughout the treatment and recovery phases to evaluate their relative drought tolerance, following the protocol described by Elasad et al. **[27]**.

3. Results

3.1. Identification and phylogenetic analysis of the cotton BRD family

To identify candidate BRD family members in cotton, we utilized 28 AtBRD protein sequences from *A. thaliana* as query probes and employed HMMER 3.0 to screen the protein datasets of four cotton species. Following verification using Pfam 31.0 and SMART databases, we recovered 69, 67, 40, and 34 BRD protein sequences from *G. hirsutum*, *G. barbadense*, *G. raimondii*, and *G. arboreum*, respectively. Detailed correspondences among gene identifiers, gene names, and coding sequence lengths are compiled in **Table S1**.

Among the 69 *GhBRD* genes identified in *G. hirsutum*, 33 were allocated to the At subgenome, while 36 were allocated to the Dt subgenome. Similarly, for the 67 *GbBRD* genes in *G. barbadense*, the respective subgenomic contributions were 35 and 32. Notably, total *BRD* gene counts in the two allotetraploid species (*G. hirsutum* and *G. barbadense*) decreased below the total number of their diploid ancestors, *G. raimondii* and *G. arboreum*. These observations suggest that genome downregulation following polyploidization is presumably achieved through the elimination of redundant loci and the refinement of regulatory networks to accommodate polyploid conditions.

To elucidate the evolutionary relationships among BRD proteins, we constructed an unrooted maximum-likelihood phylogenetic tree based on 238 BRD sequences obtained from *G. raimondii*, *G. arboreum*, *G. barbadense*, *G. hirsutum*, and *A. thaliana* (Fig. 1). There were five subfamilies (classes I-V) in which these proteins were allocated. Class V emerged as the largest group, encompassing 64 BRD members (19 GhBRDs, 19 GbBRDs, 10 GrBRDs, 9 GaBRDs, and 7 AtBRDs), whereas class III was the smallest group, with only 16 representatives (4 GhBRDs, 6 GbBRDs, 3 GrBRDs, 2 GaBRDs, and 1 AtBRD). Classes I, II, and IV comprised 57, 59, and 42 members, respectively. Each subfamily included proteins from all five species, and the distribution trends were similar across classes, indicating that BRD genes with analogous functions in cotton tend to coalesce on the same phylogenetic branches. The tree further revealed that the BRD proteins of *G. hirsutum* and *G. barbadense* share the closest evolutionary affinity. The presence of two extra BRD genes in *G. hirsutum* relative to that in *G. barbadense* indicates that these extra copies may have resulted from duplication events that occurred after the divergence of the two tetraploid lineages.

3.2. Chromosomal localization of GhBRD genes in G. hirsutum

To determine the genomic distribution of *BRD* genes on cotton chromosomes, we obtained physical location information for all the *GhBRD* genes from the CottonMD database. The results revealed that the 69 *GhBRD* genes were distributed unevenly throughout 24 chromosomes of *G. hirsutum* (Fig. 2). Among these genes, 33 were found on the At subgenome and 36 on the Dt subgenome, suggesting a symmetrical distribution between the two subgenomes that aligns with the evolutionary pattern of cotton polyploidization **[28]**. Chromosome A05 had the greatest number of *GhBRD* genes (9), followed by D05 (7). In contrast, chromosomes A03, A06, A07, A11, A13, D03, D07, and D11 each carried only a single GhBRD locus, while no *GhBRD* genes were detected on A08 or D08. Some *GhBRD* genes appear to be physically clustered; for instance, *GhBRD15*, *GhBRD16*, and *GhBRD17* on chromosome A05 display analogous motif compositions and exon-intron architectures.

3.3. Phylogenetic relationships, exon-intron organization, and conserved motifs of GhBRDs

To better understand the sequence attributes and potential functional relationships among GhBRD members, we performed domain analysis and visualization using TBtools in conjunction with SMART annotation. One BRD was present in all the GhBRD proteins (**Table S2**). To provide a more in-depth comparison, we created an unrooted phylogenetic tree of GhBRD proteins (Fig. 3A) and jointly examined their conserved motif arrangements (Fig. 3B) and exon-intron structures (Fig. 3C).

The exon counts among the 69 *GhBRD* genes varied from 1 to 22, with 42 genes having more than 8 exons (**Table S3**), a diversity that may underpin their functional versatility. Notably, *GhBRD69* itself lacks introns and consists of one single exon. Although individual *GhBRD* genes differ greatly with respect to exons and introns, those that are close in terms of phylogeny often share patterns of exon-intron organization, thus establishing a clear link between structure and evolutionary relationships.

The composition and position of conserved motifs often serve as indicators of protein function. We delineated nine conserved motifs, labeled Motif1 through Motif9. Each GhBRD protein harbors between 2 and 7 of these motifs (Fig. 3B). Proteins situated on the same branch of the phylogenetic tree displayed highly congruent motif distribution profiles, and nearly all the GhBRD proteins contained both Motif1 and Motif2, underscoring the strong conservation of these two motifs within the *G. hirsutum* BRD family (Fig. 3A and 3B). More specifically, the majority of class I members carry 3 or 5 conserved motifs, with the exceptions of GhBRD43 and GhBRD20, which contain only 2. Most class II proteins possess 6 motifs, whereas most class III members have 7; class IV proteins typically harbor 2 or 3 motifs (Fig. 3B). Despite the observable differences in exon-intron architecture and motif arrangement among the four subfamilies, members within the same branch remain structurally conserved, suggesting that BRD proteins in each branch performs relatively stable functions during cotton growth and development.

3.4. Promoter cis-acting element analysis of GhBRD genes

To elucidate the possible regulatory involvement of *GhBRD* genes in stress responses and hormone signaling, we extracted 1500 bp upstream sequences from the transcription start sites of all 69 *GhBRD* loci and screened them for stress- and hormone-associated cis-regulatory elements using the PlantCARE platform (Fig. 4). The promoter regions were found to harbor seven categories of hormone-responsive motifs: TGA-element (auxin-responsive), TCA-element (salicylic acid-responsive), P-box and GARE-motif (gibberellin-responsive), CGTCA- and TGACG-motifs (jasmonate-responsive), and ABRE (abscisic acid responsiveness). In addition, three types of stress-related elements were identified, namely, MBSs (drought response), LTRs (low-temperature response) and TC-rich repeats (defense and stress response).

All the *GhBRD* promoters also contained light-responsive motifs, with a total of 11 different variants identified: AT1-motif, ATCT-motif, ACE, Box4, G-box, GATA-motif, GT1-motif, MRE, G-box, TCT-motif and I-box. Each of the 69 *GhBRD* genes possessed at least one light-responsive element in its promoter. Furthermore, 44 genes carried ABA-responsive elements and 43 contained JA-responsive motifs (Fig. 4). The greatest number of cis-acting elements (24) was observed in *GhBRD24* and *GhBRD53*, followed by that in *GhBRD40* and *GhBRD65* (21 each). At the lower end, *GhBRD69*, *GhBRD25*, and *GhBRD56* contained only 3–5 such elements. These findings indicate that *GhBRD* genes may modulate diverse aspects of cotton growth and development through the integration of light signals and multiple phytohormone pathways.

3.5. Tissue-specific expression profiles of GhBRD genes

To analyze the spatial expression patterns of the GhBRD family, we mined publicly available transcriptomic data from *G. hirsutum* TM-1 [29]. Spatiotemporal expression analysis revealed that GhBRD family members were widely expressed in both reproductive organs (flowers and ovules) and vegetative organs (roots, stems, leaves, and fibers) and exhibited distinct expression differences (Fig. 5). Among these genes, 11 *GhBRD* genes were expressed at high levels in all the tissues, 24 *GhBRD* genes were expressed at very low levels across all the tissues, and the remaining 34 *GhBRD* genes were expressed at moderate levels in all the tissues.

The expression levels of *GhBRD58*, *GhBRD38*, *GhBRD23*, and *GhBRD69* were extremely low and barely detectable in some tissues. A pair of homologous genes (*GhBRD1*/*GhBRD34*) was highly expressed in all the tissues and exhibited similar expression patterns. The highest expression level of *GhBRD30* was detected in ovules, indicating its potential role in the early stages of seed development in cotton. *GhBRD1*, *GhBRD33*, *GhBRD34*, and *GhBRD68* were highly expressed in roots, stems, floral organs, and ovules (Fig. 5). The expression levels of *GhBRD1* and *GhBRD34* increased during the rapid elongation phase of fibers, and their expression patterns aligned with the thickening of the secondary cell wall in cotton fibers, suggesting that these genes play crucial regulatory roles in the growth and development of the associated tissues.

3.6. Silencing of GhBRD34 hinders the growth and development of cotton

The results of the expression pattern analysis revealed that *GhBRD34* is constitutively expressed, suggesting its regulatory role in plant growth and development. To confirm the biological function of *GhBRD34*, we conducted VIGS in upland cotton. Fourteen days after the silencing treatment, the newly emerged leaves of the positive control plants (clcrv:su) clearly yellowed (Fig. 6A), indicating that the VIGS system was effective. Phenotypic analysis revealed that 15 days after infiltration, the plants with clcrv:*GhBRD34* silenced were considerably shorter than the control plants with the empty vector (clcrv:00) (Fig. 6B). To investigate the underlying cause of the reduced plant height, we removed the leaves and observed that the number of internodes and leaf size were significantly reduced smaller in the clcrv:*GhBRD34* silenced plants compared with the control plants (Fig. 6C and 6D). Compared with that in the control plants, *GhBRD34* expression in the silenced plants was considerably lower according to the results of the qRT-PCR analysis (Fig. 6E). Collectively, these phenotypic and molecular data indicate that silencing of GhBRD34 expression significantly inhibits the growth and development of cotton.

3.7. Ectopic expression of GhBRD34 increases drought vulnerability and accelerates development in Arabidopsis

To further analyze the functional capacity of *GhBRD34*, we constructed a 35S promoter-driven overexpression construct and transformed it into wild-type *Arabidopsis* using Agrobacterium-mediated methods. After antibiotic screening and PCR-based confirmation, we identified homozygous lines that exhibited long-term stability and subsequently subjected them to a phenotypic study. Compared with nontransgenic plants, the overexpression plants had greater shoot height and began to flower sooner (Fig. 7A), suggesting that high *GhBRD34* levels help to accelerate both vegetative and reproductive growth.

To test whether *GhBRD34* also modulates abiotic stress responses, we subjected 4-week-old transgenic and control plants to drought by withholding irrigation for 2 weeks. Under these conditions, the wild-type plants retained their green color and sustained normal growth, whereas their overexpressing counterparts showed pronounced wilting and eventual desiccation (Fig. 7B). Upon rewatering and a subsequent 1-week recovery phase, the control plants revived rapidly, but the transgenic lines remained permanently wilted and failed to recover (Fig. 7C). These results unequivocally show that *Arabidopsis* is more susceptible to water deficit when GhBRD34 is constitutively overexpressed.

4. Discussion

BRD genes are highly conserved among plant species and play essential roles in controlling growth, development, and environmental responses. Functionally, they influence diverse processes ranging from

seed germination and leaf formation to mitotic cell cycle progression, sugar and ABA signaling, and pathogen recognition and immunity [5]. Genome-wide analyses of *BRD* genes have now been completed for several model or economically important crops, such as rice (22 genes), *Arabidopsis* (28 genes), and wheat (82 genes) [4, 30]. However, similar studies in cotton are still limited, despite its importance to the global economy. To fill this gap, we analyzed the evolutionary history and possible functions of the BRD gene family.

In this study, we first identified 69, 67, 34, and 40 *BRD* genes in *G. hirsutum*, *G. barbadense*, *G. arboreum*, and *G. raimondii*, respectively, and thoroughly examined of 238 *BRD* genes from these four cotton species and an *Arabidopsis* species. In accordance with the subfamily classification criteria of Arabidopsis, these *BRD* genes were divided into four subfamilies (I-IV) (Fig. 1). This is consistent with the classification results of other species, further confirming the widespread distribution of *BRD* genes in plants [4]. Chromosomal localization revealed that the distribution of *GhBRD* genes across chromosomes was uneven (Fig. 2). To a certain degree, this reflects the evolutionary history of the cotton BRD family. To elucidate the evolutionary relationships of the *GhBRD* gene family, we examined their phylogenetic relationships, the distribution characteristics of gene structures, and the conserved motifs (Fig. 3). The results revealed that the *GhBRD* genes can be divided into four subgroups (I-IV) on the basis of their evolutionary relationships. Moreover, all *GhBRD* genes contained one conserved motif (Motif 1) encompassing the conserved BRD, indicating that this domain has been highly conserved during cotton evolution.

Moreover, *BRD* genes belonging to the same subgroup within a species exhibit similar compositions of conserved motif. Certain *BRD* genes situated on the same chromosome also share identical conserved motifs. This phenomenon may be linked to variations in polyploidization within the plant genome. Conserved domain analysis revealed that the conserved motifs of the *GhBRD* gene family are present in nearly all the members, indicating that the domains of this gene family are highly conserved. Nevertheless, the similarity of other sequences beyond the domains is relatively low, indicating that the cotton *BRD* gene family has evolved to exhibit a certain degree of diversity. These findings align with earlier conclusions that gene structure and the distribution of conserved motifs can illuminate evolutionary relationships within gene families.

Subcellular localization prediction indicated that all the GhBRD proteins were localized in the nucleus (**Table S3**). However, these results need to be verified using fluorescent protein labeling and imaging techniques. Analysis of the cis-acting elements in the promoters revealed that, in addition to the core TATA-box and CAAT-box elements, the cis-acting elements of cotton *BRD* genes can be divided into three categories: those related to plant growth and development, those responsive to plant hormones, and those involved in stress responses (Fig. 4). All the *GhBRD* gene promoters contain numerous light-responsive elements, suggesting that the *BRD* gene family is associated with circadian rhythm regulation. Additionally, 46 *GhBRD* genes were shown to contain JA- and ABA-responsive elements, suggesting that the *GhBRD* family is crucial for biotic stress resistance. These findings align with those

of previous studies indicating that *BRD* genes play a role in biotic stress responses across various species.

This work presents a systematic analysis of the cotton *BRD* family, with an account of the number of such genes, their subfamilies, evolutionary traits, structural forms, and possible functions. The results confirmed that *GhBRD34* has significant potential value in regulating growth and drought tolerance in cotton (Fig. 6 and Fig. 7). These findings help clarify the role played by *GhBRD* genes in plant development and environmental adaptation and offer a theoretical basis for the breeding or genetic improvement of cotton. Future studies are needed to determine the exact subcellular location of *GhBRD*-related genes, the functions of their cis-acting elements, and their actual biological roles. Exploring their regulatory mechanisms in terms of stress resistance and quality formation in cotton will facilitate the practical application of research on the *GhBRD* gene family in the cotton industry.

5. Conclusions

As a vital source of both fiber and oil, cotton is the subject of this comprehensive investigation into the role of *BRD* genes under drought stress. Through a combination of genome-wide identification, we identified 210 *BRD* genes across four *Gossypium* species (*G. arboreum*, *G. raimondii*, *G. hirsutum*, and *G. barbadense*). With respect to function, silencing of *GhBRD34* in cotton significantly hindered plant growth and development. Conversely, *GhBRD34* overexpression in *Arabidopsis* not only stimulated growth but also conferred enhanced drought tolerance to the transgenic lines.

Declarations

Author contributions

H.W., C.L., and Q.Y. conceived and designed the study; H.W., X.L., Y.L., and Y.W. provided research materials and conducted the experiments; H.W., X.L., and L.X. performed data analysis; W. H. wrote the initial manuscript draft; H.W., X.W., and K.M. reviewed, edited, and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financialinterests or personal relationships that could have appeared to influencethe work reported in this paper.

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Data availability

Data are provided within the manuscript or supplementary information files.

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Tables

Table 1 is available in the Supplementary Files section.

Figures

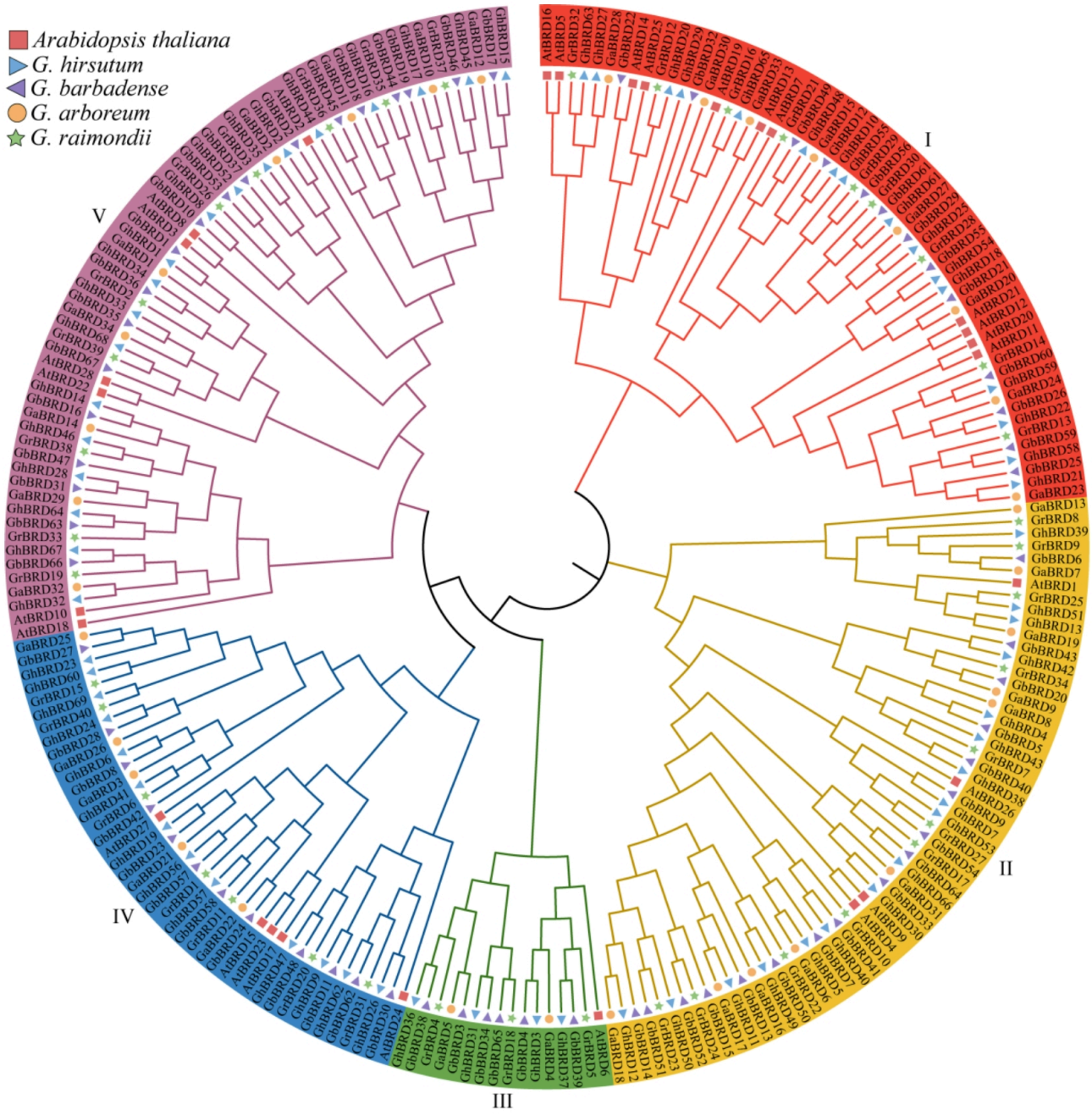


Figure 1

Phylogenetic tree of BRD family genes. The phylogenetic tree includes 238 *BRD*genes from 4 species, specifically 34 *GaBRD*genes from *G. arboreum*, 40 *GrBRD* genes from *G. raimondii*, 67 *GhBRD*genes from *G. barbadense*, 69 *GhBRD* genes from *G. hirsutum*, and 28 *AtBRD*genes from *Arabidopsis thaliana*. The tree clearly illustrates the phylogenetic relationships among these genes. The tree was generated using MEGA 11.0 software. Unique symbols indicate genes from various species: *G. hirsutum* (blue triangles),

G. barbadense(purple triangles), *G. raimondii* (green pentagrams), *G. arboreum*(yellow circles), and *A. thaliana* (red squares).

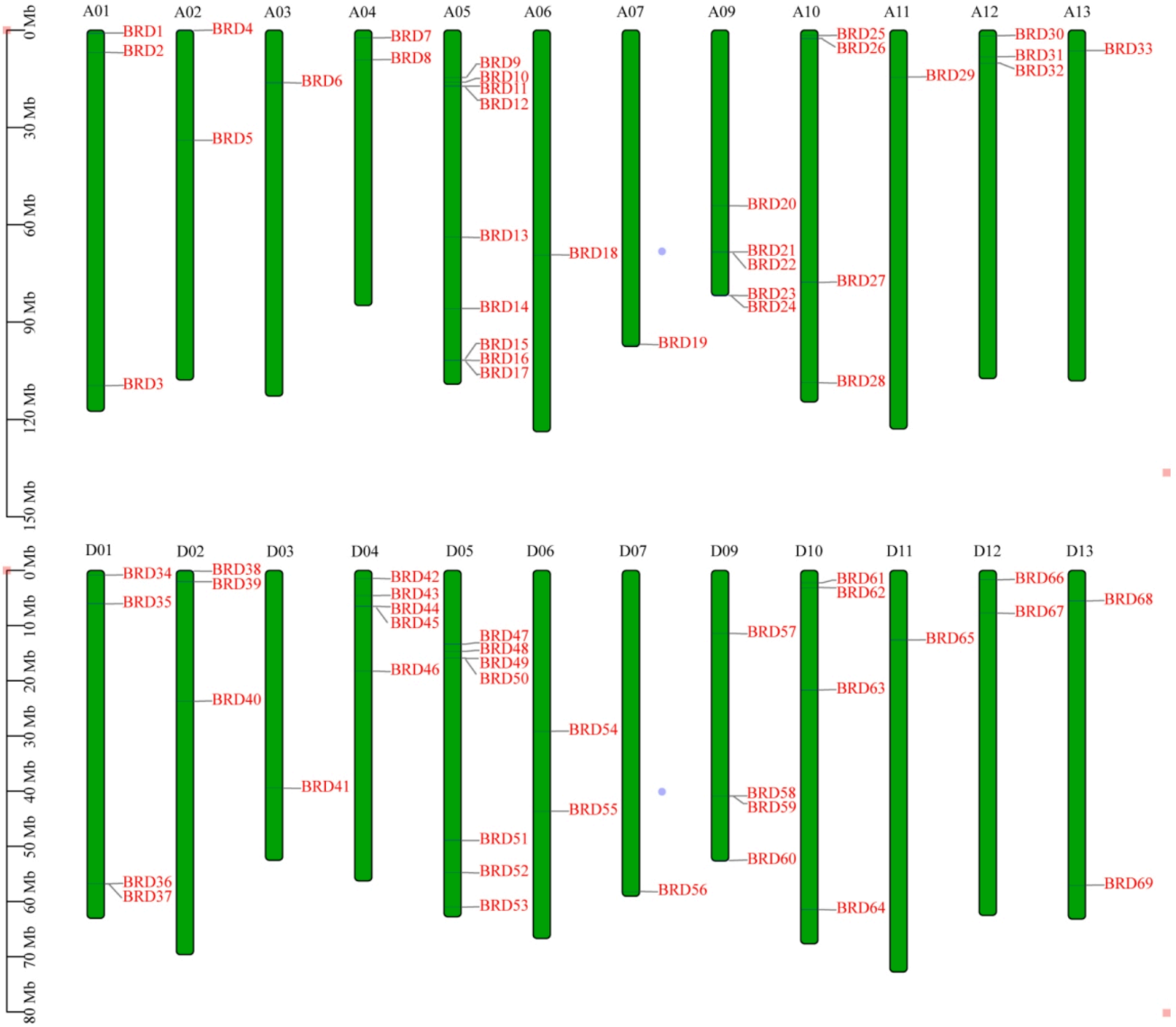


Figure 2

Chromosome location of *GhBRD* genes in *G. hirsutum*.Genes derived from the A, B, C, and D groups are indicated by pink, blue, black, and red font, respectively.

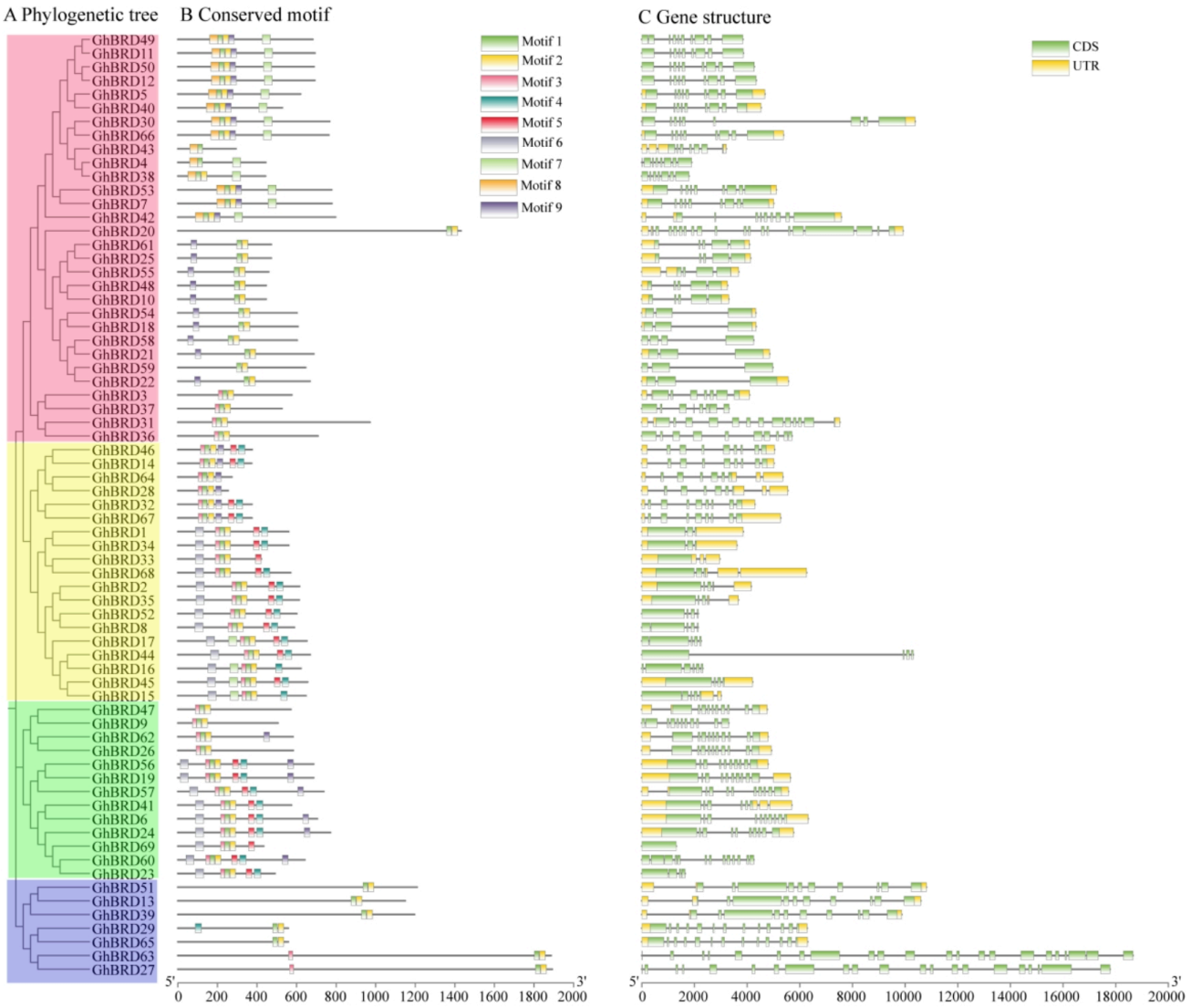


Figure 3

Characterization of gene structure, conserved motifs, and protein domains within the *GhBRD* family in *G. hirsutum*. (A) Phylogenetic relationships among GhBRD proteins were examined using the neighbor-joining method in MEGA 10.0 on the basis of full-length sequences. (B) Schematic representation of the conserved motifs in GhBRD proteins. Nine distinct motifs (1-9) are indicated by colored boxes. (C) Exon-intron organization of *GhBRD* genes. The green and yellow boxes denote coding sequences (CDSs) and untranslated regions (UTRs), respectively.

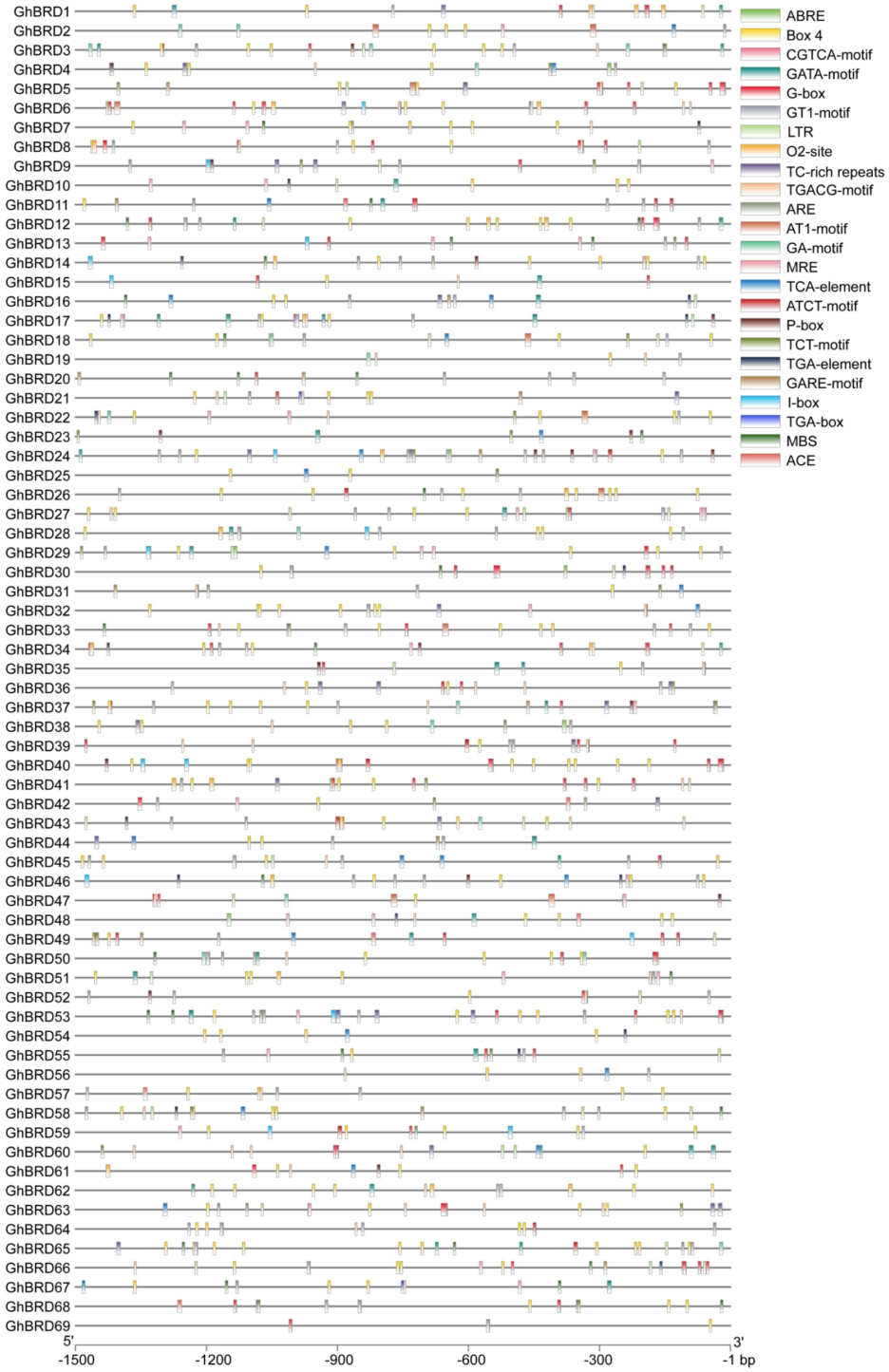


Figure 4

Prediction of cis-regulatory elements in the 1500 bp upstream regions of *GhBRD* promoters from *G. hirsutum*.Colored baxes indicate the different classes of elements found.

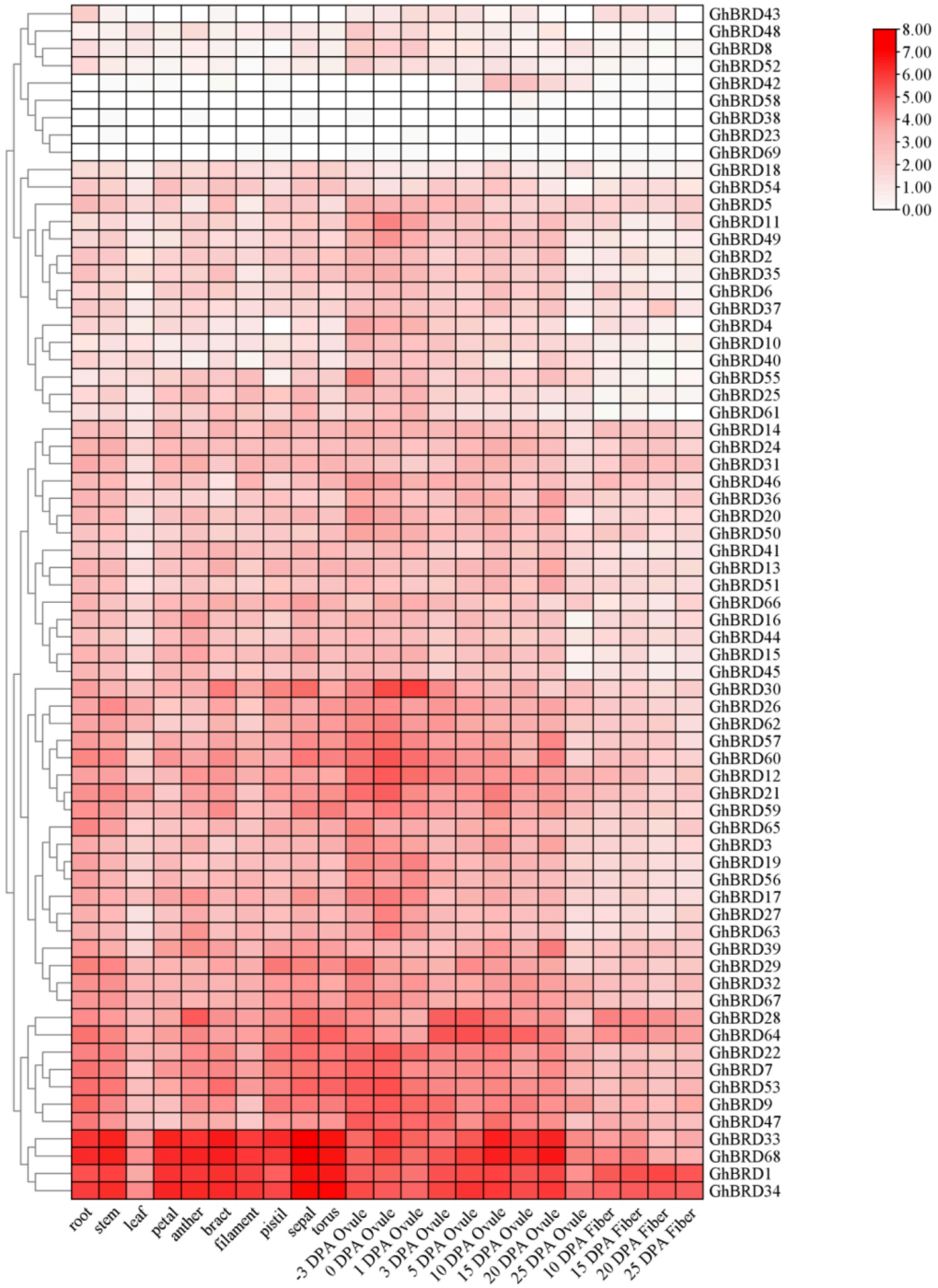


Figure 5

Tissue-specific expression analysis of 69 *GhBRD* genes in *G. hirsutum*. Samples were obtained from 10 different tissue types: Leaves, stems, roots, torus, petals, stamens, pistils, calyces, ovules (-3 to 20 DPA), and fibers (5 to 25 DPA).

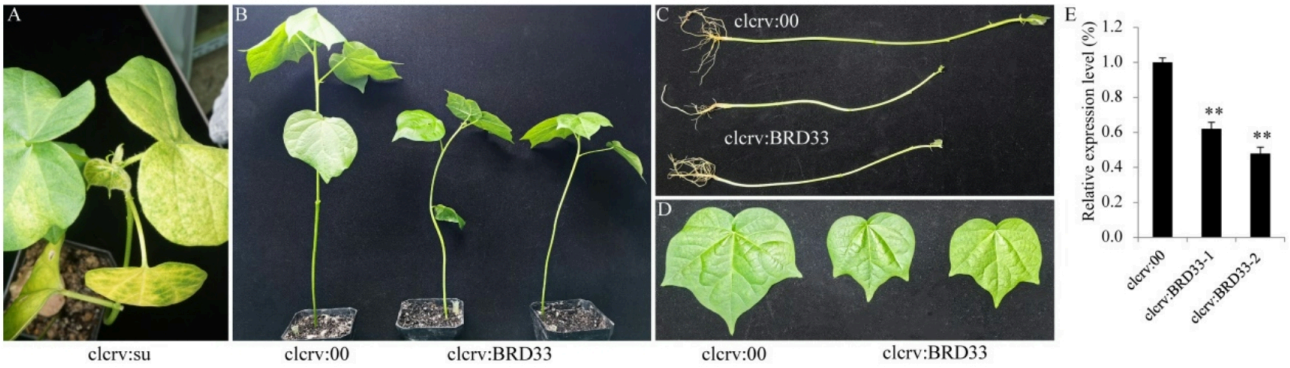


Figure 6

VIGS-mediated *GhBRD34* silencing reduces vegetative growth in cotton. (A) Newly emerged leaves of the positive control clcrv:su plants are shown in yellow; (B), (C), and (D) show the phenotypic comparisons of the whole plant, stem, and leaf, respectively, between the negative control clcrv:00 plants and clcrv:*GhBRD34* plants; (E) Quantitative assessment of *GhBRD34* transcript levels in clcrv:00 versus clcrv:GhBRD34 plants. The data represent means ± SE (n = 3 biological replicates; $p < 0.05$, $p < 0.01$; Student's t-test).

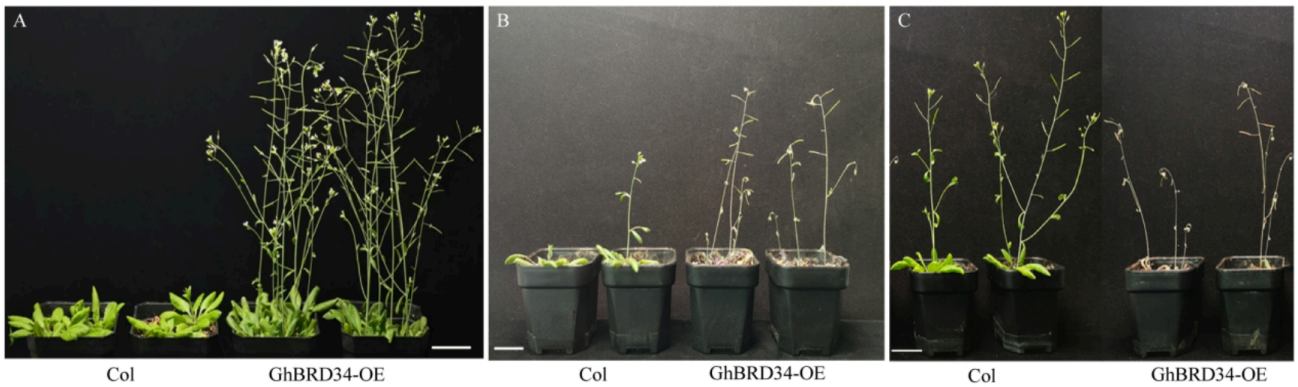


Figure 7

Ectopic expression of *GhBRD34* in *A. thaliana* enhances vegetative growth while conferring heightened susceptibility to drought stress. (A) Morphology of 4-week-old wild-type (Col-0) and *GhBRD34*-overexpressing (*GhBRD34*-OE) seedlings; scale bar = 2 cm. (B) Plant performance of Col-0 and *GhBRD34*-OE lines subjected to 1 week of sustained water withholding; scale bar = 2 cm. (C) Recovery phenotypes of Col-0 and *GhBRD34*-OE plants after 1 week of rewatering; scale bar = 2 cm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1Physicochemicalparametersof69BRDgenesinG.hirsutum.xlsx
- TableS1geneIDnumberandgenename.xlsx
- TableS2LocationofBromodomainincottonBRDproteins.xlsx
- TableS3NumbersofintronsandexonsofcottonBRDgenes.xlsx