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Original article

The *Yabby* Gene Network in Sunflower:Evolutionary Dynamics and Drought-Responsive Expression Profiling

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Abstract

The *YABBY* gene family, known for its plant-specific transcription factors, plays pivotal roles in regulating leaf polarity, floral organ development, and responses to environmental stimuli. Despite its functional importance in various plant species, a comprehensive understanding of this gene family in sunflower (*Helianthus annuus L.*) has remained elusive. In this study, we conducted a genome-wide *in silico* identification and characterization of *YABBY* genes in *H. annuus*, revealing 14 HaYABBY members distributed across 10 chromosomes. Phylogenetic analysis clustered these genes into five conserved subfamilies (FIL/YAB3, YAB2, YAB5, INO, and CRC), while gene structure and motif analyses highlighted both conserved domain architecture and subfamily-specific divergence. Promoter analysis revealed the presence of multiple stress- and hormone-responsive cis-elements, and miRNA target prediction identified HaYABBY05 and HaYABBY09 as potential post-transcriptional targets of four distinct miRNAs.

Synteny and duplication analyses suggested that segmental duplication events under purifying selection contributed to the expansion and conservation of *HaYABBY* genes. Tissue-specific expression profiling via RNA-seq demonstrated diverse expression patterns, with *HaYABBY05* exhibiting broad expression and *HaYABBY12* showing strong floral organ specificity. Under drought stress, RNA-seq and qRT-PCR analyses revealed significant cultivar- and tissue-specific expression differences between the drought-tolerant (*SUN 2235*) and drought-sensitive (*Turay*) sunflower cultivars. Notably, *HaYABBY* genes showed strong induction in the roots of SUN 2235 but were suppressed in Turay, implicating a potential role in drought adaptation.

Together, these findings provide the first comprehensive insight into the structure, evolution, and stress-responsive expression of *YABBY* genes in sunflower. This study offers valuable candidate genes and regulatory clues for improving drought resilience in sunflower breeding and sets a foundation for further functional exploration of YABBY transcription factors in crops.

Keywords: *YABBY* gene family; *Helianthus annuus*; Genome-wide analysis; Drought stress; Gene duplication; miRNA regulation; Expression profiling

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INTRODUCTION

Plants, as sessile organisms, are continuously exposed to various biotic and abiotic stressors that adversely affect their growth and productivity. Among these, abiotic stresses such as drought, salinity, and extreme temperatures significantly disrupt plant metabolism, gene expression, and signal transduction pathways, posing substantial challenges for global agriculture (Zhu, 2016). Although numerous stress-responsive genes and pathways have been identified, many remain uncharacterized, limiting our comprehensive understanding of plant adaptation mechanisms. Transcription factors (TFs) are key regulators that orchestrate gene expression networks, enabling plants to respond and adapt to environmental fluctuations (Grotewold, 2008; Iwase et al., 2009).

Interest in plant TFs has increased considerably due to their central roles in regulating stress-related genes. These factors exert control over transcription by binding to specific promoter regions of target genes and participating in signaling pathways that govern biochemical, physiological, and metabolic responses to stress (Liu et al., 2022). The *YABBY* gene family represents a small, plant-specific group of TFs exclusive to seed plants (Floyd and Bowman, 2007). Members of this family are characterized by two conserved domains: an N-terminal C2C2-type zinc finger domain and a C-terminal YABBY domain featuring a helix-loop-helix motif (Jang et al., 2004). Unlike many TF families that are broadly distributed across eukaryotes, *YABBY* genes are unique to plants and play essential roles in leaf and floral organ development, organ polarity, and meristem determinacy (Floyd and Bowman, 2007; Finet et al., 2016).

Phylogenetic analyses classify *YABBY* genes into five conserved subfamilies across seed plants: CRABS CLAW (CRC), FILAMENTOUS FLOWER (FIL)/YABBY3 (YAB3), INNER NO OUTER (INO), YABBY2 (YAB2), and YABBY5 (YAB5) (Bowman, 2000; Yamada et al., 2001). FIL, YAB3, YAB2, and YAB5 primarily contribute to lateral organ development, while CRC is crucial for carpel and nectary development (Chen et al., 1999; Siegfried et al., 1999). INO is uniquely involved in the development of the outer integument of the ovule, which ultimately gives rise to the seed coat (Villanueva et al., 1999; Filyushin et al., 2018).

Due to their pivotal developmental functions, *YABBY* genes have been characterized in a wide range of plant species. Their gene number varies across taxa—for instance, 9 genes in tomato (*Solanum lycopersicum*), 8 in rice (*Oryza sativa*), 12 in Chinese cabbage (*Brassica rapa*), and 13 in maize (*Zea mays*) (Toriba et al., 2007; Hou et al., 2019; Huang et al., 2013; Cao et al., 2015). Cotton species (*Gossypium arboreum* and *G. raimondii*) harbor 12 *YABBY* genes each, while *G. hirsutum* contains 23. Notably, 55 *YABBY* genes have been identified in seven *Magnolia* species and 54 in eight orchid species (Yang et al., 2018; Chen et al., 2020; Liu et al., 2022).

Beyond development, YABBY transcription factors also contribute to abiotic stress responses. For example, *GmYABBY10* in soybean acts as a negative regulator of drought tolerance, where its overexpression results in increased drought sensitivity (Zhao et al., 2017). In rice, *OsYABBY6* has a similar function; its downregulation enhances osmolyte accumulation and reduces water loss, thereby improving drought resistance (Zuo et al., 2024). Similarly, overexpression of *AcYABBY4* in *Arabidopsis thaliana* leads to salt stress susceptibility, evidenced by decreased root length under salt treatment (Li et al., 2019). In *Platycodon grandiflorus*, *PgYABBY5* is associated with drought response pathways (Kong et al., 2023). These studies underscore the diverse and complex regulatory roles of *YABBY* genes in stress adaptation, highlighting their potential as targets for crop improvement.

Sunflower (*Helianthus annuus L.*) is a globally significant oilseed crop valued for its adaptability to diverse environmental conditions. It is resilient against a variety of stresses, including heat, cold, drought, salinity, and heavy metal toxicity, making it an ideal candidate for studying stress tolerance mechanisms (Song et al., 2024). Despite previous studies on TF families such as MYB and bZIP in sunflower (Li et al., 2020; Rahman et al., 2023), the *YABBY* gene family has not been previously characterized in this species.

Given the agricultural importance of sunflower, this study aims to perform a comprehensive genome-wide identification and characterization of *YABBY* genes in *H. annuus*. By integrating bioinformatic analyses with transcriptomic profiling, we seek to elucidate the structural, evolutionary, and functional attributes of the *YABBY* gene family. Two Turkish cultivars—Turay (drought-sensitive) and SUN 2235 (drought-tolerant)—were subjected to drought stress, and expression analyses using RNA-seq and qRT-PCR were performed to investigate cultivar-specific transcriptional responses.

MATERIAL AND METHODS

Identification of *YABBY* Gene Family Members in *Helianthus annuus*

YABBY gene family sequences were identified in *H. annuus* genome using the Phytozome v13 database (http://www.phytozome.net) (Goodstein et al., 2012). Searches were performed using the Pfam accession number (PF04690) as a keyword and confirmed via BLASTp and Hidden Markov Model (HMM) searches in the EMBL-EBI database (http://www.ebi.ac.uk). All retrieved protein sequences were further verified for the presence of the YABBY domain using HMMER. To characterize the identified YABBY proteins, BLASTp analyses were conducted against the NCBI non-redundant database. Physicochemical properties, including molecular weight (MW) and isoelectric point (pI), were computed using the ProtParam tool available on the ExPASy server (http://web.expasy.org/protparam).

Gene Structure, Chromosomal Localization, and Conserved Motif Composition

The exon-intron organization of the *HaYABBY* genes was visualized using the Gene Structure Display Server v2.0 (GSDS; https://gsds.gao-lab.org/) (Guo et al., 2007). Chromosomal positions were determined using the genome annotation files (GFF) from Phytozome v13 and visualized via TBtools software (Chen et al., 2020). Conserved motifs among *HaYABBY* proteins were identified using the MEME Suite (v5.5.7; http://meme-suite.org/).

Phylogenetic Analysis and Multiple Sequence Alignment

Multiple sequence alignments of the *HaYABBY* proteins were conducted using ClustalW (Tamura et al., 2011). Phylogenetic relationships were inferred using the Neighbor-Joining (NJ) method with 1000 bootstrap replicates in MEGA 12. The resulting phylogenetic tree was visualized and annotated using the Interactive Tree of Life (iTOL; http://itol.embl.de/index.shtml) (Letunic and Bork, 2016).

Promoter Analysis and Subcellular Localization

Promoter regions (1500 bp upstream of the transcription start site) of *HaYABBY* genes were extracted from the *H. annuus* genome and analyzed for cis-regulatory elements using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/). Subcellular localization of each *HaYABBY* protein was predicted using the WoLF PSORT tool (https://www.genscript.com/wolf-psort.html) (Horton et al., 2007).

In Silico miRNA Target Prediction

Known *H. annuus* miRNA sequences were retrieved from the PmiREN v22.1 database (http://www.pmiren.com). miRNA target sites were predicted using the psRNATarget server (http://plantgrn.noble.org/psRNATarget) with default settings (Zhang et al., 2006). Predicted targets were validated by BLASTX against *H. annuus* expressed sequence tag (EST) databases in NCBI, using an E-value threshold of $\leq 1e^{-10}$.

Gene Duplication and Synteny Analysis

Genome and GFF3 annotation files for *H. annuus*, *Gossypium hirsutum*, and *Vitis vinifera* were downloaded from the JGI Data Portal (https://data.jgi.doe.gov/). Gene duplication events were detected using the MCScanX toolkit integrated in TBtools. Syntenic relationships between *H. annuus* and the selected species were visualized using the Dual Synteny Plotter module in TBtools. The Ka (non-synonymous), Ks (synonymous), and Ka/Ks ratios for duplicated gene pairs were calculated to assess evolutionary selection pressure. Divergence times (in million years ago, MYA) were estimated using the formula $T = Ks / (2\lambda)$, where $\lambda = 6.56 \times 10^{-9}$ (Yang et al., 2000).

Tissue-Specific Expression Profiling of *HaYABBY* Genes

Tissue-specific expression data for *HaYABBY* genes were retrieved from the NCBI Sequence Read Archive (SRA) using the following accession numbers: SRR4996822 (ligule), SRR4996800 (bract), SRR4996799 (stem), SRR4996833 (corolla), SRR4996831 (ovary), SRR4996808 (RF ovary), SRR4996821 (leaves), SRR4996828 (roots), SRR4996809 (stamen), and SRR4996814 (pistil). Transcript abundance was measured using log_2-transformed values (Büyük et al., 2016), and expression patterns were visualized via heatmaps generated in TBtools (Chen et al., 2020).

Expression Profiling of *HaYABBY* Genes Under Drought Stress

To assess the expression dynamics of *HaYABBY* genes under drought conditions, RNA-seq datasets from drought-sensitive and drought-tolerant cultivars were analyzed. The SRA accession numbers were as follows: SRR17624347 (tolerant, day 0), SRR17624344 (tolerant, day 7), SRR17624338 (tolerant, day 14), SRR17624351 (sensitive, day 0), SRR17624361 (sensitive, day 7), and SRR17624341 (sensitive, day 14) (Neupane et al., 2019). Expression levels were calculated as log_2-transformed values and visualized using heatmaps in TBtools (Büyük et al., 2016).

Plant materials, growth conditions, and drought stress treatments

Seeds of *Helianthus annuus* cultivars with contrasting drought responses—Sun-2235 (tolerant) and Turay (sensitive)—were kindly provided by the Aegean Agricultural Research Institute, Izmir, Türkiye (Celik Altunoglu et al., 2018; Ceylan et al., 2023). Following surface sterilization, the seeds were germinated and cultivated in a hydroponic system containing one-tenth strength Hoagland nutrient solution under controlled environmental conditions (25°C, 70% relative humidity, and 250 μmol m^{-2} s^{-1} light intensity).

Drought stress was imposed by complete water withholding according to the protocol described by Okay et al., (2024). Seeds of both sunflower cultivars, Sun-2235 and Turay, were transferred to weight-adjusted soil pots and grown under controlled growth chamber conditions (25°C, 70% relative humidity, 20,000 LUX light intensity, 16-hour light/8-hour dark photoperiod). Pot positions were regularly rotated to ensure uniform light exposure. After 7 days of establishment with regular watering, water was completely withheld from stress-treated plants while control plants continued to receive regular irrigation. Drought stress progression was monitored gravimetrically by measuring pot weights every 2 days. When 20% weight loss relative to initial weight was reached (9th day after water withholding), drought-stressed plants displayed characteristic stress symptoms (curved and pale leaves, reduced height, continuous weight loss), while control plants showed normal growth and increased biomass. Leaf, stem, and root tissues were harvested, immediately frozen in liquid nitrogen, and stored at -80°C for subsequent molecular analyses.

RNA Isolation, cDNA Synthesis, and Quantitative Real-Time PCR (qRT-PCR) Analysis

Total RNA was extracted from harvested plant tissues using the Favorgen Total Plant RNA Isolation Mini Kit (Biotech Corp., Ping-Tung, Taiwan), following the manufacturer's protocol. RNA quality was assessed on 1.6% agarose gel electrophoresis, while concentration and purity were determined using a NanoDrop Lite spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

First-strand complementary DNA (cDNA) was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Basel, Switzerland) in accordance with the manufacturer's instructions. Specific primers for qRT-PCR were designed using the PrimerQuest Tool (https://eu.idtdna.com/pages/tools/primerquest). Quantitative real-time PCR was conducted using iTaq Universal SYBR Green Supermix (Bio-Rad, USA) on a CFX96 Real-Time PCR Detection System (Bio-Rad, USA), following the amplification protocol previously described by Büyük et al. (2019). Three biological replicates were used for each analysis, with two technical replicates per biological replicate.

Gene expression levels were quantified using the $2^{-\Delta\Delta CT}$ method, with *actin* serving as the internal reference gene (Livak and Schmittgen, 2001). Statistical analyses were performed using GraphPad Prism 9 software. Two-way ANOVA was applied followed by Fisher's Least Significant Difference (LSD) test, with statistical significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Identification of *HaYABBY* Genes in the *Helianthus annuus* Genome

In this study, a total of 14 putative *HaYABBY* genes were identified in the *Helianthus annuus* genome using *in silico* bioinformatic approaches, as described in the Materials and Methods section. These genes were designated from *HaYABBY01* to *HaYABBY14* based on their physical locations on the chromosomes. The key features of each gene are summarized in Table 1.

The predicted *HaYABBY* proteins ranged from 94 amino acids (*HaYABBY01*) to 229 amino acids (*HaYABBY04* and *HaYABBY09*) in length. Their molecular weights (MW) were estimated to range from 10.64 to 25.19 kDa, with theoretical isoelectric points (pI) varying between 4.98 and 9.40 (Table 1). The chromosomal distribution analysis revealed that chromosomes 6, 12, 13, and 17 each contained two *HaYABBY* genes, while chromosomes 3, 4, 5, 10, 14, and 15 harbored one gene each. Collectively, the *HaYABBY* gene family members were found to be distributed across 10 of the 17 *H. annuus* chromosomes (Table 1; Figure 1).

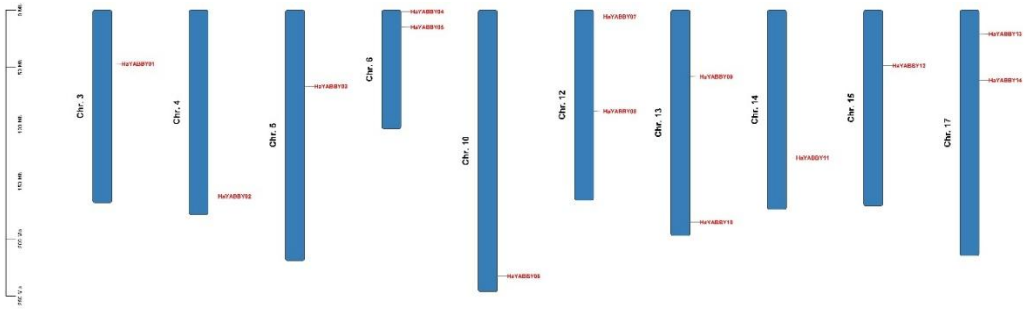

Figure 1. Chromosomal distribution of *HaYABBY* genes in *Helianthus annuus*.

A total of 14 *HaYABBY* genes were mapped onto 10 different chromosomes of the *H. annuus* genome. Chromosomes are represented as vertical blue bars labeled with their respective chromosome numbers. Gene names are displayed in red, and their physical positions are indicated along the left vertical axis in megabases (Mb). This distribution shows that the *HaYABBY* genes are unevenly dispersed throughout the genome.

Phylogenetic Analysis, Gene Structure, Conserved Motifs, and Subcellular Localization of *HaYABBY* Genes

To elucidate the evolutionary relationships of the *HaYABBY* gene family, a phylogenetic tree was constructed using 59 YABBY protein sequences from *Helianthus annuus*, *Arabidopsis thaliana*, *Solanum lycopersicum* (tomato), *Vitis vinifera* (grapevine), *Zea mays* (maize), and *Oryza sativa* (rice) (Figure 2). These reference species were selected based on their frequent use in previous studies on the *YABBY* gene family, providing a robust phylogenetic framework.

Initial analysis clustered the *HaYABBY* proteins into three major groups, which were further classified into five distinct subfamilies: **FIL/YAB3**, **YAB2**, **YAB5**, **INO**, and **CRC**, consistent with earlier reports in *Arabidopsis*, tomato, and grapevine (Zhang et al., 2019). The FIL/YAB3 subfamily was the largest, comprising seven *HaYABBY* members, suggesting its prominent role in the regulation of leaf and floral organ development in sunflower, in line with similar findings in other dicots. The YAB5 subfamily included three *HaYABBY* genes, potentially involved in organ polarity and differentiation. Both the INO and CRC subfamilies contained two members each, likely reflecting their conserved functions in ovule and carpel development, respectively.

Interestingly, no *HaYABBY* genes were classified within the YAB2 subfamily, implying either a lineage-specific loss or functional divergence in *H. annuus*. This observation supports the idea that, although the core structure of the YABBY family is generally conserved across plant species, specific subfamilies may have undergone species-specific evolutionary changes to meet developmental and environmental demands in sunflower.

Moreover, *HaYABBY* proteins were grouped into phylogenetic clades according to sequence homology, suggesting functional conservation within dicot species. Notably, monocot *YABBY* genes were absent from the YAB5 subfamily, aligning with previous evidence of evolutionary divergence between monocots and dicots in *YABBY* gene function and expansion (Romanova et al., 2021; Jie et al., 2022; Zhang et al., 2013).

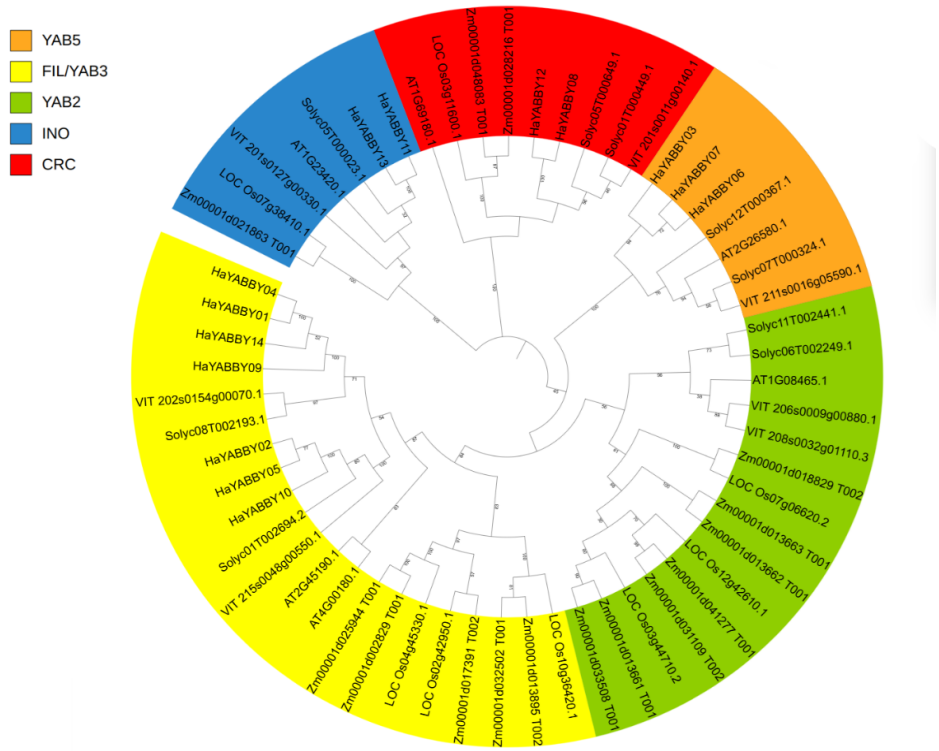

Figure 2. Phylogenetic tree of YABBY proteins from *Helianthus annuus*, *Arabidopsis thaliana*, *Solanum lycopersicum*, *Vitis vinifera*, *Zea mays*, and *Oryza sativa*. The neighbor-joining tree was constructed using 59 YABBY protein sequences and 1000 bootstrap replicates. YABBY proteins are grouped into five subfamilies: FIL/YAB3 (yellow), YAB2 (green), YAB5 (orange), INO (blue), and CRC (red). Colored blocks represent subfamily classification and highlight evolutionary relationships among dicot and monocot species. *HaYABBY* proteins are labeled and their distribution across subfamilies is shown.

According to Figure 3, the number of introns within *HaYABBY* genes ranged from 1 to 6, reflecting both conserved and variable structural characteristics across the gene family. In addition to the conserved YABBY superfamily domain—which is essential for the identification and classification of *HaYABBY* members—most proteins also contained the HMG-box superfamily domain. Notably, *HaYABBY03* was the only gene lacking this domain. This observation aligns with previous studies on the *YABBY* gene family, which have reported that although the HMG-box domain is widely conserved, certain members may lack it due to evolutionary divergence (Xia et al., 2021).

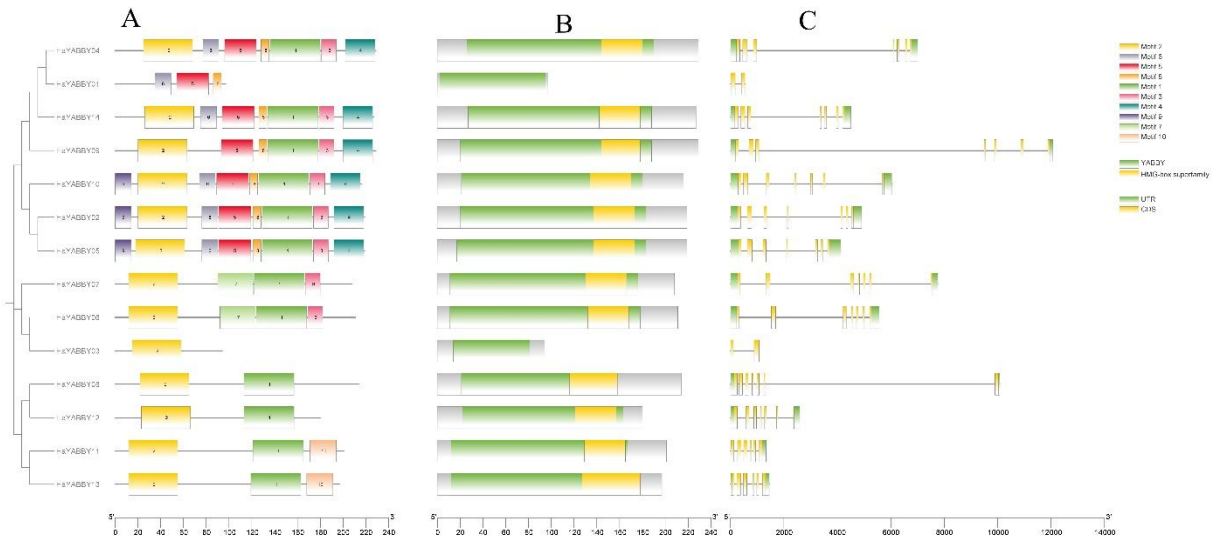

Figure 3. Structural and Functional Characterization of *HaYABBY* Genes in *Helianthus annuus*. (A) Conserved motif composition of *HaYABBY* proteins, as predicted by MEME analysis. (B) Conserved protein domains identified within *HaYABBY* members, including the YABBY and HMG-box domains. (C) Gene structure analysis showing exon-intron organization; exons are depicted as yellow boxes, and introns as black lines.

The subcellular localization of *HaYABBY* proteins was predicted using WoLF PSORT. Results indicated that 11 out of the 14 *HaYABBY* proteins are predominantly localized in the nucleus, supporting their proposed roles in transcriptional regulation. In contrast, *HaYABBY01* was predicted to be localized extracellularly, *HaYABBY03* in the cytoplasm, and *HaYABBY08* in the chloroplast. These findings are consistent with previous studies reporting nuclear localization of YABBY proteins, highlighting their conserved functions in plant development and gene expression regulation (Shen et al., 2022; Hussain et al., 2024).

Gene Duplication and Synteny Analysis of *HaYABBY* Genes

To investigate the duplication patterns of *HaYABBY* genes, both segmental and tandem duplication events were examined within the *Helianthus annuus* genome. Segmental duplication analysis revealed two gene pairs (*HaYABBY04–HaYABBY09* and *HaYABBY05–HaYABBY12*) that likely originated from segmental duplication events (Table 2), suggesting that such events contributed to the expansion of the *HaYABBY* gene family. The calculated Ka/Ks ratios for these duplicated pairs were 0.119 and 0.197, respectively—both less than 1—indicating that these gene pairs have been subject to purifying selection, thereby conserving their functional roles (Liu et al., 2022). The estimated divergence times based on synonymous substitution rates (Ks) were approximately 2.39 and 2.63 million years ago (MYA). No tandem duplication events were identified, implying that tandem duplications did not contribute to the diversification of *HaYABBY* genes in sunflower.

To further investigate the evolutionary conservation of *HaYABBY* genes, a synteny analysis was performed between *H. annuus*, *Gossypium hirsutum* (upland cotton), and *Vitis vinifera* (grapevine). The analysis identified nine orthologous *HaYABBY* gene pairs between *H. annuus* and *G. hirsutum*, and eight pairs between *H. annuus* and *V. vinifera* (Figure 4). Ka/Ks ratio calculations for these orthologous gene pairs revealed that all ratios were below 1, indicating that *HaYABBY* genes across these species have predominantly evolved under strong purifying selection, preserving their structural and functional integrity over evolutionary time.

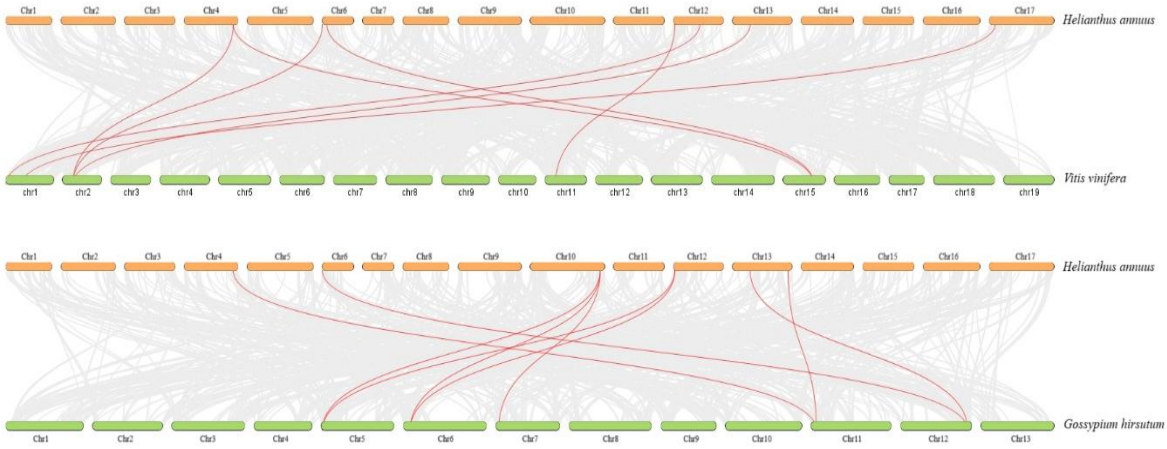

Figure 4. *Syntenic relationships of YABBY genes among Helianthus annuus, Vitis vinifera,* and *Gossypium hirsutum*. Gray shaded regions in the background denote collinear blocks across the genomes, whereas blue connecting lines indicate syntenic orthologous *YABBY* gene pairs identified between species.

Analysis of Cis-Regulatory Elements in *HaYABBY* Promoter Regions

Cis-regulatory elements (CREs) within promoter regions are critical for orchestrating gene expression in response to developmental cues and environmental stimuli (Yamaguchi-Shinozaki et al., 2005). Analysis of the *HaYABBY* promoter regions revealed a wide array of CREs, suggesting their involvement in diverse regulatory pathways. In total, 186 CREs were identified across the promoter regions of *HaYABBY* genes, highlighting the complexity of their transcriptional regulation (Figure 5).

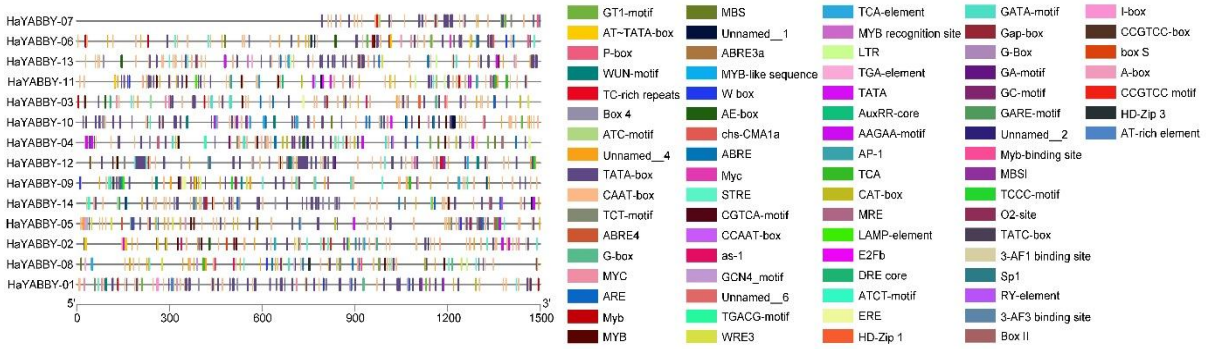

Figure 5. Cis-regulatory elements identified in the 1500 bp upstream promoter regions of *HaYABBY* genes. Different cis acting elements are shown as colored rectangles.

Light-responsive elements such as the G-box, Box 4, and GT1-motif were ubiquitously distributed, implying light-dependent regulation of *HaYABBY* gene expression (Hudson and Quail, 2003). Several hormone-responsive elements, including ABRE (abscisic acid response), TGACG-motif (MeJA response), and TGA-element (auxin response), were also present, indicating modulation by hormonal signaling pathways (Guiltinan et al., 1990; Hobo et al., 1999). Additionally, stress-related elements such as MYB, MBS, and the WUN-motif were detected, suggesting potential roles in abiotic and biotic stress responses (Abe et al., 2003; Yamaguchi-Shinozaki and Shinozaki, 2005).

Core promoter elements, including the TATA-box and CAAT-box, were consistently found across all *HaYABBY* genes, reaffirming their essential role in transcription initiation. The observed variation in the number and distribution of CREs among different *HaYABBY* genes may reflect functional divergence and differential gene regulation. Overall, these findings provide important insights into the regulatory complexity of the *HaYABBY* gene family and suggest their involvement in light perception, hormonal control, stress adaptation, and developmental regulation in *Helianthus annuus*.

miRNA Target Prediction for *HaYABBY* Genes

miRNA target prediction analysis revealed that *HaYABBY* genes are potentially regulated by four sunflower-specific miRNAs: Han-miR156b, Han-miR156c, Han-miR5709, and Han-miR5771. Among these, Han-miR156b and Han-miR156c were both predicted to target *HaYABBY05* at the same binding region, suggesting a potential cooperative or redundant regulatory mechanism. Additionally, *HaYABBY05* was also targeted by Han-miR5709, while Han-miR5771 was predicted to regulate *HaYABBY09*.

miR156 has been widely reported to play a critical role in various aspects of plant development and stress responses, supporting the likelihood of its regulatory involvement in the expression of *HaYABBY* genes (Sang et al., 2023). Although the biological functions of Han-miR5709 and Han-

miR5771 in *Helianthus annuus* have not yet been fully elucidated, their predicted interactions with *HaYABBY05* and *HaYABBY09*, respectively, suggest that these miRNAs may contribute to post-transcriptional regulation under developmental or environmental stress conditions. These results point to a complex regulatory network involving miRNA-mediated modulation of *HaYABBY* gene expression, which may fine-tune their roles in developmental processes and stress adaptation in sunflower.

Tissue-Specific Expression Patterns of *HaYABBY* Genes

To investigate the spatial expression dynamics of *HaYABBY* genes, publicly available RNA-seq datasets were analyzed across 10 different tissues of *Helianthus annuus*. A heatmap representing normalized expression levels was generated to visualize tissue-specific expression patterns.

The analysis revealed that *HaYABBY05* exhibited the highest expression levels across all examined tissues, suggesting its involvement in diverse physiological and developmental processes. In contrast, *HaYABBY13* displayed the lowest expression levels, indicating a possibly limited or highly specialized function. Interestingly, *HaYABBY12* showed marked expression in the ray floret ovary, implying a potential role in floral organogenesis and reproductive development.

These findings underscore the differential spatial regulation of *HaYABBY* genes and suggest their diverse functional roles in tissue-specific growth and organ development in sunflower (*Figure 6*).

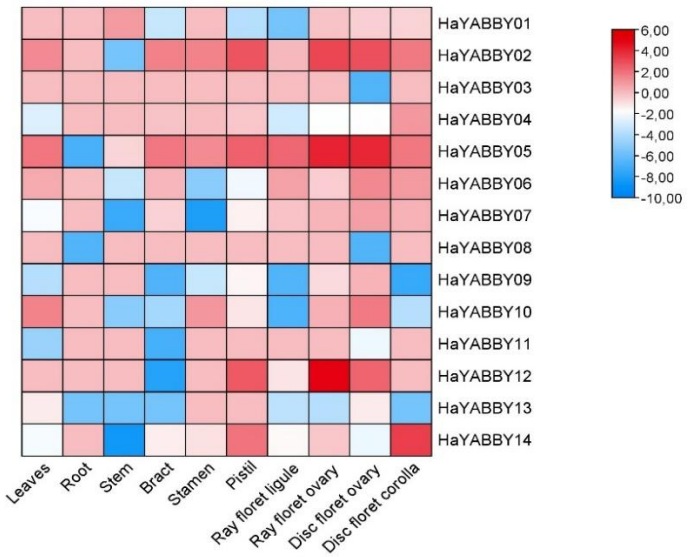

Figure 6. Heatmap of Tissue-Specific Expression Profiles of *HaYABBY* Genes in *H. annuus*. Expression data were $\log_2$-transformed based on RNA-seq datasets across various vegetative and reproductive tissues, including leaves, roots, stems, bracts, stamens, pistils, ray florets, and disc florets. Red shades indicate higher expression levels, while blue shades represent lower expression. The profiles reveal differential expression patterns, suggesting tissue-specific functional divergence among *HaYABBY* genes.

RNA-Seq and qRT-PCR Analyses of *HaYABBY* Genes Under Drought Stress

To validate the RNA-seq results and investigate the transcriptional responses of *HaYABBY* genes to drought stress, three representative genes (*HaYABBY04*, *HaYABBY05*, and *HaYABBY07*) were selected for qRT-PCR analysis. These genes were chosen based on their distinct expression profiles under drought conditions observed in the RNA-seq analysis, their predicted regulation by stress-responsive miRNAs, and the presence of stress-related cis-regulatory elements in their promoter regions. Statistical significance was evaluated using two-way ANOVA followed by Fisher's Least Significant Difference (LSD) test ($p < 0.05$), as described in the Materials and Methods section.

qRT-PCR analysis was performed on root, stem, and leaf tissues of two sunflower cultivars—SUN 2235 (drought-tolerant) and Turay (drought-sensitive)—grown under both control and drought conditions to examine genotype- and tissue-specific expression changes (Figure 7).

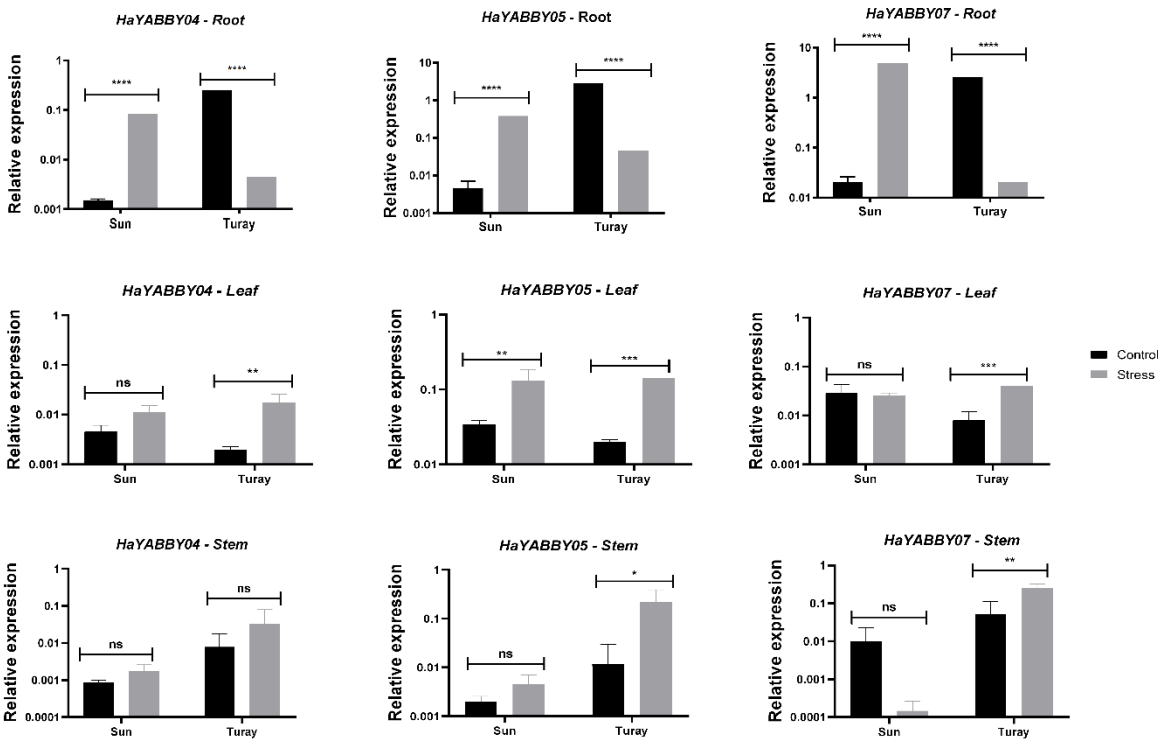

Figure 7. Relative expression levels of *HaYABBY* genes in SUN 2235 and Turay cultivars under drought stress. Gene expression levels were normalized to ACT and calculated using the $2^{-\Delta\Delta CT}$ method. Each reaction was performed with three biological and two technical replicates. Bars represent mean values ± standard error. Asterisks indicate statistically significant differences between control and drought-stressed samples (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns = not significant).

In stem tissues, *HaYABBY04* did not show statistically significant expression changes in either cultivar. In contrast, *HaYABBY05* was significantly upregulated only in Turay. *HaYABBY07* exhibited a marked induction in Turay but remained unchanged in SUN 2235, indicating a cultivar-specific stress response.

In leaf tissues, both *HaYABBY04* and *HaYABBY05* were significantly upregulated under drought conditions in both cultivars, with *HaYABBY05* showing the highest fold-change. *HaYABBY07* was significantly upregulated only in Turay, suggesting a differential transcriptional regulation between the cultivars.

Notably, root tissues showed the most pronounced divergence. All three *HaYABBY* genes were strongly upregulated in SUN 2235 under drought conditions, while their expression was markedly downregulated in the roots of Turay. This contrasting pattern suggests that activation of these genes in root tissues may contribute to enhanced drought tolerance in the tolerant cultivar.

Together, these results highlight that the expression of *HaYABBY* genes is both tissue- and cultivar-specific. Among them, *HaYABBY05* demonstrated consistent and robust upregulation across all examined tissues under drought stress, underscoring its potential role in drought response. Furthermore, the enhanced expression of these genes in the roots of the tolerant cultivar suggests their involvement in root-mediated adaptive responses to drought in sunflower.

These findings align with previous studies. For instance, soybean *GmYABBY3*, *GmYABBY10*, and *GmYABBY16* were shown to be upregulated in response to drought and other abiotic stresses (Zhao et al., 2017). Similarly, in *Rosa roxburghii*, *RrYABBY1* and *RrYABBY5* were downregulated under drought, whereas *RrYABBY2*, *RrYABBY3*, *RrYABBY4*, and *RrYABBY6* were significantly upregulated at different time points (Wen et al., 2025). These examples mirror the gene-specific and context-dependent expression responses observed in our study, further supporting the role of YABBY transcription factors in orchestrating drought adaptation in plants.

CONCLUSION

In this study, we performed the first comprehensive genome-wide characterization of the YABBY transcription factor family in *H. annuus*, integrating *in silico* identification, structural analysis, and expression profiling under drought stress. Our findings suggest that while sunflower *YABBY* genes maintain conserved roles in transcriptional regulation, they have undergone lineage-specific divergence, as evidenced by the loss of the YAB2 subfamily. The presence of diverse cis-regulatory elements and miRNA targets further indicates that these genes operate under a multi-layered regulatory network responsive to environmental stimuli.

Notably, our data establish a strong link between *HaYABBY* expression and drought stress adaptation. The contrasting expression profiles between tolerant and sensitive cultivars, particularly

the significant upregulation of key genes like *HaYABBY05* in the roots of tolerant plants, point to a genotype-dependent mechanism for drought resilience. Consequently, *HaYABBY05* emerges as a primary candidate for functional validation, and the family as a whole offers promising molecular targets for biotechnological interventions aimed at improving abiotic stress tolerance in sunflower breeding programs.

Table 1. Molecular Characteristics, Genomic Locations, and Subcellular Localization Predictions of the Identified HaYABBY Proteins in *Helianthus annuus*

Gene Name	Transcript name	Chr no.	Chr. Location	Preotein Length(aa)	pI	Molecular Weight (Da)	Subcellular Localization	NCBI Accession ID
HaYABBY01	HanXRQChr03g0067451	3	46877407-46877949	97	9,06	10727,29	Extracellular	KAJ0934931.1
HaYABBY02	HanXRQChr04g0122691	4	162728619-162733521	219	7,21	24437,79	Nuclear	XP_022035893.1
HaYABBY03	HanXRQChr05g0139121	5	66528857-66529924	94	5,28	10640,20	Cytoplasm	KAF5805244.1
HaYABBY04	HanXRQChr06g0164191	6	1146569-1153573	229	8,15	25139,60	Nuclear	XP_021969003.1
HaYABBY05	HanXRQChr06g0169631	6	14888822-14892948	219	7,21	24420,73	Nuclear	XP_021969572.1
HaYABBY06	HanXRQChr10g0315651	10	232554971-232560538	211	9,40	23622,70	Nuclear	XP_021990247.1
HaYABBY07	HanXRQChr12g0356171	12	5604453-5612212	208	9,02	23395,53	Nuclear	XP_021996680.1
HaYABBY08	HanXRQChr12g0375091	12	88466959-88477044	214	9,06	24338,20	Chloroplast	KAF5778649.1
HaYABBY09	HanXRQChr13g0395021	13	58095926-58107992	229	8,87	25195,88	Nuclear	XP_022000346.1
HaYABBY10	HanXRQChr13g0423101	13	185410574-185416614	216	6,72	24136,35	Nuclear	XP_022002694.1
HaYABBY11	HanXRQChr14g0446751	14	129027000-129028331	201	6,13	22467,35	Nuclear	KAF5801871.1
HaYABBY12	HanXRQChr15g0476711	15	48504203-48506793	180	9,28	19765,53	Nuclear	XP_022011699.1
HaYABBY13	HanXRQChr17g0539491	17	20598931-20600385	197	4,98	21878,58	Nuclear	XP_022021086.1
HaYABBY14	HanXRQChr17g0549371	17	61189446-61193955	227	8,55	25164,39	Nuclear	XP_022022911.1

Table 2. Segmentally Duplicated *HaYABBY* Gene Pairs in *Helianthus annuus* with Evolutionary Parameters (Ka, Ks, Ka/Ks, and Divergence Time Estimates)

Duplicated YABBY Gene 1	Duplicated YABBY Gene 2	Ka	Ks	Ka/Ks	Date (MYA) T=Ks/2λ
HaYABBY02	*HaYABBY05*	0.03735	0.31132	0.11997	2.39
HaYABBY06	*HaYABBY07*	0.06756	0.34213	0.19749	2.63

REFERENCES

Abe, H., Urao, T., Ito, T., Seki, M., Shinozaki, K., & Yamaguchi-Shinozaki, K. (2003). Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *The Plant Cell, 15*(1), 63–78.

Bowman, J. L. (2000). The YABBY gene family and abaxial cell fate. *Current Opinion in Plant Biology, 3*(1), 17–22.

Büyük, İ., İnal, B., Ilhan, E., Tanriseven, M., Aras, S., & Erayman, M. (2016). Genome-wide identification of salinity responsive HSP70s in common bean. *Molecular Biology Reports, 43*, 1251–1266.

Büyük, İ., İlhan, E., Şener, D., Özsoy, A. U., & Aras, S. (2019). Genome-wide identification of CAMTA gene family members in *Phaseolus vulgaris* L. and their expression profiling during salt stress. *Molecular Biology Reports, 46*, 2721–2732.

Cao, Y., Lang, Z.-H., & Wang, L. (2015). Characteristics and expression analysis of transcription factor YABBY family in maize. *Journal of Agricultural Science & Technology, 17*, 32–41.

Chen, Q., Atkinson, A., Otsuga, D., Christensen, T., Reynolds, L., & Drews, G. N. (1999). The *Arabidopsis* FILAMENTOUS FLOWER gene is required for flower formation. *Development, 126*(12), 2715–2726.

Chen, Y. Y., Hsiao, Y. Y., Chang, S. B., Zhang, D., Lan, S. R., Liu, Z. J., & Tsai, W. C. (2020). Genome-wide identification of YABBY genes in Orchidaceae and their expression patterns in *Phalaenopsis* orchid. *Genes, 11*(9), 955.

Filyushin, M. A., Slugina, M. A., Pyshnaya, O. N., Kochieva, E. Z., & Shchennikova, A. V. (2018). Structure analysis of INNER NO OUTER (INO) homologs in *Capsicum* species. *Russian Journal of Genetics, 54*, 753–757.

Finet, C., Floyd, S. K., Conway, S. J., Zhong, B., Scutt, C. P., & Bowman, J. L. (2016). Evolution of the YABBY gene family in seed plants. *Evolution & Development, 18*(2), 116–126.

Floyd, S. K., & Bowman, J. L. (2007). The ancestral developmental tool kit of land plants. *International Journal of Plant Sciences, 168*(1), 1–35.

Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N., & Rokhsar, D. S. (2012). Phytozome: A comparative platform for green plant genomics. *Nucleic Acids Research, 40*(D1), D1178–D1186.

Grotewold, E. (2008). Transcription factors for predictive plant metabolic engineering: Are we there yet? *Current Opinion in Biotechnology, 19*(2), 138–144.

Guiltinan, M. J., Marcotte, W. R., Jr., & Quatrano, R. S. (1990). A plant leucine zipper protein that recognizes an abscisic acid response element. *Science, 250*(4978), 267–271.

Guo, A. Y., Zhu, Q. H., Chen, X., & Luo, J. C. (2007). GSDS: A gene structure display server. *Hereditas, 29*(8), 1023–1026.

Guo, J., Zhou, X. T., Dai, K. L., Yuan, X. Y., Guo, P. Y., Shi, W. P., & Zhou, M. X. (2022). Comprehensive analysis of YABBY gene family in foxtail millet (*Setaria italica*) and functional characterization of SiDL. *Journal of Integrative Agriculture, 21*(10), 2876–2887.

Hobo, T., Asada, M., Kowyama, Y., & Hattori, T. (1999). ACGT-containing abscisic acid response element (ABRE) and coupling element 3 (CE3) are functionally equivalent. *The Plant Journal, 19*(6), 679–689.

Horton, P., Park, K. J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., & Nakai, K. (2007). WoLF PSORT: Protein localization predictor. *Nucleic Acids Research, 35*(Suppl. 2), W585–W587.

Hou, H., Wu, P., Gao, L., Zhang, C., & Hou, X. (2019). Characterization and expression profile analysis of YABBY family genes in Pak-choi (*Brassica rapa* ssp. *chinensis*) under abiotic stresses and hormone treatments. *Plant Growth Regulation, 87*, 421–432.

Huang, Z., Van Houten, J., Gonzalez, G., Xiao, H., & van der Knaap, E. (2013). Genome-wide identification, phylogeny and expression analysis of SUN, OFP and YABBY gene family in tomato. *Molecular Genetics and Genomics, 288*(3), 111–129.

Hudson, M. E., & Quail, P. H. (2003). Identification of promoter motifs involved in the network of phytochrome A-regulated gene expression by combined analysis of genomic sequence and microarray data. *Plant Physiology, 133*(4), 1605–1616.

Hussain, M., Javed, M. M., Sami, A., Shafiq, M., Ali, Q., Mazhar, H. S. U. D., Al-Ghamdi, K. S., El-Shehawi, A. M., Al-Zahrani, H., & Ali, D. (2024). Genome-wide analysis of plant specific YABBY transcription factor gene family in carrot (*Daucus carota*) and its comparison with *Arabidopsis*. *BMC Genomic Data, 25*(1), 26.

Iwase, A., Matsui, K., & Ohme-Takagi, M. (2009). Manipulation of plant metabolic pathways by transcription factors. *Plant Biotechnology, 26*(1), 29–38.

Jang, S., Hur, J., Kim, S. J., Han, M. J., Kim, S. R., & An, G. (2004). Ectopic expression of OsYAB1 causes extra stamens and carpels in rice. *Plant Molecular Biology, 56*, 133–143.

Kong, L., Sun, J., Jiang, Z., Ren, W., Wang, Z., Zhang, M., Xu, Y., & Xu, J. (2023). Identification and expression analysis of YABBY family genes in *Platycodon grandiflorus*. *Plant Signaling & Behavior, 18*(1), 2163069.

Letunic, I., & Bork, P. (2016). Interactive tree of life (iTOL) v3: An online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Research, 44*(W1), W242–W245.

Li, J., Liu, H., Yang, C., Wang, J., Yan, G., Si, P., Zhang, Z., & Xu, L. (2020). Genome-wide identification of MYB genes and expression analysis under different biotic and abiotic stresses in *Helianthus annuus* L. *Industrial Crops and Products, 143*, 111924.

Li, Z., Li, G., Cai, M., Priyadarshani, S. V., Aslam, M., Zhou, Q., Huang, X., Wang, X., Liu, Y., & Qin, Y. (2019). Genome-wide analysis of the YABBY transcription factor family in pineapple and functional identification of AcYABBY4 involvement in salt stress. *International Journal of Molecular Sciences, 20*(23), 5863.

Liu, H., Ye, H., Wang, J., Chen, S., Li, M., Wang, G., Zhou, Y., & Zhao, P. (2022). Genome-wide identification and characterization of YABBY gene family in *Juglans regia* and *Juglans mandshurica*. *Agronomy, 12*(8), 1914.

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *Methods, 25*(4), 402–408.

Neupane, S., Schweitzer, S. E., Neupane, A., Andersen, E. J., Fennell, A., Zhou, R., & Nepal, M. P. (2019). Identification and characterization of mitogen-activated protein kinase (MAPK) genes in sunflower (*Helianthus annuus* L.). *Plants, 8*(2), 28.

Okay, A., Kırlıoğlu, T., Durdu, Y. Ş., Akdeniz, S. Ş., Büyük, İ., & Aras, E. S. (2024). Omics approaches to understand the MADS-box gene family in common bean (*Phaseolus vulgaris* L.) against drought stress. *Protoplasma, 261*(4), 709–724.

Rahman, S., Rehman, A., Waqas, M., Mubarik, M. S., Alwutayd, K., AbdElgawad, H., Jalal, A., Azeem, F., & Rizwan, M. (2023). Genome-wide exploration of bZIP transcription factors and their contribution to alkali stress response in *Helianthus annuus*. *Plant Stress, 10*, 100204.

Romanova, M. A., Maksimova, A. I., Pawlowski, K., & Voitsekhovskaja, O. V. (2021). YABBY genes in the development and evolution of land plants. *International Journal of Molecular Sciences*, *22*(8), 4139.

Sang, Q., Fan, L., Liu, T., Qiu, Y., Du, J., Mo, B., Ma, Z., & Chen, X. (2023). MicroRNA156 conditions auxin sensitivity to enable growth plasticity in response to environmental changes in *Arabidopsis*. *Nature Communications, 14*(1), 1449.

Shen, Y., Li, X., Ma, G., Zhao, Y., Jiang, X., Gao, L., Li, C., & Liu, Y. (2022). Roles of YABBY transcription factors in the regulation of leaf development and abiotic stress responses in *Camellia sinensis*. *Beverage Plant Research, 2*(1), 1–10.

Siegfried, K. R., Eshed, Y., Baum, S. F., Otsuga, D., Drews, G. N., & Bowman, J. L. (1999). Members of the YABBY gene family specify abaxial cell fate in *Arabidopsis*. *Development, 126*(18), 4117–4128.

Song, H., Ji, X., Wang, M., Li, J., Wang, X., Meng, L., Wei, P., Xu, H., Niu, T., & Liu, A. (2024). Genome-wide identification and expression analysis of the Dof gene family reveals their involvement in hormone response and abiotic stresses in sunflower (*Helianthus annuus* L.). *Gene, 910*, 148336.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution, 28*(10), 2731–2739.

Toriba, T., Harada, K., Takamura, A., Nakamura, H., Ichikawa, H., Suzaki, T., & Hirano, H. Y. (2007). Molecular characterization of the YABBY gene family in *Oryza sativa* and expression analysis of OsYABBY1. *Molecular Genetics and Genomics, 277*, 457–468.

Villanueva, J. M., Broadhvest, J., Hauser, B. A., Meister, R. J., Schneitz, K., & Gasser, C. S. (1999). INNER NO OUTER regulates abaxial–adaxial patterning in *Arabidopsis* ovules. *Genes & Development, 13*(23), 3160–3169.

Wen, J., Luo, W., Chen, Y., An, H., Lu, M., Ma, W., & Nan, H. (2025). Identification, evolution, and function of YABBY transcription factors in *Rosa roxburghii* in response to drought stress. *Horticulture, Environment, and Biotechnology*, 1–17.

Yamada, T., Tobe, H., Imaichi, R., & Kato, M. (2001). Developmental morphology of the ovules of *Amborella trichopoda* (Amborellaceae) and *Chloranthus serratus* (Chloranthaceae). *Botanical Journal of the Linnean Society, 137*(3), 277–290.

Yamaguchi-Shinozaki, K., & Shinozaki, K. (2005). Organization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters. *Trends in Plant Science, 10*(2), 88–94.

Yang, Z., Gong, Q., Wang, L., Jin, Y., Xi, J., Li, Z., Qin, W., & Li, F. (2018). Genome-wide study of YABBY genes in upland cotton and their expression patterns under different stresses. *Frontiers in Genetics, 9*, 33.

Yang, Z., Nielsen, R., Goldman, N., & Pedersen, A. M. K. (2000). Codon-substitution models for heterogeneous selection pressure at amino acid sites. *Genetics, 155*(1), 431–449.

Zhang, B., Pan, X., Cobb, G. P., & Anderson, T. A. (2006). Plant microRNA: A small regulatory molecule with big impact. *Developmental Biology, 289*(1), 3–16.

Zhang, S., Wang, L., Sun, X., Li, Y., Yao, J., Nocker, S. V., & Wang, X. (2019). Genome-wide analysis of the YABBY gene family in grapevine and functional characterization of VvYABBY4. *Frontiers in Plant Science, 10*, 1207.

Zhang, X. L., Yang, Z. P., Zhang, J., & Zhang, L. G. (2013). Ectopic expression of BraYAB1-702, a member of YABBY gene family in Chinese cabbage, causes leaf curling, inhibition of development of shoot apical meristem and flowering stage delaying in *Arabidopsis thaliana*. *International Journal of Molecular Sciences, 14*(7), 14872–14891.

Zhao, S. P., Lu, D., Yu, T. F., Ji, Y. J., Zheng, W. J., Zhang, S. X., Du, H., & Cui, X. Y. (2017). Genome-wide analysis of the YABBY family in soybean and functional identification of GmYABBY10 involvement in high salt and drought stresses. *Plant Physiology and Biochemistry, 119*, 132–146.

Zhu, J. K. (2016). Abiotic stress signaling and responses in plants. *Cell, 167*(2), 313–324.

Zuo, J., Wei, C., Liu, X., Jiang, L., & Gao, J. (2024). Multifunctional transcription factor YABBY6 regulates morphogenesis, drought and cold stress responses in rice. *Rice, 17*(1), 69.