

# Whole-genome Identification and Expression of Lateral Organ Boundaries Domain Genes under Biotic and Abiotic Stresses in *Helianthus annuus*

Yingying ZHOU<sup>1</sup>, Juanjuan LI<sup>1</sup>, Kangni ZHANG<sup>1</sup>, Qian HUANG<sup>1</sup>, Jun ZHAO<sup>2</sup>, Weijun ZHOU<sup>1,\*</sup>

<sup>1</sup> Institute of Crop Science, Zhejiang University, Hangzhou 310058, China

<sup>2</sup> Inner Mongolia Agricultural University, Hohhot 010010, China

## INTRODUCTION

The lateral organ boundaries domain (LBD) protein, a family of plant-specific transcription factors, plays a crucial role in plant growth, phytohormone induction, and the response to abiotic stress. In this study, a comprehensive analysis was conducted to identify a total of 47 *LBD* genes in the sunflower (*Helianthus annuus* L.) whole-genome. By employing AlphaFold3, we have conducted a systematic investigation into the structures and functions of the *LBD* gene family in sunflowers. We also used Network analysis performed via WGCNA and enrichment analysis conducted through KEGG to indicated that the *LBD* gene family demonstrates significant involvement in regulating the growth, development, and different stress responses of sunflowers. This study provides a good basis for further investigation of the biological functions and evolution of *HaLBD* genes.

## MATERIALS AND METHODS

### Identification and Phylogenetic analysis

Ensembl genome database, TAIR, HMM3.0 (PF03195), BLAST, SMART, CDD v3.20, MEGA v11, iTOL

### Sequence Analysis

ExpAsy, CELLO v2.5, MEME v5.4.1, PlantCARE, TBtools

### Expression Patterns and Co-expression Analyses

RNA-seq data for sunflower tissues (SRP092742), under abiotic stresses (PRJNA869183), response to resistance against *Orobanche cumana* (PRJNA706194)

WGCNA (v1.72) in R, Cytoscape v.3.10.1, AlphaFold3, PyMOL

## RESULTS AND DISCUSSION

In this study, a comprehensive analysis was conducted to identify a total of 47 *LBD* genes in the *H. annuus* whole-genome. These genes were subsequently phylogenetically grouped into two distinct clusters (Class I and Class II) along with *LBDs* from *Arabidopsis* and *Oryza sativa*.

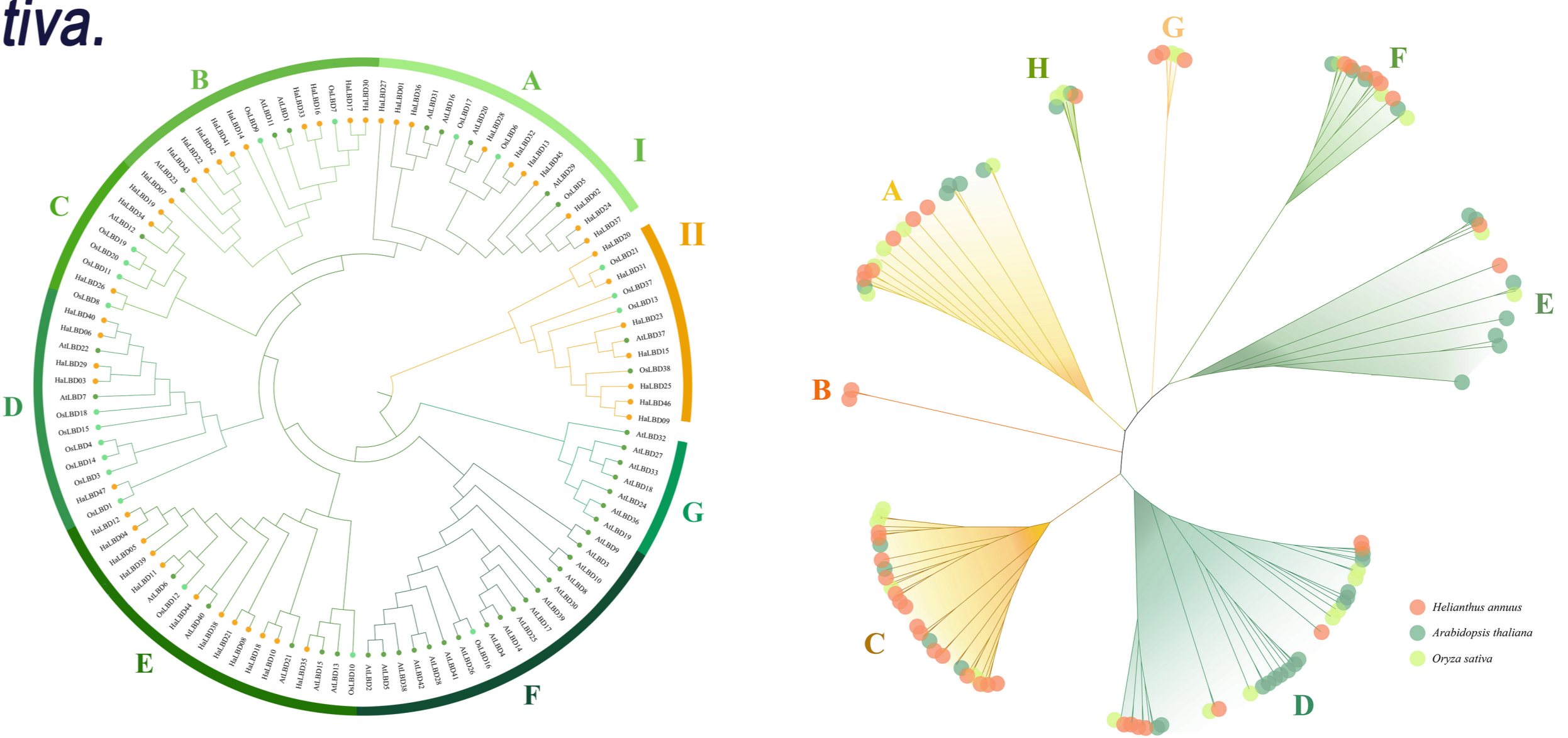
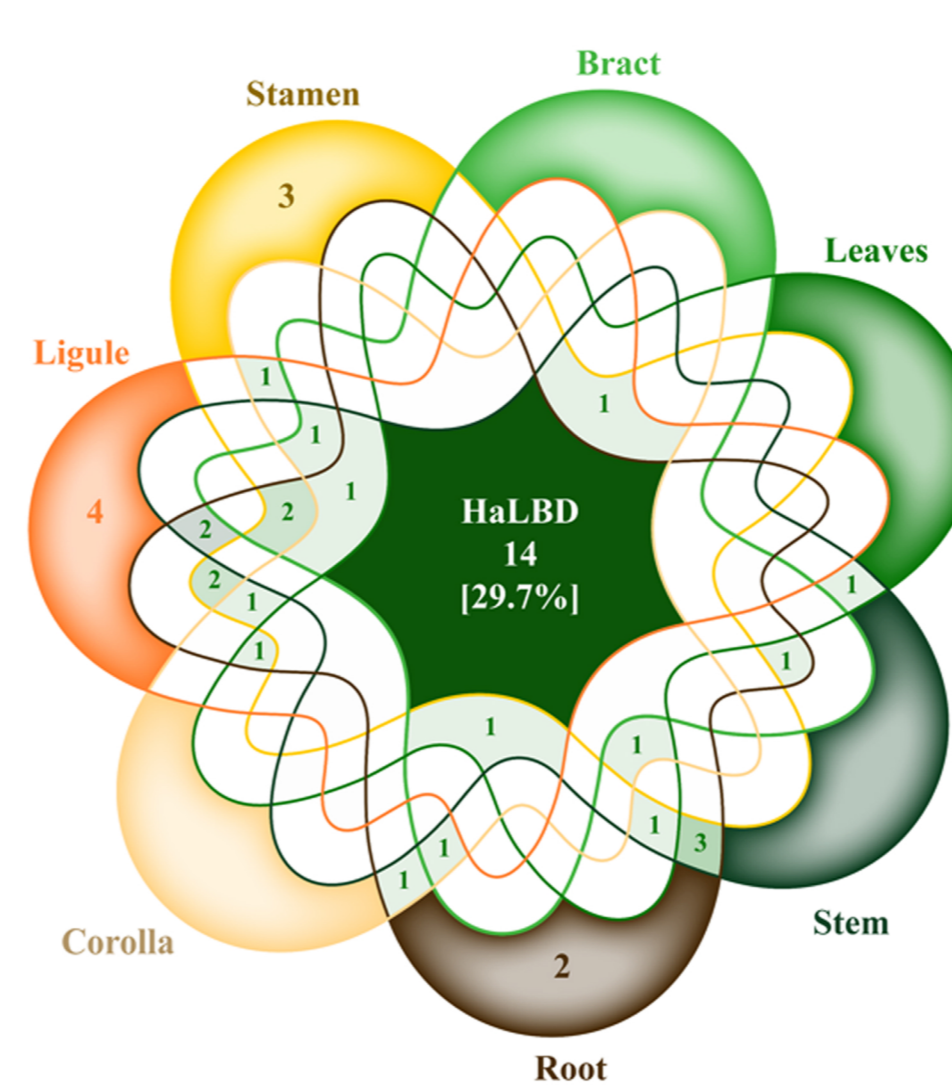


Fig. 1 Phylogenetic tree and unrooted neighbor-joining tree depicting *LBD* family members in *A. thaliana*, *H. annuus* and rice.



The expression patterns of *HaLBD* genes demonstrate limited tissue specificity. 14 *HaLBD* genes are expressed across the seven tissues, The root had the highest proportion of expressed genes. Transcriptomic data analysis revealed that both biotic and abiotic stresses significantly influence the expression of *LBD* genes, consistent with findings from cis-acting element analyses.

Fig. 2 Expression profiles of *HaLBD* genes among 7 tissues. The Venn diagram is employed to illustrate the overlap of genes expressed across distinct tissues

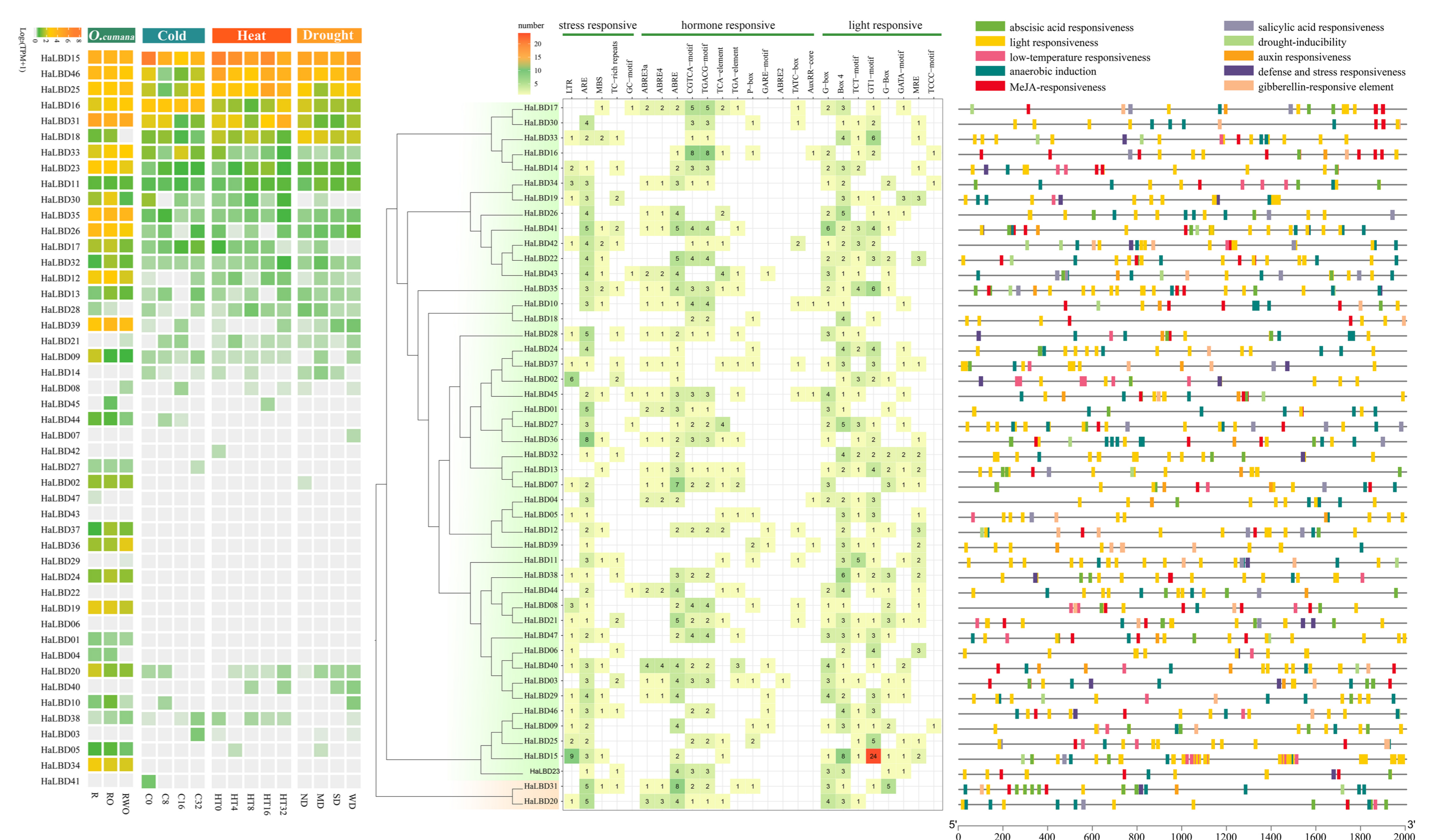
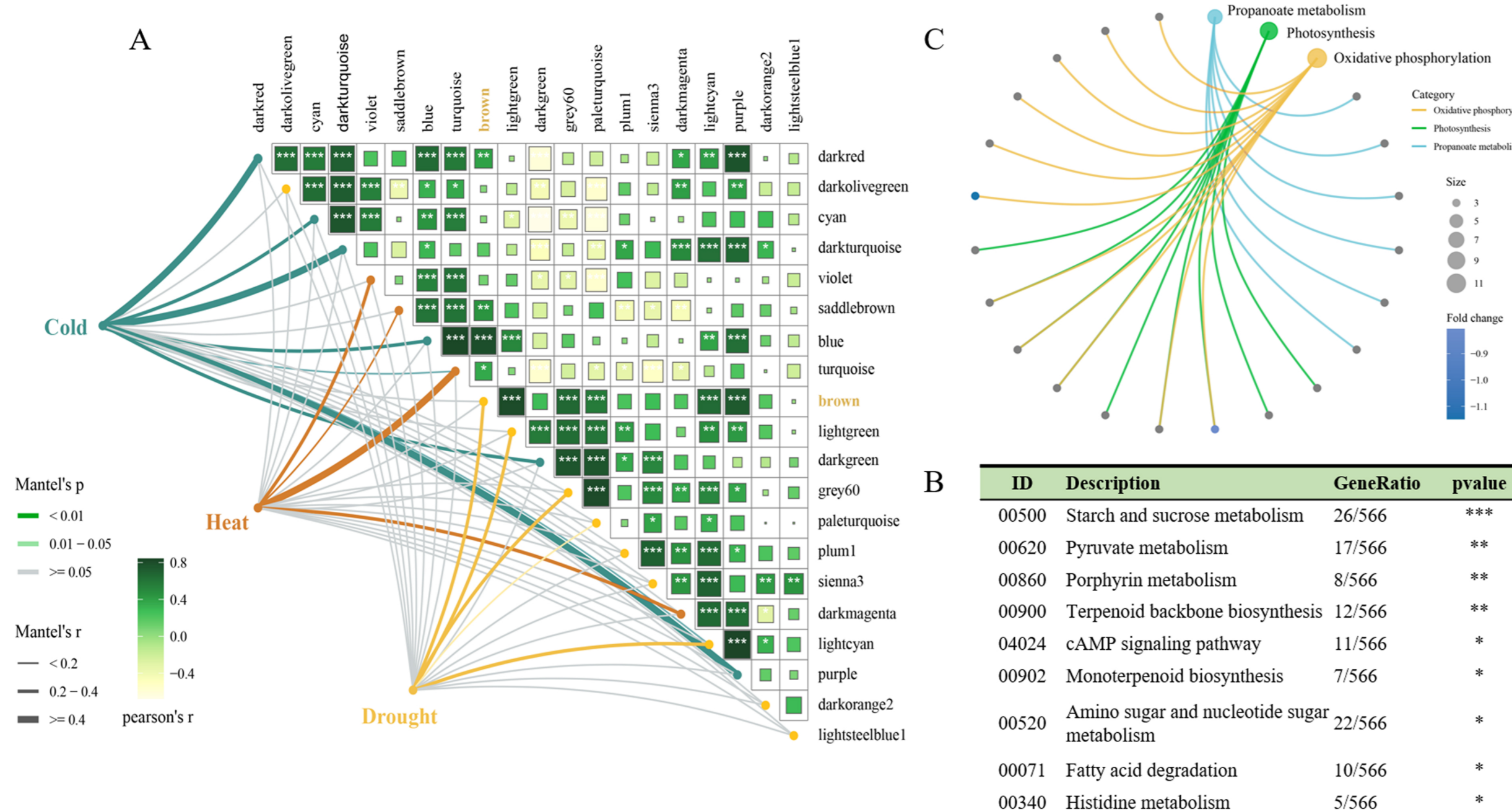


Fig. 3 The RNA-Seq analysis of *HaLBD* genes was performed under various stress conditions, including heat, cold, drought, and infestation by the parasitic plant *Orobanche cumana*. The distribution of cis-acting elements in the promoter region of sunflower *LBD* genes.



Co-expression and interaction analyses suggest that *LBD* genes may help plants resist stress through interactions with *F-BOX*, *CBD*, *CRK*, *G-LecRLK*, *ECH*, and *JIP* genes, which are involved in pathogen recognition, response, and stress resistance.

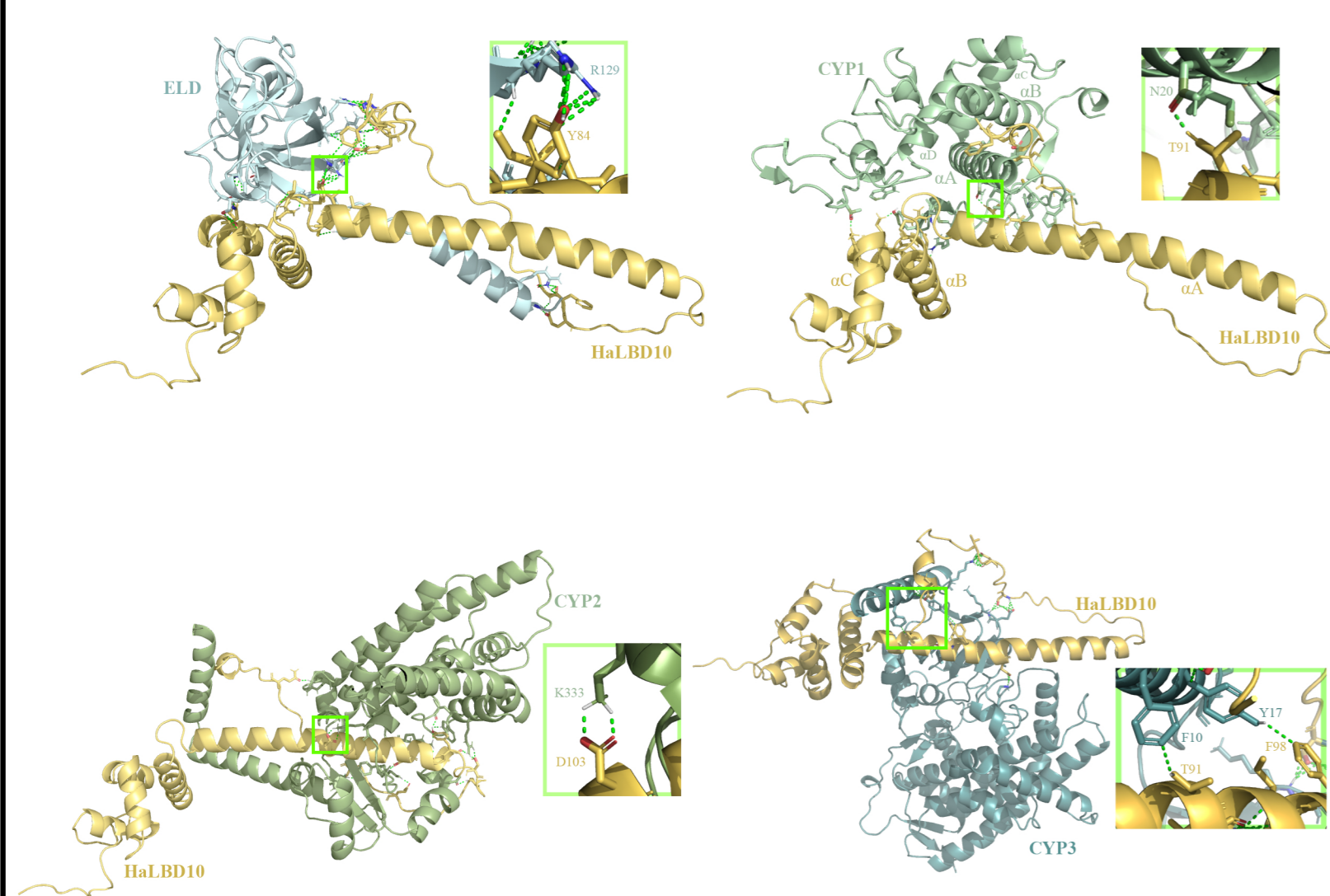


Fig. 4 Functional enrichment of genes co-expressed with *HaLBD* based on WGCNA. Co-expression and structural prediction of *HaLBD10* with key resistance genes.