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Research Article

Computational Insights into *Lateral Organ Boundary Domain (LBD)* Transcription Factors in Chickpea (*Cicer arietinum* L.) Reveal Their Roles in Defense Under Biotic and Abiotic Stress Responses

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is an important legume crop, cultivated worldwide due to its high nutritional importance and domestic demand. Due to low genetic diversity, its productivity is limited by both biotic and abiotic stresses. Despite its importance, the study of transcription factors underlying different stresses remains unstudied. In this study, whole-genome analysis of LBD transcription factor in *C. arietinum* revealed 48 putative *CaLBDs* genes, distributed across all 8 chromosomes. Comparative phylogeny with *Oryza sativa* and *Arabidopsis thaliana* classified LBD transcription factors into 5 groups (**I**-a, **I**-b, **I**-c, **I**-d, and **II**-a), based on sequence homology and conserved domains. Variation in intron/exon count across *CaLBD* genes indicated structural diversity, while conserved motifs suggested functional roles in biotic and abiotic stress responses due to their characteristic LBD domain. Synteny analysis uncovered the evolutionary relationships of *C. arietinum* with *A. thaliana* and *Medicago truncatula.* Gene duplication analysis demonstrated both tandem and segmental duplications expressing their roles in LBD gene family expansion. Transcriptomic profiling of *CaLBD* genes under *Phytophthora* root rot treatment revealed strong upregulation of *CaLBD38* and *CaLBD46*. Under *Fusarium* wilt stress, *CaLBD46* was highly expressed, followed by *CaLBD14* and *CaLBD38*. Under the salt and drought stress conditions, *CaLBD38* and *CaLBD46* expressed the strongest upregulation. This genome-wide study provides a foundation and valuable resources for future genetic studies and serves as a base for development of stress-resilient cultivars with enhanced resistance through advanced molecular breeding programs.

Keywords: Abiotic stresses, Biotic stresses, *Cicer arietinum* L., Computational insights, Expression Analysis, LBD Family, Phylogenetic Analysis

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a cool season, self-pollinated leguminous crop cultivated globally in arid and semi-arid regions (Singh *et al.*, 2024). It is a member of Fabaceae family, which is the third largest family of flowering plants comprising 751 genera and approximately 19,000 known species (Xu & Deng, 2017). Its genome size was reported as 738Mb, containing 28,269 genes (Varshney *et al.*, 2013). Chickpea is believed to have originated in the central region of the Fertile Crescent's (in modern Syria, Turkey and Iran) (Zhang *et al.*, 2024). Chickpea is a rich source of proteins (18.2%-20.5%), carbohydrates (56.8–61.0%), vitamins, fibers, minerals (11.0-22.1%) and fats (3.7-4.1%), fulfilling dietary requirement of both humans and livestock (Irum *et al.*, 2025). Based on different characteristics such as seed shape, flower color, and seed size, it is categorized into two major groups, desi and kabuli (Sari *et al.*, 2024).

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Although it is widely cultivated, chickpea has a narrow genetic diversity due to domestication bottlenecks and its self-pollinated nature (Shanaz *et al*., 2025). Transcription factors (TFs) are regulatory proteins that control gene expression by binding to DNA and play an important role in many physiological processes (Oksuz *et al*., 2023). They are involved in developmental processes such as signal transduction, cell morphogenesis, and environmental stress response by regulating gene expression (Huang *et al*., 2021). Among these TFs, the LBD proteins are plant-specific transcription factors often recognized by their highly conserved LOB (Lateral Organ Boundaries) domain and generally composed of a conserved N-terminal region and a variable C-terminal section. The structural domain of LBD family is located at the N-terminal region and includes several key motifs such as a leucine zipper-like motif ($LX_6LX_3LX_6L$) for protein dimerization, the GAS motif (Gly-Ala-Ser), a zinc finger-like motif ($CX_2CX_6CX_3C$) for DNA binding (Zhang *et al*., 2025). There are two classes of LBD gene family based on evolutionary studies and sequence similarity (Class I and Class II). If class I members contain zinc finger-like motifs, GAS clusters, and leucine zipper-like helical motifs, they can be categorized into five groups (I-A, I-B, I-C, I-D, and I-E), while class II LBD members, which lack leucine zipper-like structural domains, are classified into two groups (II-A and II-B) (Kong *et al*., 2017). The first discovery of LBD gene family happened during a gene expression analysis in the shoot apex of *Arabidopsis* seedlings (Liang *et al*., 2022). There are different number of identified LBD gene family members across plant species, such as *Arabidopsis thaliana* (43) (Shuai *et al*., 2002), *Oryza sativa* (31) (Zhao *et al*., 2023), *Malus domestica* (58) (Wang *et al*., 2013), *Zea mays (*44) (Zhang *et al*., 2014), *Vitis vinifera* (40) (Cao *et al*., 2016), and *Morus notabilis* (31) (Luo *et al*., 2016).

LBD proteins are plant-specific TFs containing a conserved LBD domain and are potentially involved in lateral organs development and different metabolic processes, such as anthocyanin synthesis and nitrogen metabolism (Xu *et al*., 2016). The existing studies confirmed their active roles in growth regulation and development in plants. For example, *AtLBD16*, *AtLBD18*, and *AtLBD29* play an active role in regulation of lateral root initiation in *Arabidopsis* (Lee *et al*., 2019). LBD proteins are also involved in root and pollen development (Kim *et al*., 2016), pathogen like responses (Huang *et al*., 2020), lateral organ development (Zhang *et al*., 2020), and secondary xylem and phloem development (Rubin *et al*., 2009). Flower-developing LBD proteins have also been observed to have role in floral scent biosynthesis (Peng *et al*., 2025), besides stress tolerance and hormone signaling functions (Li *et al*., 2025).

Despite extensive research on other transcription factors and the significance of chickpea as an important legume crop, studies on the LBD gene family remain limited. To compensate for this research gap, we conducted a detailed study for the identification of LBD gene family in *C. arietinum* and performed different bioinformatics analyses under multiple biotic and abiotic stresses. These analyses included comparative phylogeny, genomic localization, synteny, Intron/Exon structure, gene duplication, domain, cis-regulatory, and gene expression profiling. Therefore, this study provides a complete detail of LBD gene family in chickpea and their responses against various biotic and abiotic stresses. It not only provides a comprehensive genetic resource for future studies on molecular genetics of chickpea but also contributes significantly to developing new resistant varieties through molecular breeding techniques.

MATERIALS AND METHODS

Database searches for sequence retrieval of *Cicer arietinum*

To retrieve LBD protein sequences and to identify members of LBD transcription factor family in *C.arietinum*, the Plant Transcription Factor Database (https://planttfdb.gao-lab.org/family.php?fam=LBD) was utilized (Jin *et al*., 2016). Several physiochemical properties of *CaLBD* protein sequences were also retrieved from Plant TFDB such as Isoelectric points (PI), protein lengths and molecular weights (MW). For obtaining the coding and genomic sequences of *CaLBD* proteins in FASTA format, the BLAST tool available on the NCBI (National Center for Biotechnology Information) https://www.ncbi.nlm.nih.gov/ was utilized. NCBI was further utilized to determine the genomic information of *CaLBD* genes.

Chromosomal Localization, Gene Structure, and Conserved Motif Characterization of *CaLBD* Genes

The NCBI database was utilized to determine the chromosomal positions of *CaLBD* proteins. The chromosomal mapping was visualized using TBtools-II software (Chen *et al*., 2023). The intron/exon distribution was demonstrated using the Gene Structure Display Server (GSDS) v2.0 (https://gsds.gao-lab.org/) (Hu *et al*., 2015) by uploading coding and corresponding genomic sequences. We also identified the conserved motifs within the protein sequences using the MEME suite (v5.5.8) (https://meme-suite.org/meme/tools/meme) with default parameters except for the number of motifs, which were set to ten (Bailey *et al*., 2009).

Multiple Sequence Alignment, Comparative Phylogenetic and Domain Analyses of LBD Proteins in *C. arietinum*

Evolutionary relationships among *C. arietinum*, *A. thaliana*, and *O. sativa* were investigated after retrieving their protein sequences from Plant TFDB. The putative *CaLBD* proteins were given names from *CaLBD01-CaLBD48* based on their chromosomal positions. ClustalW feature of MEGA11 was employed for Multiple Sequence Alignment (Larkin *et al*., 2007; Tamura *et al*., 2021). The classification of LBD genes in *C. arietinum* was based on evolutionary relationships with the LBD members in *A. thaliana* based on pairwise distances, using Neighbor-Joining method in MEGA11 (Saitou & Nei, 1987).

To evaluate the significance of each node of the phylogenetic tree, the preset parameters such as poisson model, and pair-wise deletion were enabled along with bootstrap value of 1000. The phylogenetic tree was visualized using iTOL v7.0 (https://itol.embl.de/) (Letunic & Bork, 2024). The *CaLBD* sequences were submitted to the Conserved Domain Database (CDD) at NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) for identification of conserved domains and to generate an output data file (Marchler-Bauer *et al*., 2015). The visualization of conserved domains of each *CaLBD* gene was carried out using TBtools-II.

Cis-regulatory Elements Identification and Protein-Protein Interaction among *CaLBD* Genes

To identify the cis-regulatory elements within promoter regions of *CaLBD* genes, we retrieved 1000 base pairs upstream regions of each gene and uploaded them to the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot *et al*., 2002). Tab-delimited files, containing cis-regulatory element data, were generated for each *CaLBD* gene and subsequently visualized using TBtools-II software. Protein-protein interaction analysis was carried out through the STRING search tool v12.0 (http://string-db.org/) (Szklarczyk *et al*., 2025).

Following the enabling of "multiple protein by sequence" option, amino acid sequences of *CaLBD* transcription factors were provided as input. *Cicer arietinum* was chosen as the reference organism and all parameters were maintained at their default configuration. The resulting interaction networks were predicted based on different factors including gene neighborhood, gene fusion, experimental data, co-expression, co-occurrence, and text mining evidence. Additionally, k-means clustering was performed by setting the number of clusters to three to further explore functional protein modules.

Comparative Synteny and Gene Duplication Analysis of LBD Members from *C. arietinum* and Related Plant Species

The evolutionary relationships of *C. arietinum* with *A. thaliana* and *Medicago truncatula* were investigated using the MCScanX feature of TBtools-II (Tang *et al*., 2008). The genomic files of *C. arietinum* and reference plant species were used as input for this purpose. The output files were later visualized using dual synteny plot feature of TBtools-II. Furthermore, the advanced circos feature was utilized to visualize gene duplication events among *CaLBD* genes.

Transcriptomic Analysis of *CaLBDs* Responses to Various Biotic and Abiotic Stresses

The SRA repository (https://www.ncbi.nlm.nih.gov/sra) available on NCBI was utilized to retrieve transcriptomic data of *CaLBD* genes under accession numbers SRP396207, SRP485731, and SRP603604 corresponding to BioProjects PRJNA870250, PRJNA1068660, and PRJNA1296865,respectively. These BioProjects contain 11, 6, and 26 runs, encompassing 57.23 Gb, 42.37 Gb and 39.46 Gb of experimental data respectively. To perform read mapping of reference genome of *C. arietinum* (NCBI RefSeq assembly: GCF_000331145.2), the Galaxy platform (https://usegalaxy.org/) was used with Bowtie2 with preset parameters (De Almeida *et al*., 2016). The Feature Counts tool of Galaxy server was utilized to calculate FPKM values of all *CaLBD* genes (Liao *et al*., 2014). The expression profiles of all *CaLBD* genes were visualized by generating a heatmap using TBtools-II.

RESULTS

Identification and Characterization of LBD Family Members in *C. arietinum*

In this study, 68 LBD genes were identified from the genomic dataset of *C. arietinum*. Altogether, 20 genes were marked as redundant and deleted to avoid duplication. The remaining 48 genes were considered candidate genes, and their nomenclature was developed as (*CaLBD01*-*CaLBD48*) according to their positions in ascending order on different chromosomes by following Jia *et al*. (2022). Among the candidate *CaLBD* TFs, the smallest protein was *CaLBD27* with 126 amino acids and *CaLBD40* was the largest protein, encompassing 312 amino acids. The average length of *CaLBDs* was 215.72 amino acids, indicating a typical range size as reported in LBD of other plant species. These proteins had isoelectric points, ranging from 4.42 (*CaLBD35*) to 8.643 (*CaLBD34*), with an average value of 7.06 which showed that *CaLBD* proteins had both acidic and basic properties which may influence their behavior in DNA binding. Furthermore, the molecular weights were observed from 14204.3Da to 35616.6Da, with a calculated mean of 24494.81Da. This indicated their expected molecular size which justifies their functional roles as regulatory proteins.

Table 1. Different characteristics of 48 *CaLBD* genes identified from the whole genome data of *Cicer arietinum*.

TF ID	Given names	Protein length (aa)	Molecular weight (Da)	Isoelectric point (pI)	Gene symbol	Chr No.	Gene start	Gene end	Exon count
XP_004488806.1	CaLBD01	143	15930.2	8.4931	LOC101492597	1	41974834	41978470	2
XP_004487250.1	CaLBD02	197	22436.4	5.0372	LOC101508383	1	50452639	50455173	2
XP_012570417.1	CaLBD03	232	26666.5	6.6968	LOC101498778	1	52525430	52531227	7
XP_004489213.1	CaLBD04	129	14540.5	7.8643	LOC101508089	2	321603	324790	4
XP_004516674.1	CaLBD05	263	29278.7	8.4075	LOC101508034	2	4131867	4133514	2
XP_004489657.1	CaLBD06	222	24110.9	7.931	LOC101493585	2	5773889	5775945	2
XP_004490703.1	CaLBD07	230	25244.4	7.4637	LOC101514968	2	41128612	41130559	2
XP_004490916.1	CaLBD08	206	22891.5	6.2681	LOC101508415	2	43620372	43621714	2
XP_004491022.1	CaLBD09	191	21660.5	7.7019	LOC101513557	2	46311094	46311890	1
XP_004491008.1	CaLBD10	168	18949.4	6.6535	LOC101509246	2	46432773	46434088	2
XP_004491050.1	CaLBD11	199	22150.2	8.1133	LOC101495510	2	46716932	46719073	3
XP_004491406.1	CaLBD12	156	17999.4	8.2741	LOC101507470	2	50929929	50930893	2
XP_012568000.1	CaLBD13	243	26367.5	5.7403	LOC105851536	3	1539338	1540506	2
XP_012568012.1	CaLBD14	303	3064.3	7.7386	LOC101511599	3	1540604	1542219	2
XP_004491980.1	CaLBD15	166	18402.5	5.2311	LOC101490681	3	33761505	33763431	5
XP_004492662.2	CaLBD16	182	20302.2	7.8833	LOC101508422	3	60009330	60011645	2
XP_004492639.1	CaLBD17	222	23370.5	8.139	LOC101500615	3	60029238	60033888	2
XP_004493763.1	CaLBD18	219	24439.4	7.6951	LOC101510680	3	69054527	69056170	2
XP_004493764.1	CaLBD19	214	24198.3	6.137	LOC101511002	3	69062110	69063365	2
XP_004495114.1	CaLBD20	229	25609.4	5.2274	LOC101505857	4	1257172	1258600	2
XP_004496332.1	CaLBD21	231	25341.2	6.9698	LOC101508655	4	10332793	10334502	2
XP_004497094.1	CaLBD22	214	23166.5	8.2085	LOC101499577	4	16130813	16132130	2
XP_004497769.1	CaLBD23	281	31492.2	6.3903	LOC101493950	4	46155571	46157772	2
XP_004500020.1	CaLBD24	272	30041.3	4.9727	LOC101488980	5	49114646	49115993	2
XP_004500442.1	CaLBD25	177	19877.6	5.6634	LOC101504817	5	57137831	57140218	2
XP_004500506.1	CaLBD26	199	22231.4	8.1593	LOC101504923	5	59169298	59170023	1
XP_004500573.1	CaLBD27	126	14204.3	6.6486	LOC101498950	5	59262447	59262827	1
XP_004515643.2	CaLBD28	132	14533.6	8.4706	LOC101510141	5	60486750	60488832	2
XP_004501886.1	CaLBD29	198	21119.7	7.2429	LOC101513806	5	61970853	61973259	2
XP_004502131.1	CaLBD30	154	17693.1	7.7282	LOC101508147	5	62845331	62846315	2
XP_004502856.1	CaLBD31	240	26654.3	9.0244	LOC101488992	5	69420065	69421570	3
XP_004505394.1	CaLBD32	296	33700.7	8.551	LOC101500246	6	16232231	16235238	2
XP_004505337.1	CaLBD33	172	18716	7.8931	LOC101509427	6	18131323	18134696	2
XP_004505626.1	CaLBD34	202	23290.1	8.643	LOC101497215	6	21534807	21537019	2
XP_004505954.1	CaLBD35	202	22416.3	4.4285	LOC101500987	6	66157216	66159197	2
XP_004508794.1	CaLBD36	302	34192.1	5.3166	LOC101497462	7	8704752	8709099	3
XP_004515877.1	CaLBD37	190	20963.5	6.0953	LOC101507273	7	9654114	9656169	2
XP_004509073.1	CaLBD38	235	25538.1	7.6885	LOC101509129	7	12924107	12925333	2
XP_004509216.1	CaLBD39	292	33054.2	7.0408	LOC101500386	7	14104134	14105090	1
XP_004509168.1	CaLBD40	312	35616.6	6.919	LOC101511490	7	14108235	14110191	2
XP_004509801.2	CaLBD41	259	29387.6	5.3354	LOC101506682	7	21658969	21660825	3
XP_004511083.1	CaLBD42	221	24596.3	8.3929	LOC101510654	7	46913201	46916339	4
XP_004510943.1	CaLBD43	216	23715.8	8.0802	LOC101509036	7	50228229	50234541	2
XP_004510942.1	CaLBD44	230	24204.2	8.1475	LOC101508720	7	50271768	50276816	2
XP_012574097.1	CaLBD45	258	29594.1	6.7249	LOC101515615	7	58160454	58162100	2
XP_004511855.1	CaLBD46	234	25590	8.0864	LOC101498138	8	3678807	3680004	2
XP_004511963.1	CaLBD47	307	34099	6.8267	LOC101503029	8	4401901	4405582	8
XP_004511965.1	CaLBD48	298	32834.6	6.3629	LOC101503584	8	4415924	4420105	4

Genomic Localization of LBD Encoding Genes in *C. arietinum*

The genome dataset for *C. arietinum*, retrieved from NCBI, revealed the mapping of *CaLBD* genes on all 8 chromosomes. This allocation of *CaLBD* genes was found to be uneven on different chromosomes (Figure 1). Each of

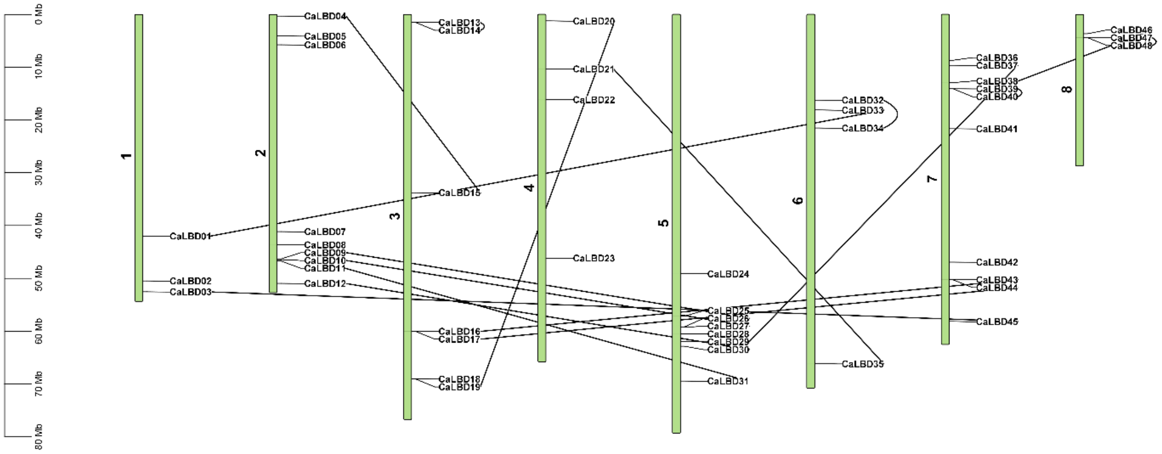


Figure 1. Genomic mapping of 48 *CaLBD* genes was visualized using TBtools-II. The 8 chromosomes of *C. arietinum* are represented by light green colors bars. The exact locations of *CaLBD* genes are indicated by dark lines. Dark lines correspond to gene duplication events among *CaLBD* genes. The scale on the left side of the figure, ranging from 0 to 80 Mb, represents the chromosome lengths in megabases (Mb).

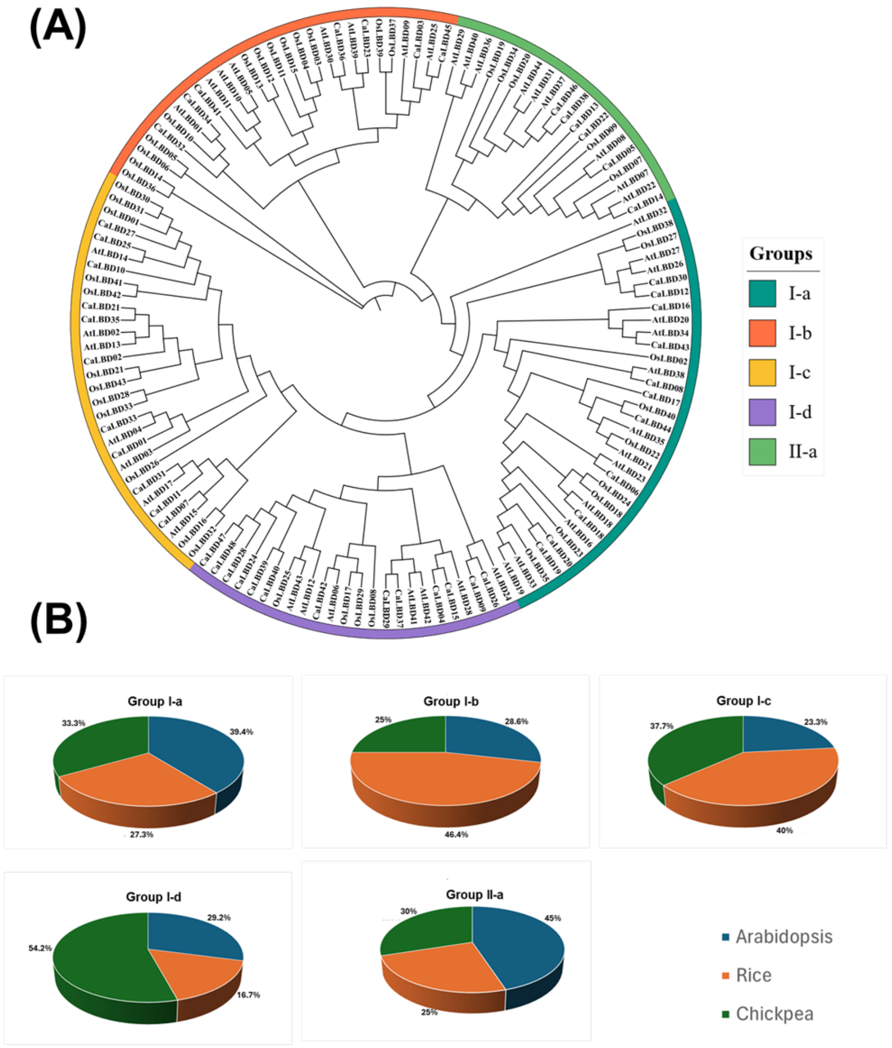


Figure 2. (A) Comparative phylogenetic analysis of 135 LBD members from *C. arietinum*, *A. thaliana* and *O. sativa*. The tree was computed using the Neighbor-joining method in MEGA11 and its visualization was done using iTOL v7.0. To illustrate evolutionary relationships of these species, the *CaLBD* proteins were classified into 5 distinct groups and visualized by distinct colors. (B) The visualization of LBD members' ratio of *C. arietinum*, *A. thaliana* and *O. sativa* in each group.

chromosomes 1 and 8 had the lowest number of genes (3, 6.25%). Chr 4 and 6 each contained 4 genes (8.33%), followed by chr 3 and 5 which demonstrated 7 and 8 *CaLBD* genes, respectively. Chr 2 had 9 *CaLBD* genes (18.75 %) whereas the highest number of genes (10, 20.83%) was observed on chr 7.

Multiple Sequence Alignment, Comparative Phylogenetic and Domain analyses of LBD Proteins in *C. arietinum*

A comparative phylogenetic analysis was performed to determine the evolutionary relationships among *CaLBD*s and the well-characterized LBD members of *A. thaliana* and *O. sativa*. In total, 135 proteins from these species were used to construct an unrooted neighbor-joining (NJ) tree (Figure 2). The LBD members were categorized into two main classes (I and II) which were further distributed into 5 sub-groups (I-a, I-b, I-c, I-d, and II-a). Among these, group I-a was the largest, comprising 33 LBD members, whereas group II-a was the smallest with 20 members only. Additionally, groups I-b, I-c, and I-d consisted of 28, 30, and 24 members, respectively. The grouping of LBD members of these species is totally based on their structural and functional similarity. For example, members of Class I are involved in stress signaling and organ development. Similarly, Class II members participated in metabolic regulation. So, the clustering of *CaLBD* genes with LBD members of *A. thaliana* and *O. sativa* shows structural similarity and functional divergence among them.

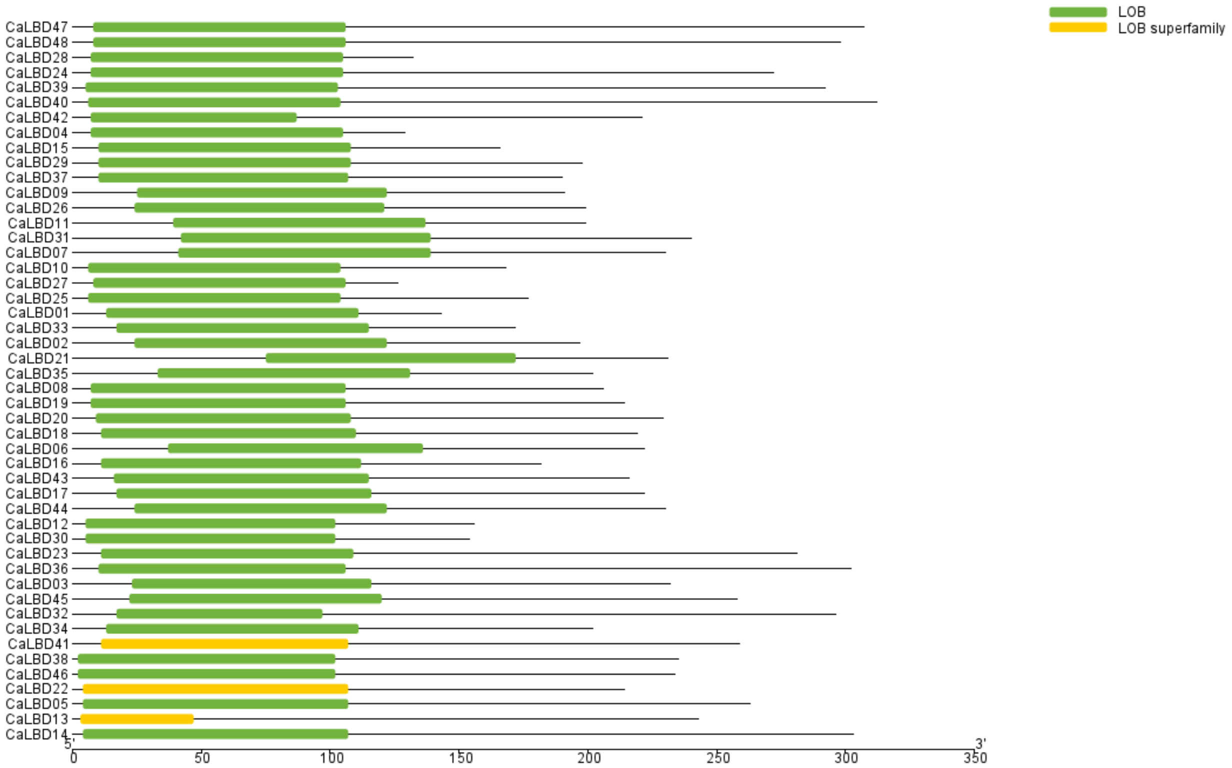


Figure 3. Conserved domain regions of *CaLBD* proteins, represented by different colors. LOB and LOB superfamily domains were detected and visualized using TBtools-II. The scale (0-350) below corresponds to the amino acid length of *CaLBD* proteins.

Two conserved domains, LOB and LOB superfamily, were identified through NCBI's Conserved Domain Database (CDD) and were subsequently visualized using TBtools-II (Figure 3). The LOB domain was conserved in majority of *CaLBD* genes. However, three genes including *CaLBD22*, *CaLBD13*, and *CaLBD41* expressed the conservation of the LOB superfamily domain instead of the LOB domain, indicating structural or functional divergence of these genes.

Gene Structure and Conserved Motif Analyses

The pattern of introns and exons provides insights into gene structure and highlights their evolutionary conservation (Figure 4B). Among 48 *CaLBD* genes, 14 genes were intron-less. A total of 29 genes had only single intron.Only 5 *CaLBD* genes contained 2 introns, indicating highest intron count in this gene family. Length variation in *CaLBD* gene structures indicates differences in intron–exon distribution and overall gene structure among *CaLBD* genes. For the identification of conserved motifs within 48 *CaLBD* genes, the MEME suite online tool was employed, which resulted in the identification of 10 conserved motifs (0-10) (Figure 4A).

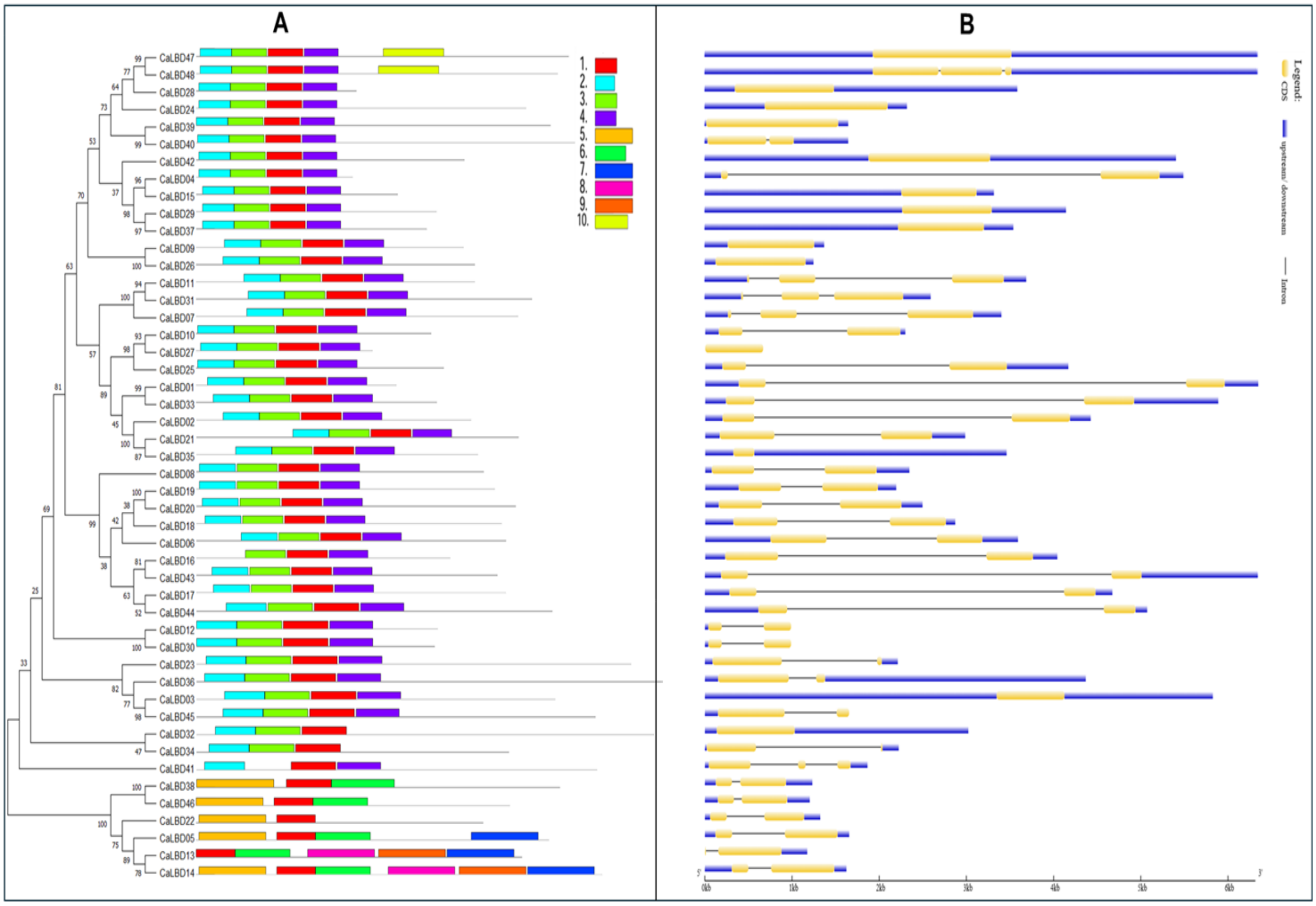


Figure 4. (A) The motif distribution across all *CaLBD* genes detected using MEME suite (v5.5.8). Visualization of motifs distributed across target regions in correspondence to the phylogenetic tree. (B) Intron/Exon distribution of *CaLBD* genes aligned with the phylogenetic tree. The visualization of CDS, regulatory regions and introns is performed by yellow, blue and grey colors, respectively. The length of intron/exons is proportional to the scale attached below.

Motif 1 was the most consistent motif, identified in all *CaLBD* proteins suggesting its central role in functional regulation, followed by motif 2 and 3 which were detected in 41 *CaLBD* proteins, respectively. Motif 4 was identified in 40 proteins, while motifs 5 and 6 both were detected in 5 *CaLBD* proteins. The rest of motifs were found in a limited number across *CaLBD* proteins. Overall, the motifs length ranged from 11 (Motif 4) to 50 (Motif 2). This variation in number and distribution of motifs suggests its structural divergence and functional specialization throughout the chickpea genome. Different attributes of all identified motifs are given below in Table 2.

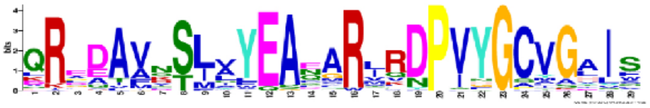
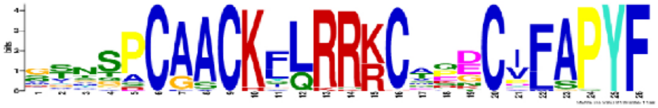
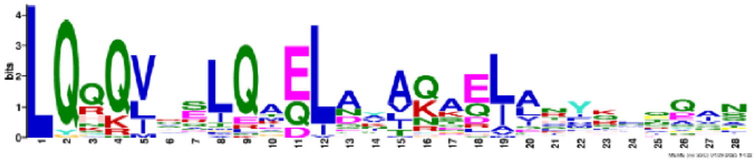
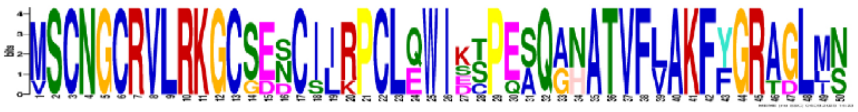
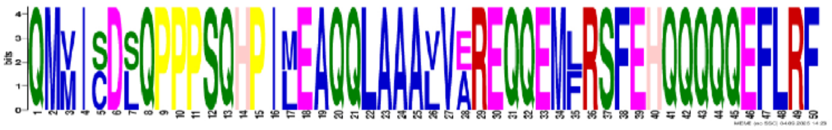
Synteny Analysis and Gene Duplication Analysis

A synteny block is a region of genome in which genes are conserved in a sequence across other genomes (Liu *et al*., 2018). In this research, a synteny analysis of *C. arietinum* was performed with two other species, such as *A. thaliana* and *M. truncatula*. Very strong syntenic connections were identified between *C. arietinum* and *M. truncatula* which indicated a close evolutionary relationship between them. However, a few syntenic regions were found with *A. thaliana.* Gene duplication played a crucial role in the expansion of gene families in plants, leading to the evolution of new gene functions and improving environmental adaptability (Ai *et al*., 2022). In this study, we discovered 17 duplicated gene pairs, of which 13 pairs were segmentally duplicated and 4 pairs (*CaLBD13*/*CaLBD14*, *CaLBD32*/*CaLBD34*, *CaLBD39*/*CaLBD40* and *CaLBD47*/*CaLBD48*) exhibited tandem duplication. These duplicated pairs were widely located across all chromosomes, with the highest number (7) on chromosome 7.

Cis-Regulatory Elements Prediction and Protein-Protein Interaction Network Analysis

Cis-regulatory elements, located within the promoter region, are non-coding DNA sequences that modulate gene expression by interacting with transcription factors during developmental and environmental changes (Ali *et al*., 2024; Sharif *et al*., 2025). In this study, a total of 54 cis-regulatory elements were identified (Figure 7). Among these, the most abundantly found elements were TATA-box and CAAT box, serving as core promoter elements. Several elements including GT-1 motif, Box 4, G-box, TCT-motif, and GA-motif were involved in light responsiveness. ABRE and TCA motif elements were linked to Abscisic acid and salicylic acid responsiveness, respectively. Gibberellin responsive elements comprised the P-box, TATC-box, and GARE-motif, while TGACG-motif and CGTCA-motif elements played their roles in MeJA-responsiveness. Additionally, the MBS element and TC-rich repeats elements were responsible for drought inducibility and defense responses, respectively.

Table 2. Different characteristics of all 10 motifs identified by using MEME suite in *CaLBDs*.

Motif Logos	**E-value**	**Sites**	**Width**
	8.0e-390	60	15
	4.3e-448	13	50
	8.00e-156	40	15
	2.70E-89	13	11
	3.40E-80	9	26
	6.20E-95	8	38
	3.50E-82	13	21
	4.90E-59	13	15
	8.00E-62	5	49
	4.00E-52	15	17

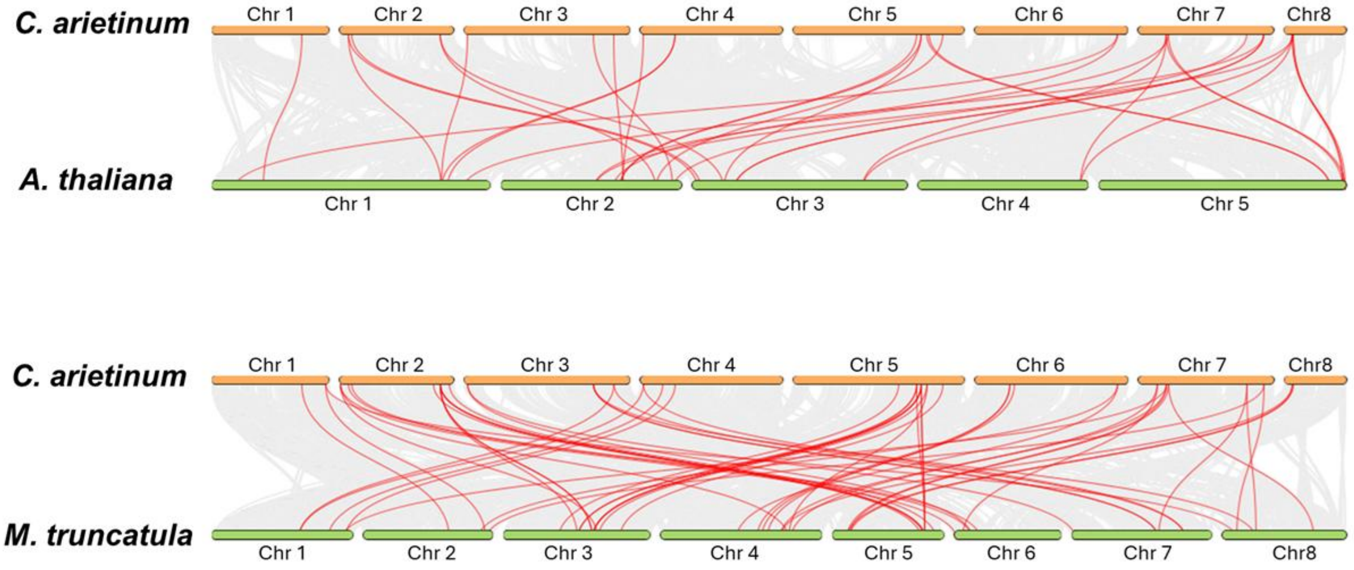


Figure 5. The dual synteny plot in TBtools-II illustrated syntenic regions between *C. arietinum*, *A. thaliana*, and *M. truncatula*. Red lines indicate homologous syntenic regions between *C. arietinum* and the other species, while grey lines represent syntenic blocks within the *C. arietinum* genome.

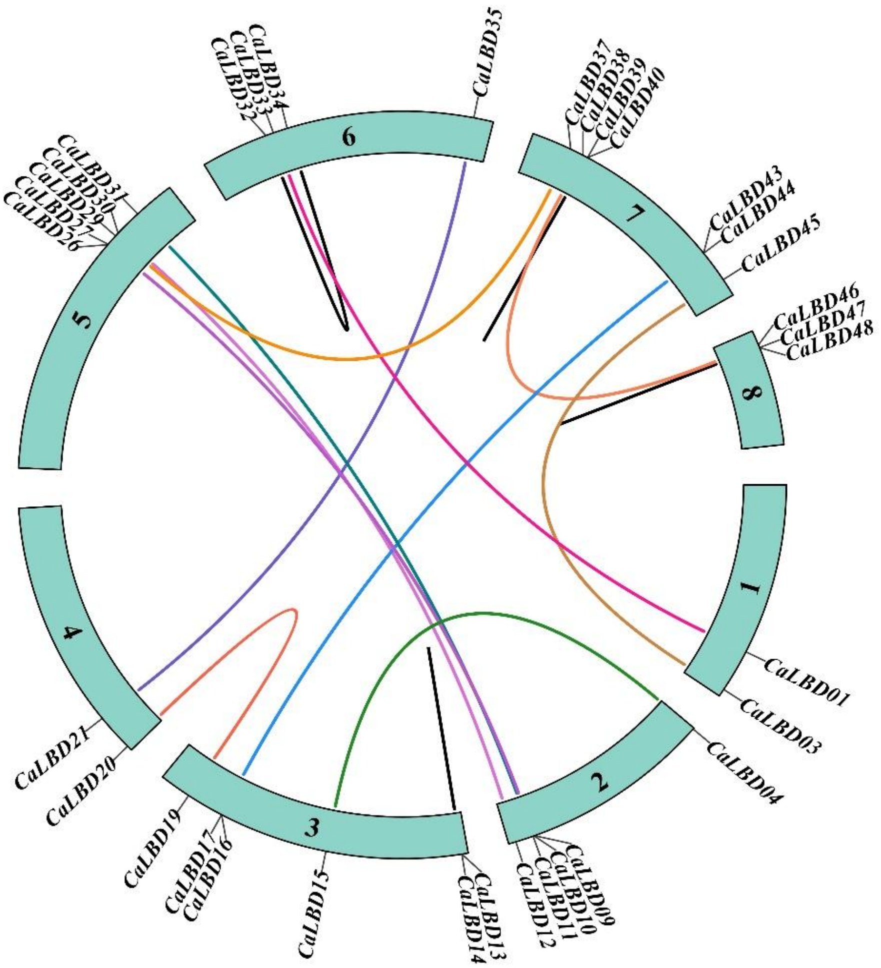


Figure 6. The illustration of tandem and segmental duplications within *C. arietinum* using Circos plot. Tandems and duplications among the 48 *CaLBD* genes are visualized by distinct colors across different *C. arietinum* chromosomes. The colored boxes ranging from 1 to 8 represent the chromosomes of *C. arietinum*.

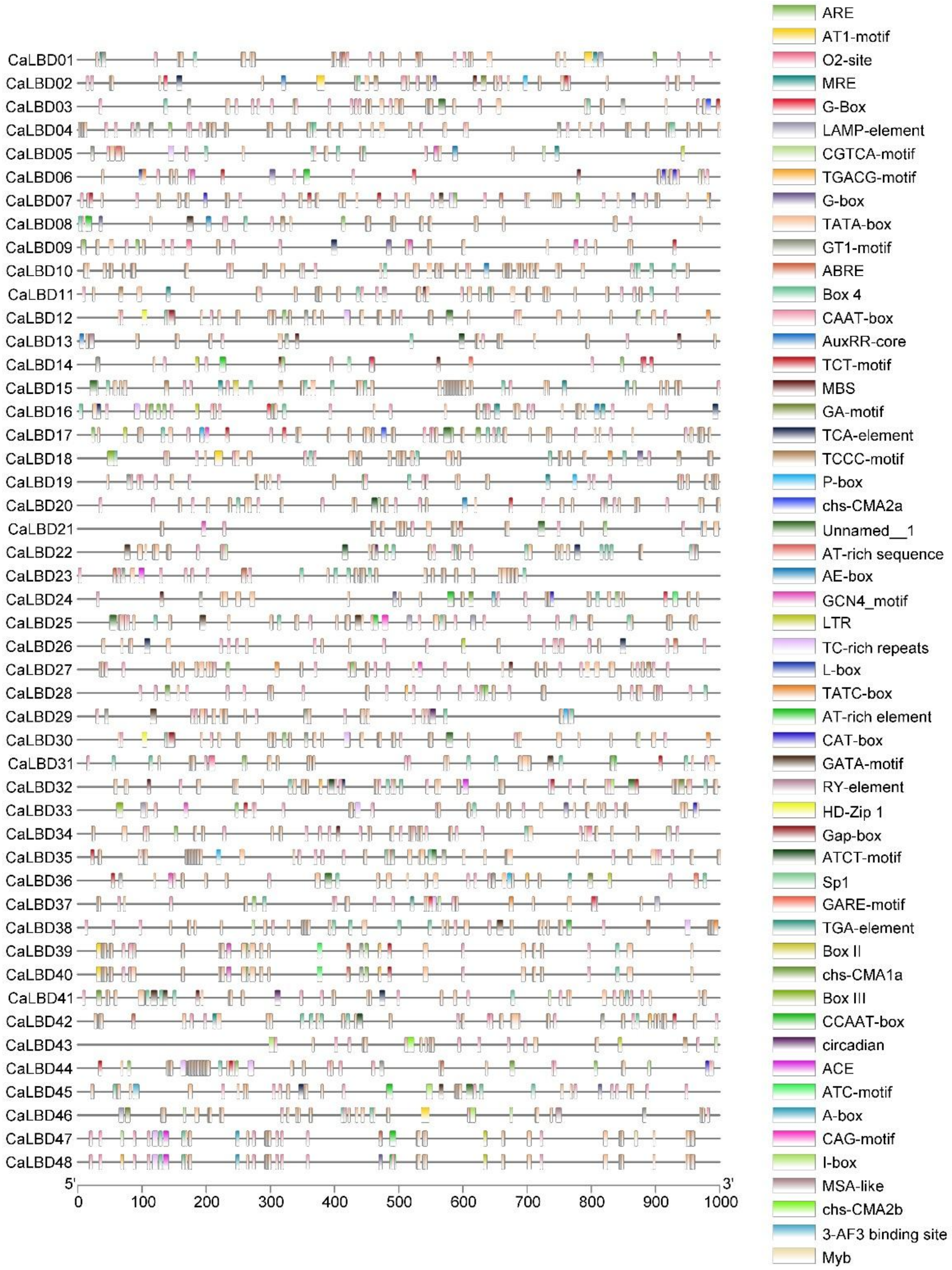


Figure 7. Graphical visualization of predicted cis-regulatory elements in *CaLBD* proteins generated using TBtools-II. TATA-box and CAAT box were the most prevalent cis-regulatory elements among *CaLBD* genes.

Protein-Protein Interactions

The analysis of protein-protein interactions resulted in 14 interaction networks, among which *CaLBD41* was identified as central hub protein, displaying strong direct associations with several members of the *CaLBD* family, including *CaLBD05*, *CaLBD08*, *CaLBD09*, *CaLBD16*, *CaLBD17*, *CaLBD18*, *CaLBD19*, *CaLBD20*, *CaLBD24*, *CaLBD26, CaLBD27*, and *CaLBD43*. This central positioning suggests its potential role in mediating functional links within the *CaLBD* gene family. Additionally, a limited interaction network was observed among *CaLBD12*, *CaLBD22*, and *CaLBD32*, highlighting their important role in maintaining secondary interactions within the gene family.

Gene Expression Profiling under Different Biotic and Abiotic Stresses

LBD TFs played crucial role in biotic and abiotic stress responses in plants as reported in previous studies by Xu *et al*. (2021), Liu *et al*. (2019), and Yu *et al*. (2020). In the present study, the effect of biotic and abiotic stresses was studied across all 48 *CaLBD* genes. *CaLBD46* was strongly upregulated under *Phytophthora* root rot treatment, followed by *CaLBD14* and *CaLBD38*. In addition, several genes including *CaLBD06*, *CaLBD09, CaLBD10, CaLBD28,* and *CaLBD35* remained neutral. Under treatment with *Fusarium oxysporum* wilt, *CaLBD46* was highly induced, followed by *CaLBD38* and *CaLBD14*. Furthermore, many genes such as *CaLBD24*, *CaLBD34*, *CaLBD39* and *CaLBD41* showed downregulation. Under salt and drought stress conditions, *CaLBD38* and *CaLBD46* showed the strongest upregulation. A few genes such as *CaLBD13* and *CaLBD14* showed a minimal upregulation. The genes including *CaLBD39, CaLBD12, CaLBD30, CaLBD04, CaLBD32, CaLBD34*, and *CaLBD41* showed strongest downregulation in both abiotic stress conditions. While rest of *CaLBD* genes showed neutral expression.

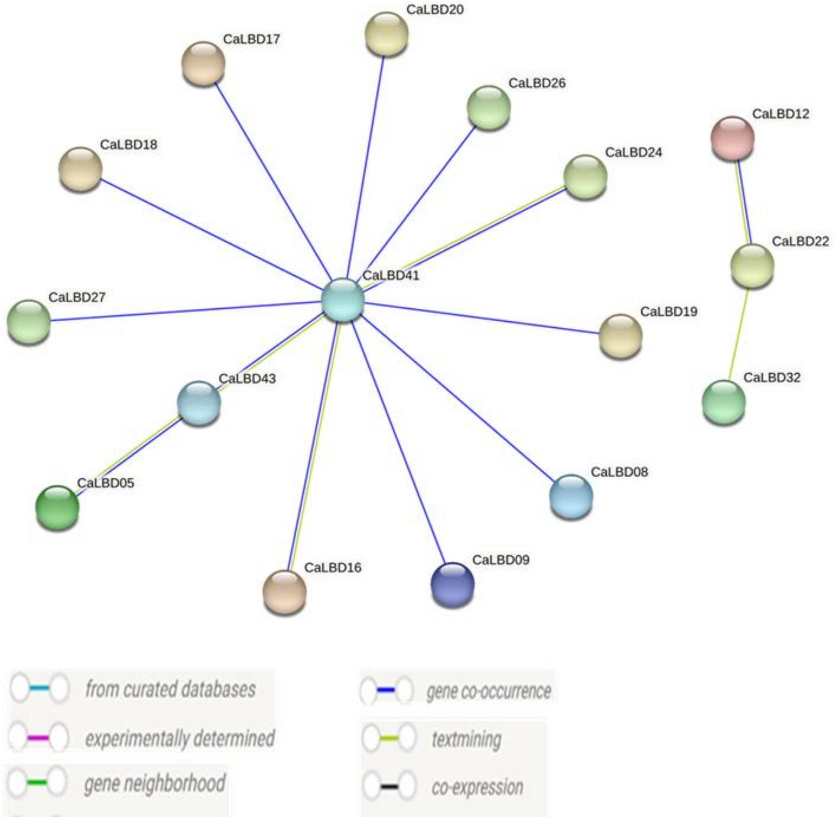


Figure 8. Mapping of protein-protein interaction among *CaLBD* proteins generated using the STRING database. Nodes represent individual proteins while edges correspond to known or predicted interactions.

DISCUSSION

Chickpea (*Cicer arietinum* L.) is a highly valued and widely cultivated pulse crop worldwide, with both desi and kabuli varieties (Zhang *et al*., 2024). It is an important legume that is rich in nutrients like proteins, complex carbohydrates, and essential minerals, ensuring a healthy and balanced diet (Felisiak *et al*., 2024). LBD transcription factors are a family of genes found only in plants that regulate lateral root formation and influence the overall plant growth and development (Sun *et al*., 2025). LBD transcription factors have been identified through genome-wide analyses across different plant species. The determination of 42 LBD genes in *Arabidopsis thaliana* provides the basis for the functional studies of this gene family (Shuai *et al*., 2002). Among cereals, *Zea mays* contained 45 LBD genes (Wang *et al*., 2025), *Oryza sativa* possessed 36 genes (Zhao *et al*., 2023), polyploid crop *Triticum aestivum* had 90 *LBD* genes (Wang et al., 2021) and in dicots, *Cucumis melo* had 40 LBD genes (Tufekci, 2024). These findings provided a comparative basis for the understanding of the evolution of LBD genes across plant species. *CaLBD* genes perform several biological functions. For instance, *CaLBD29* is involved in alteration of leaf size and shape and causes male and female sterility. Furthermore, *CaLBD19* acts as a positive regulator of the gene network controlling root crown formation. *CaLBD47* regulates the distal-proximal patterns of petals in plants.

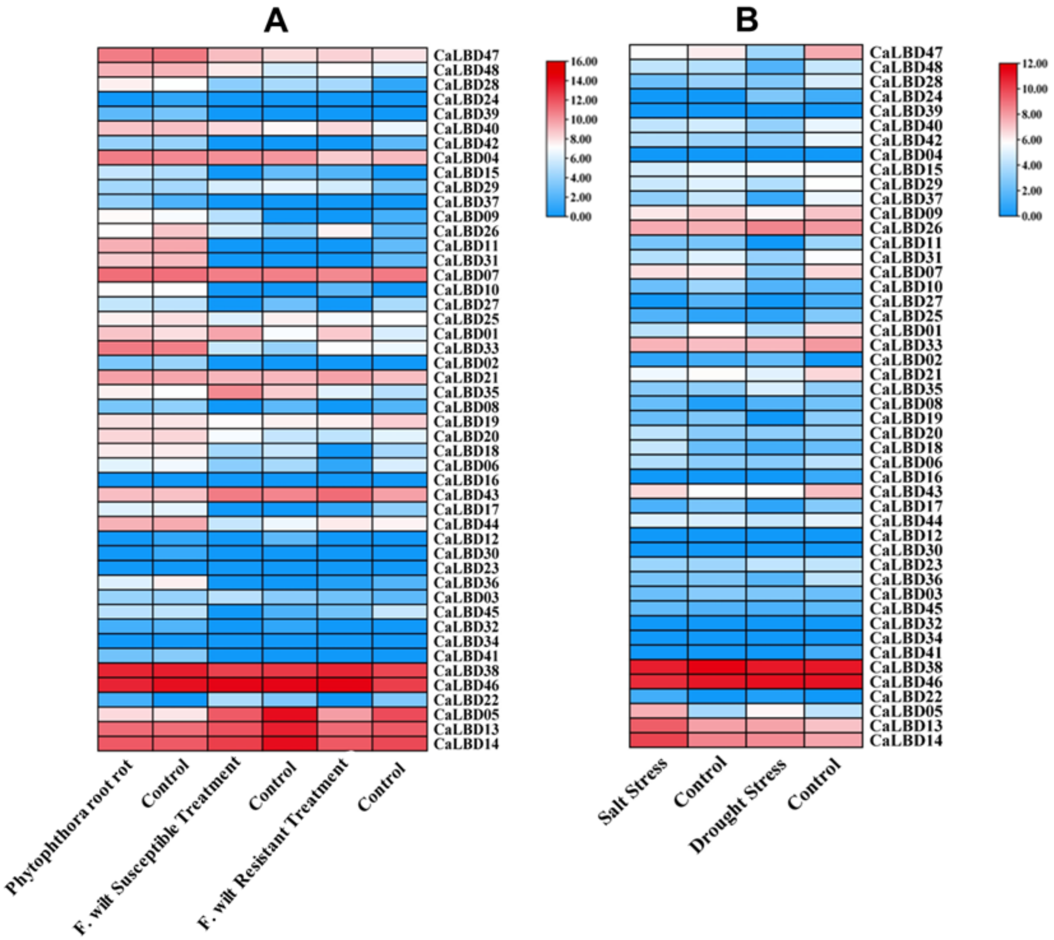


Figure 9. Gene expression analysis of 48 CaLBD genes under different Biotic and Abiotic stresses. (A) Transcriptomic profiling of CaLBD genes under biotic stresses of Phytophthora root rot and Fusarium wilt. (B) CaLBD genes expression under abiotic stresses of salt and drought. Rows represent genes while columns correspond to Biotic stresses of Fusarium wilt and Phytophthora root rot and Abiotic stresses of Drought and Salt along with their controls. Expression levels are highlighted by distinct colors.

Being in nucleus, *CaLBD20* is actively involved in lateral root formation and is regulated by the transcriptional activators ARF19 and ARF7. Whereas *CaLBD28* controls the adaxial-abaxial polarity of leaves and is involved in repression of homeobox gene BP. These findings highlight the crucial roles of *CaLBD* genes in regulating plant development and morphology. A total of 48 LBD genes were identified in *C. arietinum*, distributed non-uniformly across all its 8 chromosomes. This non-uniform distribution is common in transcription factor families. For instance, 90 LBD genes were distributed across all 20 chromosomes in soybean (Yang *et al*., 2017). In the comparative phylogenetic analysis of *CaLBDs* with *AtLBDs* and *OsLBDs*, the two main classes (I and II) were revealed, which were further divided into five distinct subgroups (I-a, I-b, I-c, I-d, II-a). Among all the species, Class-I had the highest number of LBD members which align with recent studies by Feng *et al*. (2022), Grimplet *et al*. (2017), and Ma *et al*. (2023), who also identified the predominance of class-I LBD genes in *Arabidopsis*, *Glycine max*, and *Phoebe bournei*, respectively.

The *CaLBD* genes showed a simple intron/exon structure, with 29 genes contained one intron, 14 genes lacked introns, and only 5 genes had two introns. Notably, all class-II genes had only one intron, which contrasted with the findings of Xu *et al*. (2021), who found no introns in the class-II LBD genes of *T. aestivum*. These results suggested a conserved gene structure, possibly linked to plant-specific regulations. Motif analysis identified ten motifs in *CaLBD* transcription factors, with motif 1 found in all members, followed by motifs 2 and 3. These findings were aligned with previous research by Huang *et al*. (2021) and Teng *et al*. (2018), who also found similar motif patterns.

Gene duplication analysis identified 17 *CaLBD* duplicated gene pairs, with 13 segmental duplications and 4 tandem duplications. The prevalence of segmental duplications suggested their key roles in the expansion of the LBD gene family in *C. arietinum.* These findings align with the previous study by Tian *et al*. (2022), which showed both types of gene duplication, with segmental duplication being more frequent. Synteny analysis revealed strong syntenic relationship between *CaLBD* genes and *M. truncatula* because both species belong to the same Fabaceae family (Subramanya *et al*., 2025), whereas weaker connection with *A. thaliana* was observed. A similar strong syntenic blocks between *C. arietinum* and *M. truncatula* was found by Varshney *et al*. (2013) who further observed its fragmented synteny with non-legumes. Overall, these blocks played an important role in facilitating the orthologs identification among the species (Kong *et al*., 2017).

Different cis-regulatory elements known for their active role in stress adaptation and transcriptional regulation were detected in promoter region of *CaLBD* genes. The presence of light responsive elements such as G-box and related elements highlighted their participation in photo-regulation and light mediated processes of development. A similar enrichment of light responsive elements was found by Wang *et al*. (2025), who found these elements in the promoters of *ZmLBD* genes. The presence of elements such as MBS and TC-rich repeats indicated the role of *CaLBD* genes in regulating responses against drought and pathogen stresses. Furthermore, different hormonal cis-regulatory elements indicated the role of phytohormones in *CaLBD* gene expression.

Transcriptomic profiling of *CaLBD* genes was performed under biotic (*Phytophthora* and *Fusarium oxysporum*) and abiotic (drought and salt) stress conditions. The highest expression of abiotic stress was observed in *CaLBD38* and *CaLBD46*. Under biotic stress conditions, *CaLBD46* showed significant upregulation followed by *CaLBD14* and *CaLBD38*. These results expressed the active response of *CaLBD46* and *CaLBD38* against biotic and abiotic stress conditions. This may be due to the presence of specific cis-regulatory elements, such as methyl jasmonate (MeJA), which are known to mediate resistance against heavy metals, drought, salinity, and pathogen stress (Yu *et al*., 2018).

CONCLUSION

This study addressed the critical research gap of lack of study on LBD transcription factor family in *Cicer arietinum* especially focusing on their functional roles against multiple biotic and abiotic stresses. A total of 48 candidate *CaLBD* genes were identified, distributed across all 8 chromosomes. These were classified into 2 groups and further subdivided into 5 subgroups by comparative phylogeny, revealing their evolutionary relationships with model plants such as *Oryza sativa* and *Arabidopsis thaliana.* Different other bioinformatics analyses were performed including synteny, gene structure, domain, gene duplication and gene expression analysis. Both types of duplications, i.e., tandem and segmental duplications were observed, expressing their roles in expansion of LBD gene family. Under the biotic stresses of *Phytophthora* root rot and *Fusarium* wilt, the transcriptomic profiling of *CaLBD* genes revealed the significant upregulation of *CaLBD46,* followed by *CaLBD14* and *CaLBD38.* Furthermore, under abiotic conditions of drought and salt stress, *CaLBD38* and *CaLBD46* expressed their strong upregulation. These findings show the role of LBD transcription factors against multiple biotic and abiotic stresses and are a valuable contribution to the existing literature about the molecular genetics of chickpea. Overall, this study not only provides valuable resources for future genetic studies about the molecular genetics of chickpea but also an asset for the development of stress tolerant varieties of chickpea through advanced molecular breeding and genetics programs.

AUTHOR CONTRIBUTIONS

Mubashir Sharif: Writing – original draft, Writing – review & editing, Formal analysis. Maria Riaz: Writing – original draft, Writing – review & editing. Shafqat Ali: Writing – review & data analysis. Aqsa Junaid: Validation, Writing – review & editing. Abdullah Bin Talat: Writing – review & editing. Qasim Ali: Writing – review & editing. Zaheer Ahmad: Writing – review & editing. Muhammad Annas Shahid: Conceptualization, Supervision, Resources, Validation.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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