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# Genome-Wide Identification of the *As2/LOB* Gene Family in *Elymus sibiricus* and the Expression Profiles Characterization

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**Abstract:**

**Background:** The *As2/LOB* gene family plays critical roles in regulating plant growth, development, and abiotic stress responses. Despite this gene family has been characterized in multiple plant species, it remained unstudied in the perennial allotetraploid grass *Elymus sibiricus*, an important species for grassland restoration and forage production on the Qinghai-Tibet Plateau.

**Results:** We identified 64 *As2/LOB* genes in *E. sibiricus* and systematically characterized their chromosomal distribution, gene structure, conserved motifs, cis-acting elements, and phylogenetic relationships. Phylogenetic analysis classified these proteins into seven distinct subfamilies. Gene structure analysis revealed the presence of conserved domains and motifs characteristic of the As2/LOB family, underscoring both functional conservation and subfamily-specific divergence. Tissue-specific expression profiling across roots, stems, leaves, and spikes at multiple developmental stages revealed pronounced spatial and temporal patterns, with several subfamily members showing preferential enrichment in organs that directly determine plant architecture, nutrient acquisition, and photosynthetic capacity—key traits contributing to forage biomass and yield. In parallel, expression analysis under indole-3-acetic acid (IAA) treatment, a central phytohormone regulating organogenesis and cell elongation, further implicated these genes in auxin-mediated developmental networks underlying vegetative and reproductive growth. Promoter analysis identified numerous hormone-responsive and stress-responsive cis-acting elements, suggesting potential regulatory

roles in hormone signalling and environmental adaptation. Chromosomal localization and synteny analysis indicated that the *As2/LOB* genes were unevenly distributed across the 14 chromosomes and identified multiple syntenic gene pairs, indicating that gene duplication events have contributed to family expansion. In addition, expression profiling under salt, drought, and ABA treatments revealed differential regulation of several members, suggesting their auxiliary roles in stress adaptation that may help stabilize biomass production under unfavorable environments.

**Conclusion:** This study provides the first comprehensive genomic resource for the *As2/LOB* gene family in *E. sibiricus*, revealing their expansion via duplications and functional diversification in organ development and biomass-related growth. Notably, *EsLOB52* was consistently induced across IAA, salt, drought, and ABA treatments, highlighting its potential as a candidate integrator of growth regulation and stress adaptation. These findings establish a foundation for molecular breeding aimed at improving both biomass yield and stress resilience in *E. sibiricus*.

**Keywords：** *Elymus sibiricus*; *As2/LOB* gene family; evolutionary relationships; gene expression; abiotic stress

## 1. Introduction

*Elymus sibiricus* is a perennial allotetraploid grass species (2n = 4x = 28, StSt HH) widely distributed across cold regions of Eurasia and is of considerable ecological and economic importance on the Qinghai-Tibet Plateau[1]. It is widely utilized for grassland restoration and livestock forage production, owing to its high genetic diversity, nutritional value, and notable stress tolerance, including cold and drought resistance[2]. Despite these valuable traits, the primary breeding objectives for *E. sibiricus* remain centered on improving biomass yield—particularly aboveground productivity—and enhancing yield stability under the harsh environmental conditions characteristic of high-altitude regions. With the recent release of a reference genome for *E. sibiricus* L. cv. Chuancao No. 2[3], genomic resources are now available to dissect the molecular basis of these complex agronomic traits. However, systematic functional characterization of genes governing growth, development, and biomass accumulation in this species remains largely lacking.

The *As2/LOB* gene family comprises a group of plant-specific transcription factors that play critical roles in regulating organ boundary formation, lateral root development, and leaf morphogenesis[4-10]. These developmental processes are of particular significance because they directly determine key agronomic traits: leaf size and morphology influence photosynthetic area and carbon assimilation efficiency; root architecture determines nutrient and water uptake capacity; and tillering patterns shape overall plant architecture and resource allocation[11].Coll

ectively, these traits constitute the fundamental determinants of biomass accumulation and yield in forage grasses. In model plants such as *Arabidopsis thaliana*, the founding member *AS2* interacts with *AS1* to repress *KNOX* gene expression, thereby regulating leaf adaxial-abaxial polarity and lateral organ formation [12-14]. *LOB* domain proteins also mediate lateral root and floral organ development by controlling cell division patterns at organ boundaries[15]. Throughout plant development, *As2/LOB* genes function from embryogenesis to maturity: during early embryogenesis, they help establish proper morphological patterns by precisely controlling cell division and differentiation; in the vegetative phase, they fine-tune stem elongation, root development, and lateral branching, thereby shaping overall plant architecture[6].; during reproductive growth, they contribute to floral organ formation by maintaining the floral meristem and initiating floral organ primordia[15].

In addition to their established roles in development, accumulating evidence has implicated *As2/LOB* genes in abiotic stress responses, positioning this family as a compelling candidate for understanding the growth–defense trade-off—the physiological balancing mechanism by which plants modulate growth and stress adaptation to optimize survival and productivity under fluctuating environments[16]. This family enhances stress resilience through multiple mechanisms, including regulation of stomatal movement, root system remodeling, osmoprotectant synthesis, and ion homeostasis, thereby mitigating the negative impacts of drought and salinity on growth and biomass production [17, 18]. Genome-wide analys

es have revealed that *As2/LOB* genes exhibit stress-responsive regulation in species such as *Hordeum vulgare* and *Asparagus officinalis*[19, 20]. These observations suggest that *As2/LOB* genes may function as integrative nodes that coordinate developmental programming with environmental signaling, making them attractive targets for breeding programs aimed at achieving both high biomass yield and stress resilience.

Despite the well-documented functions of *As2/LOB* genes in other plant species, their diversification, expression patterns, and physiological roles in *E. sibiricus* remain completely unexplored. To address this gap, we performed a genome-wide identification and comprehensive characterization of the *As2/LOB* gene family in *E. sibiricus*. Our analysis encompasses phylogeny, conserved domains, gene structures, chromosomal localization, cis-regulatory elements, and cross-species synteny. Particular emphasis was placed on tissue-specific expression profiling across different organs and developmental stages to infer their potential involvement in organ development and biomass-related growth regulation. In parallel, we investigated their transcriptional responses to IAA, ABA, salt, and drought treatments, to explore their auxiliary roles in stress adaptation. This study provides a foundational genomic resource for the *As2/LOB* family in *E. sibiricus* and establishes a basis for future functional studies and molecular breeding efforts aimed at improving both biomass productivity and environmental adaptability in this important forage grass.

## 2. Materials and Methods

## 2.1 Plant Materials and Methods

*Elymus sibiricus* materials were provided by the Academy of Animal Science and Veterinary Medicine, Qinghai University. Plants were cultivated in controlled-environment chambers at the Chinese Academy of Agricultural Sciences under 16-h/8-h light/dark cycles (25°C/18°C), 250 μmol photon $m^{-2}$ $s^{-1}$ light intensity, and 70% relative humidity. Two-week-old seedlings were subjected to phytohormone (IAA and ABA) and abiotic stress (salt and drought) treatments. Gene expression analysis was conducted on samples collected from seven critical growth stages—seedling stage, tillering stage, jointing stage, booting stage, heading date, flowering date, and maturity period—and five tissue types: roots, base, stem, leaves, and spikes. For each combination of stage and tissue, three independent biological replicates were analyzed. For stress treatments, seedlings received: 0.1 mM IAA, 0.1 mM ABA, 200 mM NaCl (salt), and 20% PEG6000- (drought). Samples were collected at 0, 3, 6, 12, 24, 48, 72, 120, and 288 hours post-treatment with three biological replicates. Heatmaps were generated using TBtools v2.225 software.

## 2.2 Identification Analysis of *As2/LOB* Gene Family

Forty-two *A. thaliana As2/LOB* protein sequences were retrieved from TAIR（https://www.arabidopsis.org/）. *E. sibiricus* gene sequences were translated to proteins using TBtools v2.225. Candidate proteins were identified through BLASTP alignment against *A. thaliana* reference sequences （E-value＜$1e^{-5}$）with duplicate removal[3]. Separately, the *As2/LOB* HMM profile from Pfam（http://pfam.

xfam.org/） screened predicted *E. sibiricus* proteins. Results from BLAST and HMM searches were intersected, followed by SMART domain verification to exclude sequences lacking the *As2/LOB* domain, yielding non-redundant *E. sibiricus As2/LOB* proteins. Physicochemical properties were predicted using TBtools v2.225[21].

### 2.3 Conserved Motifs and Gene Structure Analysis

Gene structures of *E. sibiricus As2/LOB* family members were analyzed using TBtools v2.225. Conserved protein motifs were identified via MEME（http：//meme.nbcr.net/meme） with a maximum of eight motifs, followed by visualization of results in TBtools v2.225.

### 2.4 Chromosomal Localization Analysis of *As2/LOB* Gene Family

Gene density data for *E. sibiricus* chromosomes were extracted using the chromosomal heatmap function in TBtools v2.225. Chromosomal positions of *As2/LOB* gene family members were mapped based on the *E. sibiricus* genome database, with distribution patterns visualized through the Gene Location Visualize from GTF/GFF tool in TBtools v2.225.

### 2.5 Promoter Analysis of the *As2/LOB* Gene Family

Promoter regions (1000 bp upstream of translation start sites) for *E. sibiricus As2/LOB* genes were extracted from whole-genome data using TBtools v2.225. Cis-acting elements were annotated via PlantCARE（http://bioinformatics.psb.ugent.be/webtools/plantcare/html/）[22], followed by manual curation to remove non-functional elements. Spatial distribution and functional classification of predicte

d cis-elements were visualized using TBtools v2.225.

### 2.6 Synteny and Homologous Gene Pairs

Intra-species synteny of *E. sibiricus As2/LOB* genes and inter-species synteny among *E. sibiricus*, *A. thaliana*, *B. distachyon*, *H. vulgare*, and *O. sativa* were analyzed using MCscanX in TBtools v2.225. Resulting syntenic relationships were visualized via integrated Circos plotting.

### 2.7 Evolutionary Analysis of the *As2/LOB* System

*A. thaliana As2/LOB* protein sequences were retrieved from TAIR（(https://www.arabidopsis.org/）, while other plant sequences were obtained from NCBI（https://www.ncbi.nlm.nih.gov/）. Multiple sequence alignment of *As2/LOB* proteins was performed using MUSCLE in MEGA-X, followed by phylogenetic tree construction via the Neighbor-Joining（NJ） method in MEGA v7.0. Final tree visualization and annotation were implemented in iTOL（https://itol.embl.de/）.

### 2.8 The secondary and tertiary structure of proteins

Protein secondary structures were predicted using SOPMA (https://npsa.lyon.inserm.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html), while tertiary structures were modeled with SWISS-MODEL（https://www.swissmodel.expasy.org/）.

### 2.9 Total RNA Extraction and qPCR Analysis

Total RNA was extracted using Trizol reagent (Invitrogen,Carlsbad,CA,USA) according to the manufacturer's protocol, followed by genomic DNA removal with DNase I (Promega,Madison,WI,USA). First-strand cDNA was synthesized from 1 μg total RNA using M-MLV reverse transcriptase (Promega,Madison,WI,U

SA). RT-qPCR was performed on a CFX96 Touch™ system (Bio-Rad) under the following conditions: 95°C for 2 min; 40 cycles of 95°C for 15 s and 60°C for 40 s; followed by melt curve analysis (95°C for 15 s, 60°C for 15 s). The Medicago sativa actin gene served as internal control, with relative expression calculated by the 2 -ΔΔCt method. All experiments included three biological and technical replicates, with primers designed using Primer 5.0 software.

### 2.10 Data Analysis

Statistical analyses were performed using one-way ANOVA in SPSS (v23.0), with data presented as mean ± standard deviation. Graphical analyses employed GraphPad Prism 10.1.1, with statistical significance defined at $P < 0.05$.

## 3.Results

### 3.1 Identification and Physicochemical Properties of the *As2/LOB* Gene Family

In this study, the physicochemical properties of the *As2/LOB* transcription factor family in *E. sibiricus* were systematically analyzed.yrevealing significant heterogeneity among family members. A total of 64 *As2/LOB* genes were identified in the *E. sibiricus* genome and designated *EsLOB1–EsLOB64* according to their chromosomal locations. Analysis of the encoded proteins (Table 1) showed that their amino acid lengths ranged from 100 to 466 residues, with predicated molecular weights (MW) varying from 10.69 to 51.49 kDa (average MW: 25.56 kDa). Theoretical isoelectric points (pI) ranged from 4.58 (*EsLOB41*) to 9.83 (*EsLOB26*), including 34 acidic proteins (pI < 7) and 30 basic Proteins. The c

oexistence of acidic (e.g.,EsLOB14, pI=4.95) and basic (e.g., *EsLOB26*) subgroups is consistent with the functional diversification reported for the LOB family in *A. thaliana* [4].

The instability index, an indicator of protein stability (with lower values reflecting higher stability), ranged from 29.72 to 78.26. Notably, approximately 64% of the members exhibited instability indices above 50 (e.g., *EsLOB38* = 78.26), suggesting that the majority of EsLOB proteins may be relatively unstable.[23]. The aliphatic index, which reflects the relative volume of aliphatic side chains and is associated with thermostability, ranged from 50.31 to 91.80. Grand averages of hydropathy (GRAVY) values varied from -0.69 to 0.14. based on GRAVY values, six proteins —*EsLOB6*, *EsLOB10*, *EsLOB20*, *EsLOB27*, *EsLOB40* and *EsLOB53* were predicted to be hydrophobic, whereas the remaining 58 proteins were hydrophilic. The presence of higher aliphatic indices (e.g., *EsLOB5* = 91.80) may indicate enhanced thermostability [24], while strongly negative GRAVY values (e.g., *EsLOB52*, GRAVY = -0.69) suggest hydrophilic characteristics potentially associated with cytoplasmic localization. Collectively, these results highlight considerable structural diversity within the EsLOB protein family which may underpin their functional differentiation and adaptive roles in *E. sibiricus* structural adaptability [25].

### 3.2 Phylogenetic Analysis of *As2/LOB* Gene Family

To explore the evolutionary relationships of *As2/LOB* gene family members in *E. sibiricus* and other plant species, a phylogenetic tree were constructed based

on 187 amino acid sequences, including 64 from *E. sibiricus*, 32 from *A. t haliana*, 28 from *Brachypodium distachyon*, 29 from *H. vulgare*, and 34 from *Oryza sativa* (Figure 1). Phylogenetic analysis classified the As2/LOB protein s into seven distinct subfamilies —*class Ia, class Ib, class Ic, class Id, class I e, class IIa,* and *class IIb*. Among the 64 *E. sibiricus* members, *class Ia* comp rised 16 members, *class Ib* 14 genes, *class Ic* 7 genes, *class Id* 7 genes, *clas s Ie* 8 genes, *class IIa* 8 genes, and *class IIb* 4 gens. Multiple sequence align ment revealed high sequence similarity between several *E. sibiricus* genes (*Es LOB21, EsLOB56, EsLOB32, EsLOB10, EsLOB9*) and their putative orthologs i n *B. distachyon* (*Bd4-2, Bd1-2, Bd4-5, Bd2-4, Bd2-1*) and *H. vulgare* (*HvLBd3 H-1, HvLBd4H-3, HvLBd5H-1, HvLBd1H-1, HvLBd1H-4*). This close phylogenet ic relationship suggest that suggested that *As2/LOB* proteins within the same su bfamily across these grass species may have conserved biological functions.

### 3.3 Conserved Motifs and Gene Structure Analysis of *As2/LOB* Gene Famil y

To further explore the functional diversification of *As2/LOB*-related candidate g enes in *E. sibiricus*, conserved motifs and gene structures were systematically analyzed (Figure 2). Among the 64 identified genes, , 49 contained motifs 1-3, of which 43 also harbored motif 5. Eleven genes possessed motifs 1, 4, 5, 6, and 7, and seven of these additionally contained motif 8. Motif 1 was pred ominantly located at the N-terminus, whereas motifs 5 and 7 were primarily di stributed at the C-terminus. As shown in Figure 2B, all 64 As2/LOB proteins

contained the conserved *LOB* domain. Notably, EsLOB31 harbored both the *LOB* domain and the PRK10856 conserved domain, suggesting potential divergence. Gene structure analysis revealed that members of the *As2/LOB* familyshared relatively conserved exon-intron organization patterns, although substantial variation was observed in gene length and exons-intron number . Specifically, 24 genes contained both untranslated regions (UTRs) and coding sequences (CDSs), whereas 40 genes were composed exclusively of CDS regions. The number of exons per gene ranged up to five. Collectively, these findings indicate a high degree of structural conservation within the *As2/LOB* gene family, accompanied by ostructural variation that may contribute to functional differentiation. in *E. sibiricus*. The conserved domains, motifs composition, and gene organization collectively suggest that these genes play vital roles in diverse biological processes and possess significant evolutionary and regulatory relevance.

### 3.4 Chromosomal localization and synteny analysis of the *As2/LOB* gene family

Chromosomal localization analysis of *E. sibiricus As2/LOB* genes revealed an uneven distribution across its 14 chromosomes(Figure 3A), ranging from one gene each on chromosomes 7 and 8 to 11 genes on chromosome 14. Relatively gene-dense regions were observed on Chr05, Chr06, Chr12, and Chr14. Notably, two local gene clusters (*EsLOB44–EsLOB 48* and *EsLOB59–EsLOB64*) were identified on Chr12 and Chr14, respectively, suggesting that tandem duplication events contributed to local gene expansion. These results indicate that structu

ral variation, particularly gene duplication, has played a critical role in the evol utionary diversification of the EsLOB gene family, thereby providing a theoreti cal foundation for further exploring of its regulatory functions in plant develo pment and responses to environmental stimuli. To further elucidate the expansio n mechanisms of the *As2/LOB* family, segmental duplications were analyzed within the *E. sibiricus* genome. A total of 30 segmental duplicated gene pairs were identified (Figure 3B), indicating that segmental duplication has been the predominant driver of family expansion, especially on chromosomes 3, 4, 5, 6, and 14. Furthermore, comparative synteny analyses were conducted between *E. sibiricus* and four representative species, including *A. thaliana*, *B. distachyon*, *H. vulgare*, and *O. sativa* (Figure 3C). Nine homologous gene pairs were detec ted ed between *E. sibiricus* and *A. thaliana*, distributed across chromosomes 5, 6, 9, 10, 13, 14. In contrast, 42, 51, and 48 orthologous gene pairs were ident ified between *E. sibiricus* and *B. distachyon*, *H. vulgare*, and *O. sativa*, respect ively. These orthologous relationships were mainly distributed on chromosomes 3, 4, 5, 6, 9, 10, 12, 13, and 14, demonstrating a high level of gene collineari ty and evolutionary conservation between *E. sibiricus* and other grass species.

### 3.5 Functional Analysis of Cis-Elements in the Promoter Region of the *As2/LOB* Gene Family

To elucidate the potential regulatory mechanisms underlying *As2/LOB* gene func tion in *E. sibiricus*, we identified cis-acting elements within the 2,000 bp upstr eam promoter regions of all 64 genes using PlantCARE (Figure 4; Supplement

ary Table 1). The identified cis-elements were classified into four major categories based on their annotated functions: Hormone Response, Development-related, Abiotic Stress Response, and Biotic Stress Response.

Notably, development-related cis-elements were broadly distributed across the *EsLOB* promoters (Figure 4). In particular, CAT-box, a motif associated with meristem expression, was detected in 20 gene promoters, while GC-motif was identified in 22 genes. MSA-like elements, which are involved in cell cycle regulation, were found in 13 gene promoters, and RY-element, associated with seed-specific regulation, was present in 13 genes. In addition, NON-box (meristem-specific activation) was detected in *EsLOB22*, and circadian control-related elements were identified in four genes (*EsLOB15*, *EsLOB31*, *EsLOB46*, *EsLOB51*) (Supplementary Table 1). The widespread presence of these development-related cis-elements—particularly those associated with meristem activity, cell division, and seed development—suggests that *EsLOB* genes are potentially involved in the regulation of organ growth and development. Given that meristem activity determines leaf initiation, tillering capacity, and root branching, and cell cycle regulation directly impacts organ size and biomass accumulation, these observations point to a potential role for *EsLOB* genes in modulating plant architecture and yield-related traits in E. sibiricus.

Among hormone-responsive elements, auxin-related motifs (AuxRR-core and AuxRE) were detected in five *EsLOB* genes (*EsLOB1*, *EsLOB56*, *EsLOB60*, *EsLOB61*, and *EsLOB64*). Given that auxin is a central regulator of organogenesis, l

ateral root initiation, and leaf morphogenesis, the presence of these elements suggests that these genes may participate in auxin-mediated developmental programs that shape plant architecture and influence biomass production. In addition, ABRE motifs (ABA-responsive) were the most abundant hormone-related elements, occurring 133 times across 49 genes, while gibberellin-responsive elements (GARE-motif in 14 genes and TATC-box in 4 genes) were also detected.

For stress-associated cis-elements, drought-inducible MBS elements were detected in 19 gene promoters, low-temperature-responsive LTR elements in 31 genes, wound-responsive WUN-motif in *EsLOB20*, and anaerobic induction ARE elements in 31 genes (Supplementary Table 1). The distribution of these stress-related elements overlapped extensively with development-related elements in several genes, suggesting a potential role for these *EsLOB* members in coordinating growth and stress adaptation.

The distribution of cis-acting elements varied markedly among *EsLOB* genes. Seven genes contained only one functional category of cis-element, sixteen genes possessed two categories, and twenty-seven genes harbored three categories. Notably, *EsLOB19*, *EsLOB34*, *EsLOB35*, *EsLOB40*, *EsLOB42*, *EsLOB48*, *EsLOB53*, *EsLOB56*, *EsLOB59*, *EsLOB61*, and *EsLOB62* possessed a combination of Hormone Response, Development-related, and Abiotic Stress Response elements. The co-occurrence of these three categories within the same promoters suggests that these genes may function as integrative hubs, potentially mediating the trade-off between growth and stress adaptation—a critical determinant of ecologic

al fitness in *E. sibiricus*, which must maintain productivity under the harsh con ditions of the Qinghai-Tibet Plateau. These candidates, particularly those harbori ng Development-related and auxin-responsive cis-elements, represent promising t argets for molecular breeding aimed at optimizing biomass yield without compr omising stress resilience.

### 3.6 Secondary and tertiary structure Analysis of As2/LOB proteins

The secondary and tertiary structures of EsLOB proteins were predicated to ch aracterize their structural features (Figure 5, Table 2). Overall, EsLOB proteins exhibited a high proportion of intrinsically disordered regions, with α-helices r epresenting the predominant structural elements. Secondary structure predictions of the 64 *EsLOB* proteins indicated that β-turn structures were generally rare, ranging from 0 to a maximum of 4.26%, and 56% of the proteins lacked dete ctable β-turn entirely. This pattern may reflect either a systematic bias in pred iction algorithm or, more plausibly, the intrinsic structural properties of the LO B domain, which is primarily composed of α-helices and loops[10, 26]. Rand om coils constituted the major secondary structure (44.64–71.20%; with a mean >60%), indicating extensive intrinsically disordered regions (IDRs) [27]. Such disorder is a common feature of plant transcription factors and is thought to fa cilitate dynamic protein-protein interactions and flexible transcriptional regulatio n[28]. In contrast, α-helices accounted for 20.45-52.98% of residues（mean ~3 6%）and showed considerable interprotein variability, suggesting functional dive rgence and differences in binding-specificity among family members [29]. . E

xtended β-turn strands were limited (generally <5%; mean 2.6%), although slightly elevated proportions were observed in *EsLOB63, EsLOB64 EsLOB3,* and *EsLOB48* (7.00–8.03%), confirming that β-turn are not major conformational feature of this family [30]. Collectively, high disorder and variable α-helices defines the structural core of the EsLOB family. This conformational plasticity may underlie their multifunctional regulatory roles in plant growth and development[27, 28]. Structurally, the conserved *As2* domain is located at the N-terminus, whereas the C-terminus contains the LOB domain, which is enriched in basic residues and forms a helix-loop-helix (HLH) structure essential for protein-protein interactions.

### 3.7 Tissue-specific expression analysis of *As2/LOB* genes

To investigate whether *As2/LOB* gene family members in *E. sibiricus* function as general housekeeping genes or exhibit tissue-specific regulatory roles, representative genes from each subfamily were analyzed across 7 developmental stages (seedling, tillering, jointing, booting, heading date, flowering date, and maturity) and five tissues (roots, stem base, stem, leaves, and spikes) (Figure 6). The results revealed pronounced tissue specificity and developmental stage-dependent expression patterns. Class Ia members (*EsLOB3*, *29*, *44*, *49*, *53*, *58*, *59*, and *60*) displayed predominantly root-enriched expression during the tillering stage. Relative to seedling-stage roots (normalized to 1.0), *EsLOB29*, *EsLOB58*, and *EsLOB60* were markedly induced in tillering roots (6.51-, 4.29-, and 5.42-fold, respectively) and remained elevated at maturity (2.06–2.69-fold). In contrast,

*EsLOB3* and *EsLOB49* exhibited relatively stable root expression across all stages (1.0–2.38-fold), suggesting functional divergence within the subfamily in regulating root development and lateral root initiation. *Class Ib* genes (*EsLOB6*, *7*, *12*, *24*, *32*, *36*, *42*, and *50*) showed extreme leaf-predominant and stage-dependent expression. Among all members of this gene family, ***EsLOB36*** exhibited the highest transcript abundance. It was highly expressed in leaves at both the tillering （23.47-fold）and maturity （23.96-fold）stages, while low transcript levels were also detected in stem bases(0.27-fold) and stems (0.12-fold) during the tillering stage. This expression pattern suggests that **EsLOB36** is not only a key positive regulator of leaf development but may also contribute to the coordinated regulation of aboveground vegetative tissues during plant vegetative growth. , *EsLOB12* peaked in flowering-stage leaves (6.28-fold), whereas *EsLOB50* was elevated in tillering (5.93-fold) and mature leaves (3.82-fold). Other members (*EsLOB6*, *7*, *24*, *42*) maintained moderate leaf expression (2.14–4.38-fold) across stages. *Class Ic* members (*EsLOB15*, *20*, *23*, *27*, and *52*) exhibited dual enrichment in roots and leaves. *EsLOB15*, *EsLOB20*, and *EsLOB27* peaked in roots of tillering-stage (9.29-, 6.22-, and 6.13-fold, respectively) representing the highest root expression observed at this stage. They also displayed high expression in seedling (8.91-fold) and flowering-stage (7.21-fold) leaves, indicating potential cross-tissue regulatory functions. Within *Class Id*, *EsLOB34* was enriched in roots of tillering-stage (3.89-fold), whereas *ESLOB61* was upregulated in roots of flowering-stage (3.50-fold) and mature spikes (2.20-fold). *Class Ie*

genes displayed a diffuse expression profile, with *EsLOB19* showing a modest upregulation in roots of tillering-stage (3.08-fold). The *Class II* subfamily displayed significant functional divergence. In *Class IIa*, *EsLOB2* was specifically activated in spikes of flowering-stage (3.56-fold). *EsLOB21* showed bimodal peaks in leaves of flowering-stage (6.59-fold) and roots of tillering-stage (5.28-fold). The Class IIb member *EsLOB51* maintained sustained high expression in roots during tillering (6.77-fold), heading (5.15-fold), and maturity stages (5.15-fold), suggesting a constitutive role in root function distinct from the stage-specific pattern observed in*Class Ia*. *ESLOB1* was enriched in leaves during flowering (5.70-fold) and maturity stages (4.68-fold). Overall, these results demonstrate substantial functional differentiation among As2/LOB subfamilies, with distinct spatial and temporal expression patterns indicative of specialized regulatory roles in root development, leaf growth, and reproductive organ formation.

### 3.8 Expression Profile of *As2/LOB* in Response to Abiotic Stress

To evaluate the involvement of *As2/LOB* genes in abiotic stress responses, gene expression patterns were quantified following hydroponic treatment of two-week-old *E. sibiricus* seedlings with 0.1 mM auxin (IAA), 0.1 mM abscisic acid (ABA), 200 mM NaCl (salt stress), or 20% PEG6000 (drought stress). Samples were collected at 3, 6, 12, 24, 48, 72, 120, and 288 h, with three biological replicates per time point.

Indole-3-acetic acid (IAA) regulates organogenesis and cell elongation. Here, we performed a temporal expression analysis following IAA treatment in *E. sibir*

*icus*, which revealed a complex *As2/LOB*-mediated regulatory network for biom ass. Individual members exhibited distinct, subfamily-specific expression dynami cs that appear to coordinate fundamental cellular processes such as division, el ongation, and differentiation. Following IAA treatment, several genes (*EsLOB3*, *29*, *24*, *42*, *27*, *31*, *2*, *21*, *47*, and *51*) displayed a transient early up-regulation followed by down-regulation.Notably, the timing of repression was subfamily-d ependent: Class Ia and Ic members declined during 72–120 h, whereas Class I b, IIa, and IIb members decreased earlier(12–24 h). Conversely, another group (*EsLOB44, 53*, *59*, *6*, *7*, *32*, *46*, *14*) showed initial reduction at 3–6 h followe d by up-regulation. A third group (*EsLOB49*, *36*, *50*, *20*, *23*, *52*, *61*, *19*, *33*, *4*, and *56*) exhibited sustained upregulation. Among these, *EsLOB36*, *EsLOB23*, *EsLOB52*, *EsLOB61*, *EsLOB19*, and *EsLOB56* were particularly responsive, with 3.97-, 3.07-, 3.08-, 2.18-, 5.52-, and 5.36-fold increases, respectively (Figure 7).

These findings indicate that during the early treatment phase (3 – 12 h), most g enes were rapidly activated, with transcript levels significantly above the 0 h c ontrol, reflecting a swift positive response to auxin signaling. During the mid-tr eatment phase (24 – 72 h), these genes maintained consistently high transcript l evels, with some members reaching peak expression; no family-wide repression was observed. During the late treatment phase (120 – 288 h), most genes remai ned highly expressed, without a general downward trend or adaptive downregul ation. Overall, these results demonstrate that IAA effectively activates transcripti

on of the Class I LOB gene family in *E. sibiricus*. The temporal activation pa ttern of this family suggests its involvement in auxin-mediated regulation of do wnstream growth and developmental processes.

Under ABA treatment, several genes including *EsLOB1, EsLOB14, EsLOB20–2 1, EsLOB24, EsLOB27, EsLOB42, EsLOB47, EsLOB49, EsLOB51, EsLOB53, E sLOB56,* and *EsLOB60* and *EsLOB61*, displayed transient upregulation followed by repression (Figure 7). Notably, *EsLOB18* and *EsLOB36* were remarkably upr egulated by 3.34- and 3.88-fold, respectively. The ABA-responsive expression pattern of *EsLOB52* was consistent with its drought-induced expression, sugge sting participation in ABA-dependent stress signaling pathways.

Under NaCl stress, most *As2/LOB* genes displayed progressive downregulation. However, *EsLOB2, EsLOB7, EsLOB12, EsLOB18, EsLOB27, EsLOB29, EsLOB3 3–34, EsLOB44, EsLOB50–51, EsLOB58–59,* and *EsLOB61* exhibited transient upregulation prior to decline. In contrast, *EsLOB14, EsLOB20, EsLOB52,* and *EsLOB60* showed sustained upregulation (Figure 8), suggesting their potential i nvolvement in salt adaptation and stress-responsive regulatory pathways. Under PEG-induced drought stress, most genes showed early upregulation followed by gradual repression. Notably, *EsLOB2, EsLOB6,* and *EsLOB52* were significantly induced (7.98-, 4.36-, and 6.97-fold, respectively; Figure 8), indicating key role s in osmotic stress adaptation, possibly through root development modulation.

## 4. Discussion

The *AS2/LOB* gene family, also known in the literature as the *LBD* (LATERAL

ORGAN BOUNDARIES DOMAIN) family based on its conserved domain, comprises plant-specific transcription factors with critical functions in organ development and stress responses. In this study, we performed the first genome-wide identification of this family in *Elymus sibiricus*, identifying 64 *EsLOB* members and systematically characterising their gene structures, conserved motifs, chromosomal distributions, cis-acting elements and expression profiles. As a plant-specific transcription factor family, these *AS2/LOB* (*LBD*) genes regulate multiple developmental processes from seedling establishment to reproductive maturity, with core functions in organ boundary formation and lateral organ development – processes that directly determine leaf area, root architecture, tillering patterns and reproductive output, collectively constituting the developmental basis of forage biomass accumulation [4-6]. In addition, this family is deeply involved in phytohormone signalling and abiotic stress responses, suggesting a pivotal role in coordinating the growth-defence trade-off [16，17].

Family expansion and structural divergence provide the foundation for functional diversification. The predicted *EsLOB* proteins range from 100 to 466 amino acids, with molecular weights of 10.69–51.49 kDa and theoretical isoelectric points (pI) ranging from 4.58 to 9.83. Among these, 34 proteins were acidic (pI < 7) and 30 were basic (pI > 7). Compared with *O. sativa* (35 genes)[31], *Z. mays* (43 genes)[32], and *H. vulgare* (24 genes)[19], the *AS2/LOB* family in *E. sibiricus* is considerably larger, implying a positive correlation between family size and genome complexity. The allotetraploid nature (StStHH) likely contribute

d to this expansion through sub-genome additive effects. Conserved motif analysis showed that motifs 1–3 constitute the core domain, consistent with the canonical *LOB* protein architecture. Promoter analysis revealed abundant hormone-responsive (ABRE, GARE-motif, AuxRE) and abiotic stress-responsive (MBS, LTR) cis-elements, but their distribution varied markedly among subfamilies, indicating differential hormonal and stress regulation that may mediate adaptive phenotypic plasticity in *E. sibiricus* under diverse habitats[33, 34].

Phylogeny and chromosomal distribution reveal functional divergence between Class I and Class II, with implications for biomass. Phylogenetic analysis with *A. thaliana*, *B. distachyon*, *H. vulgare*, and *O. sativa* classified the 64 *EsLOB* proteins into seven subfamilies (Class Ia–Ie, Class IIa–IIb). These genes are unevenly distributed across the 14 chromosomes, with chromosome 14 harbouring the most members (11 genes), most of which belong to Class Ia. This clustering suggests that chromosome 14 may be a hotspot for functional diversification of the *EsLOB* family. Functionally, Class I genes are widely involved in leaf development (including morphogenesis and polarity establishment) and lateral root growth. For example, Arabidopsis AS2 interacts with AS1 to regulate leaf axis formation (adaxial-abaxial, medial-lateral and proximal-distal axes), thereby modulating cell proliferation and leaf symmetry – processes that directly determine leaf area and photosynthetic capacity, the morphological basis of biomass accumulation[12-14，46].

In this study, expression analysis revealed that Class Ib *EsLOB* genes exhibit characteristic leaf-preferential and developmental stage-dependent expression patterns, suggesting that this subfamily plays central roles in leaf morphogenesis, vegetative growth[35], and leaf development in forage grasses. These findings indicate that Class Ib *EsLOB* genes are likely key regulators coordinating the spatiotemporal development of leaves.

Overall, all Class Ib members were preferentially expressed in leaf tissues, although their transcript abundances varied markedly across the tillering, flowering, and maturity stages. This dynamic expression pattern indicated that these genes may be not function redundantly; rather, they may have undergone functional diversification to establish a coordinated regulatory network in which individual members contribute to distinct aspects of leaf development at specific developmental stages.

Leaf size is a key agronomic trait that determines photosynthetic efficiency and forage yield. Previous studies in *Elymus sibiricus* have demonstrated that leaf area and photosynthetic rate are both strongly and positively correlated with aboveground biomass and hay yield[36], with leaf morphological traits explaining a substantial proportion of phenotypic variation in forage productivity. Studies on perennial grasses adapted to alpine environments have further shown that a larger effective leaf area enhances canopy interception of photosynthetically active radiation[37, 38], increases carbon assimilation, and promotes the accumulation of aboveground biomass, thereby providing a fundamental basis for high forage productivity. At the molecular level, genes involved in leaf morphogenesis have been shown to regulate leaf area by

controlling cell proliferation and expansion[39, 40], ultimately influencing canopy photosynthetic capacity and final biomass production. Based on these findings, we speculate that Class Ib *EsLOB* genes may regulate leaf cell proliferation and expansion at different developmental stages, thereby modulating leaf size and total leaf area. Such regulation is likely to affect photosynthetic carbon assimilation and assimilate accumulation, ultimately influencing key yield-related traits, including tiller number and aboveground biomass, and consequently contributing to forage yield formation in *E. sibiricus*[41, 42]. These findings suggest that Class Ib *EsLOB* genes are closely associated with leaf development in *E. sibiricus* and provide a valuable framework for identifying candidate regulators of leaf morphogenesis. However, their precise biological functions, downstream regulatory networks, interacting proteins, and potential roles in modulating photosynthetic performance and stress tolerance remain largely unknown. Future studies integrating gene overexpression and loss-of-function analyses with transcriptomic approaches will be required to elucidate the molecular mechanisms by which Class Ib *EsLOB* genes regulate leaf development and plant architecture. Such studies will facilitate the identification of key genetic resources for improving plant architecture, enhancing photosynthetic efficiency, and increasing forage yield in *E. sibiricus*.

In contrast, Class II genes mainly participate in hormone signalling and metabo lic regulation, particularly auxin and cytokinin pathways. Representative exampl es include Arabidopsis *ASL9/LBD3*, which promotes cytokinin-induced secondar y root growth and maintains auxin/cytokinin balance during apical hook formati

572 on and lateral root development – its overexpression suppresses primary root
573 elongation and lateral root formation[4, 43-45]; and *ASL3/LBD25*, which acts in
574 both auxin signalling and photomorphogenesis and serves as a substrate recept
575 or of the CUL4-RING E3 ligase, negatively regulating ABA-mediated seed ger
576 mination and seedling establishment[46]. These phenotypes illustrate the diverse
577 roles of Class II genes in hormone-driven organogenesis and environmental res
578 ponses.
579 Gene duplication events drove family expansion and may contribute to biomass
580 stability. Intra-genomic synteny analysis identified 30 segmentally duplicated gene
581 pairs, indicating that segmental duplication is the predominant driver of *EsLOB* family
582 expansion, especially on chromosomes 3, 4, 5, 6 and 14. Cross-species synteny showed
583 high collinearity between *E. sibiricus* and *B. distachyon* (42 pairs) and *H. vulgare* (51
584 pairs), far exceeding that with *A. thaliana* (9 pairs), reflecting conserved evolutionary
585 relationships among monocots. Such uneven distribution and abundant collinear pairs
586 suggest that expansion via segmental and tandem duplications likely promoted
587 functional diversification and neofunctionalisation. This may support stable biomass
588 accumulation under fluctuating environments – when some members are suppressed by
589 stress, redundant or neofunctionalised paralogues can compensate, buffering the impact
590 on growth and development[47].
591 IAA-induced expression and homologous gene analysis support the involvement
592 of *EsLOB* genes in auxin-mediated organ development affecting biomass. The
593 phytohormone auxin (IAA) is a central regulator of plant height, tillering and lateral

root formation, directly influencing biomass allocation. In this study, *EsLOB36*, *EsLOB23*, *EsLOB52*, *EsLOB61*, *EsLOB19* and *EsLOB56* were significantly up-regulated under exogenous IAA treatment. In model plants, the core auxin signalling component *ARF7* directly activates *LBD16*, *LBD18* and *LBD29*, which are essential for lateral root initiation and development [48]. Furthermore, *LBD29* interacts with *MYB2* and *MYB108* to induce *CDEF1* expression and up-regulate the auxin influx carrier gene *LAX3*, promoting cell separation and lateral root emergence[49]. In our phylogenetic tree, *EsLOB21* and *EsLOB56* cluster with Arabidopsis *AtLBD29* (*ASL16*), suggesting they are putative orthologues of *AtLBD29* in *E. sibiricus*. Protein sequence alignment confirmed that both *EsLOB21* and *EsLOB56* contain the canonical *LOB* domain ($CX_2CX_6CX_3C$ zinc-finger motif and $LX_6LX_3LX_6L$ leucine-zipper-like region), with their C-terminal regions sharing >45% sequence similarity with *AtLBD29*. This conserved IAA-ARF-LBD regulatory module has been validated in diverse species. In Panax ginseng, IAA enhances the transcriptional activity of *PgARF*, which acts synergistically with *PgLBD18* to optimize lateral root architecture[50]. In the monocot crop Setaria italica, *SiLBD21* is strongly induced by IAA in roots and stem nodes, and its ectopic expression promotes lateral root formation and tiller production by repressing the tillering regulator *SiMOC1*[48]. In Solanum lycopersicum, transcriptomic analysis of early lateral root formation revealed that *LBD16* is a key component of the auxin signalling module IAA14-ARF7/ARF19-LBD16-CDKA1 that regulates lateral root initiation[11]. Therefore, the IAA-induced *EsLOB21* and *EsLOB56* likely act through similar

616 mechanisms to regulate lateral root development in *E. sibiricus* – by expanding root
617 absorption area and improving water/nutrient use efficiency, they indirectly promote
618 above-ground biomass accumulation. Moreover, the up-regulation
619 of *EsLOB7*, *EsLOB23* and *EsLOB36* under IAA also suggests their involvement in
620 stem elongation and leaf expansion, synergistically enhancing overall plant biomass.
621 As a plant-specific transcription factor family, *LBD* proteins also contribute to
622 photosynthetic efficiency and biomass accumulation by regulating nitrogen metabolism
623 and carbon partitioning. In Arabidopsis, the nitrate-inducible *LBD37*, *LBD38* and
624 *LBD39* act as negative regulators of anthocyanin biosynthesis and repress multiple
625 nitrogen-responsive genes, including key genes required for nitrate uptake and
626 assimilation [6]. This regulatory cascade alters nitrate content, protein, amino acid and
627 starch levels, thereby affecting nitrogen-related growth and biomass accumulation [6].
628 Similarly, *ASL3/LBD25* acts as a key growth regulator downstream of auxin and
629 modulates photomorphogenic processes [10,38].
630 Stress-responsive expression patterns identify *EsLOB52* as a potential hub integrating
631 growth and defence. Under ABA treatment, *EsLOB18* and *EsLOB36* were remarkably
632 up-regulated (3.34- and 3.88-fold, respectively), consistent with the ABA-inducible
633 pattern of *ASL19/LBD30* in Arabidopsis[51]. Under salt
634 stress, *EsLOB14*, *EsLOB20*, *EsLOB52* and *EsLOB60* showed sustained up-regulation,
635 suggesting their roles as positive regulators of salt adaptation. Under PEG-simulated
636 drought, *EsLOB2* (7.98-fold), *EsLOB6* (4.36-fold) and *EsLOB52* (6.97-fold) were
637 strongly induced. Notably, *EsLOB52* was consistently up-regulated under all four

treatments (IAA, salt, ABA and drought), a unique expression pattern that strongly implicates it as a molecular hub integrating auxin signalling with multiple abiotic stress pathways. Under normal conditions, it may participate in auxin-driven root architecture to promote nutrient uptake; under stress, it may rapidly respond to ABA and orchestrate downstream defence genes, enhancing tolerance without completely sacrificing growth. However, the effects of *AS2/LOB* genes on biomass can be double-edged. Ectopic or overexpression can incur growth costs – for example, *StAS2-15* overexpression in potato accelerates metabolism, leading to premature leaf senescence[52]. and in tomato, mis-expression of certain *LBD* genes affects flowering time and fruit development[11]. In our tissue-specific profiles, several Class Ia members (e.g., *EsLOB58*) showed decreasing root expression from tillering to maturity (from 4.29-fold to 2.06-fold), suggesting that developmental programmes fine-tune the spatiotemporal expression of these genes to optimise resource allocation – high expression during vigorous growth promotes root expansion, while down-regulation at maturity shifts resources to above-ground biomass accumulation. Future functional studies via gene editing of *EsLOB52* and other core candidates will be essential to clarify their molecular switch mechanisms in the growth-defence balance.

In summary, this study provides a comprehensive bioinformatic characterisation of 64 *AS2/LOB* family members in *E. sibiricus*, revealing their expansion through segmental duplication and functional divergence between Class I and Class II in organ development and hormone responses. Tissue-specific expression profiles, IAA/ABA/stress induction patterns and orthologue analysis collectively demonstrate

that this family deeply influences forage biomass by regulating root architecture, leaf morphogenesis and stress adaptability. In particular, the multi-stress inducibility of *EsLOB52* positions it as a promising candidate for integrating growth and stress adaptation. These findings provide valuable gene resources and a theoretical foundation for future functional validation and molecular breeding aimed at improving both biomass yield and environmental resilience in *E. sibiricus*. Subsequent work will employ gene knockout/overexpression and transcriptomic approaches to dissect the molecular pathways through which these genes modulate biomass, as well as their sub-genome expression biases in the allotetraploid context.

## 5. Conclusions

This study presents the first genome-wide analysis of the *As2/LOB* gene family in *E. sibiricus*, identifying 64 members classified into seven subfamilies. Segme ntal and tandem duplications were identified as major drivers of family expansi on. Tissue-specific expression profiling revealed pronounced spatial and tempora l patterns, with several members preferentially enriched in roots, leaves, and sp ikes—organs that directly determine plant architecture, nutrient uptake, photosyn thetic capacity, and reproductive output, all fundamental to forage biomass and yield. Promoter analysis supported these observations, identifying widespread de velopment-related cis-elements (CAT-box, GC-motif, MSA-like, RY-element) and auxin-responsive elements (AuxRR-core, AuxRE) in multiple *EsLOB* promoters, suggesting their potential involvement in auxin-mediated growth regulation and biomass accumulation. These findings establish a foundation for molecular bre

682 eding aimed at improving biomass productivity in *E. sibiricus*.

683 **Abbreviations：**

684 ZF ICG LZL MW ABA

685 ZF zinc finger

686 ICG internally conserved glycine-containing

687 LZL leucine zipper-like

688 MW molecular weights

689 ABA abscisic acid

690 **Supplementary Information：**

691 **Supplementary Material 1: Supplementary table 1.** Classification of *LOB* cis

692 -acting elements

693 **Supplementary Material 2: Supplementary table 2.** Cis-acting element motif

694 **Supplementary Material 3: Supplementary table 3.** Primer design for *LOB* g

695 ene family

696 **upplementary Material 4: Supplementary table 4.** Conserved domain of the

697 *LOB* gene family

698 **Supplementary Material 5: Supplementary table 5.** List and detailed informa

699 tion of identified *LOB* genes in *Elymus sibiricus*

700 **Supplementary Material 6: Supplementary table 6.** Gene sequences for the

701 *LOB* phylogenetic tree

702 **Ethics approval and consent to participate**

703 This article does not contain any studies involving human participants or anima

lsperformed by the authors. These methods were carried out by relevant guideli nes andregulations. *Elymus sibiricus* used in this study is a prevalent wild spec ies in the Qinghai area. Based on international standards, all the experimental r esearch and fieldstudies on plants, including the collection of plant material we re approved by Instituteof Animal Sciences. These materials are stored in Instit ute of Animal Sciences of Chinese Academy of Agricultural Sciences.

**Consent for publication**

Not applicable.

**Availability of data and materials：**

The genome sequences of *A. thaliana*, *B. distachyon*, *H. vulgare*, and *O. sativa* were downloaded from phytozome database (https://phytozome-next.jgi.doe.gov /). The datasets supporting the results of this article are included in the article and Additional files.

**Competing Interests**

The authors declare no competing interests.

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## Figure Legends:

Figure 1. Phylogenetic tree of *As2/LOB* proteins in *E. sibiricus*, *A. thaliana*, *B. distachyon*, *H. vulgare*, and *O. sativa*. Note: Colors denote species: green = *A. thaliana*; purple = *B. distachyon*; blue = *H. vulgare*; red = *O. sativa*; yello w = *E. sibiricus*.

Figure 2. (A) Conserved motifs analysis of the *As2/LOB* gene family; (B) *As2/ LOB* protein conserved structural domains; (C) Gene structure analysis of the *A*

884 *s2/LOB* gene family.

885 Figure 3. （A）Chromosomal locations of the *As2/LOB* gene family (B) Intra-s
886 pecies syntenic analysis of *E. sibiricus As2/LOB* family (C) Inter-species synten
887 ic analysis between *E. sibiricus* and four representative plants Note: Gene dens
888 ity is depicted in a color gradient, with blue indicating regions of lower gene
889 density and red indicating regions of higher gene density. Gray lines indicate c
890 ollinear relationships between *E. sibiricus* and other species; red lines denote h
891 omologous *As2/LOB* gene pairs.

892 Figure 4. Distribution of cis-acting elements in *As2/LOB* gene family promoter
893 s.

894 Figure 5. Tertiary structure of *As2/LOB* protein

895 Figure 6. Tissue-specific expression patterns of the *As2/LOB* gene family in *E.*
896 *sibiricus*

897 Figure 7. Expression dynamics of *As2/LOB* genes under ABA and IAA treatme
898 nts in *E. sibiricus*. Time-course analysis (0, 3, 6, 12, 24, 48, 72, 120, and 288
899 h post-treatment) showing relative expression levels. Values represent mean ±
900 SD of three biological replicates. Statistical significance determined by one-way
901 ANOVA: *P < 0.05; **P < 0.01.

902 Figure 8. Expression dynamics of *As2/LOB* genes under salt and drought stress
903 in *E. sibiricus*. Time-course analysis (0, 3, 6, 12, 24, 48, 72, 120, and 288 h
904 post-treatment) showing relative expression levels. Values represent mean ± SD
905 of three biological replicates. Statistical significance determined by one-way AN

OVA: *P < 0.05; **P < 0.01.

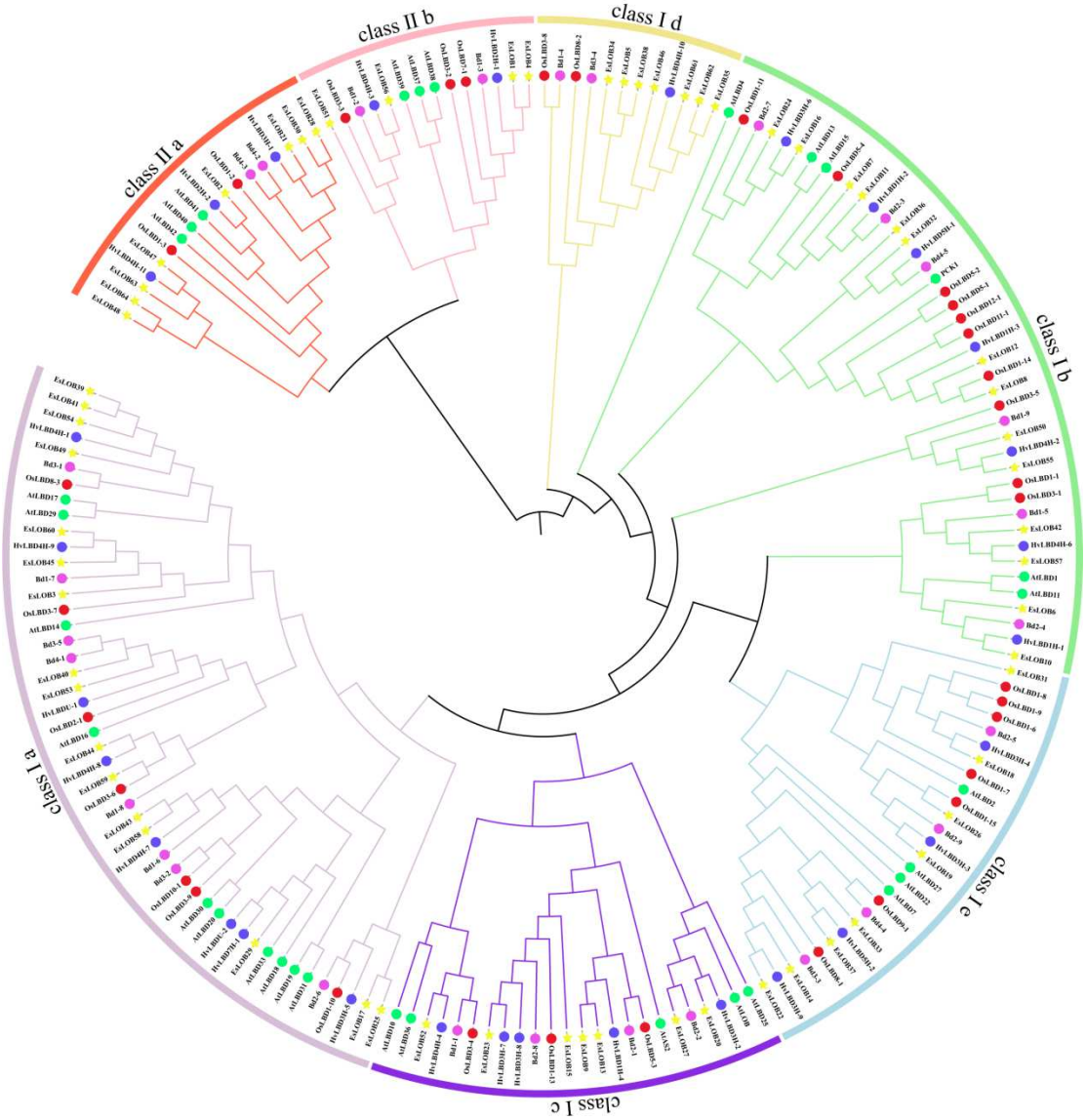



Figure 1. Phylogenetic tree of *As2/LOB* proteins in *E. sibiricus*, *A. thaliana*, *B. distachyon*, *H. vulgare*, and *O. sativa*. Note: Colors denote species: green = *A. thaliana*; purple = *B. distachyon*; blue = *H. vulgare*; red = *O. sativa*; yello w = *E. sibiricus*.

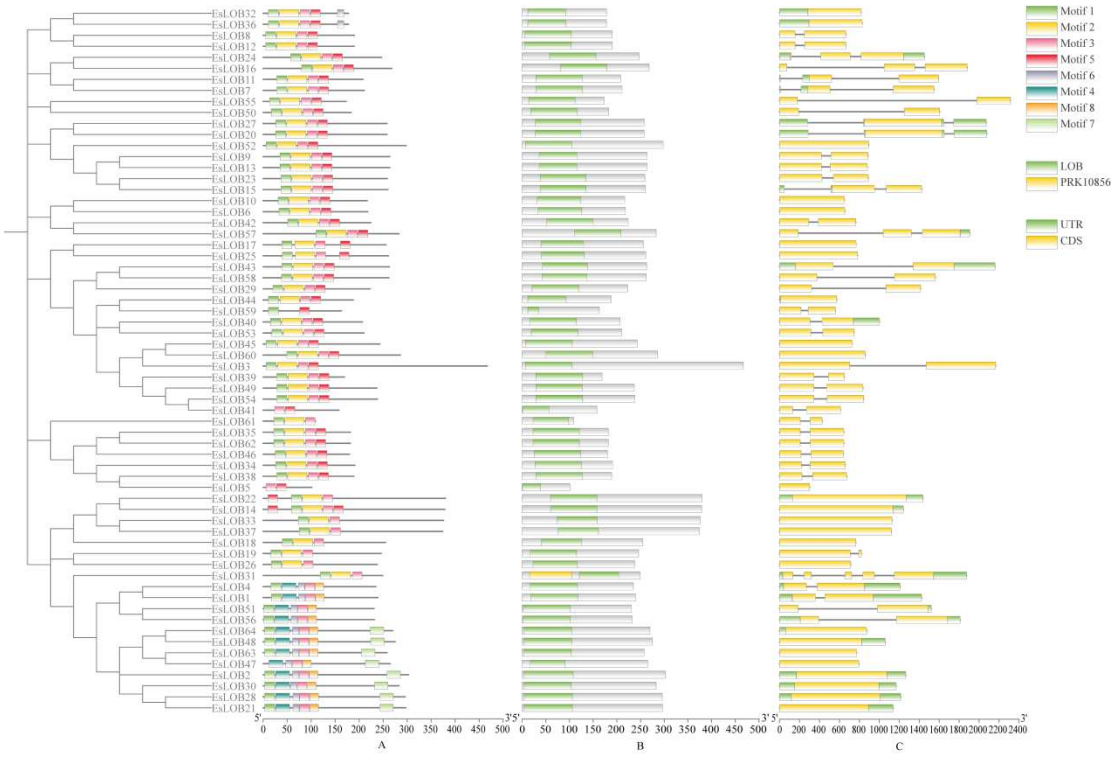



Figure 2. (A) Conserved motifs analysis of the *As2/LOB* gene family; (B) *As2/LOB* protein conserved structural domains; (C) Gene structure analysis of the *As2/LOB* gene family.

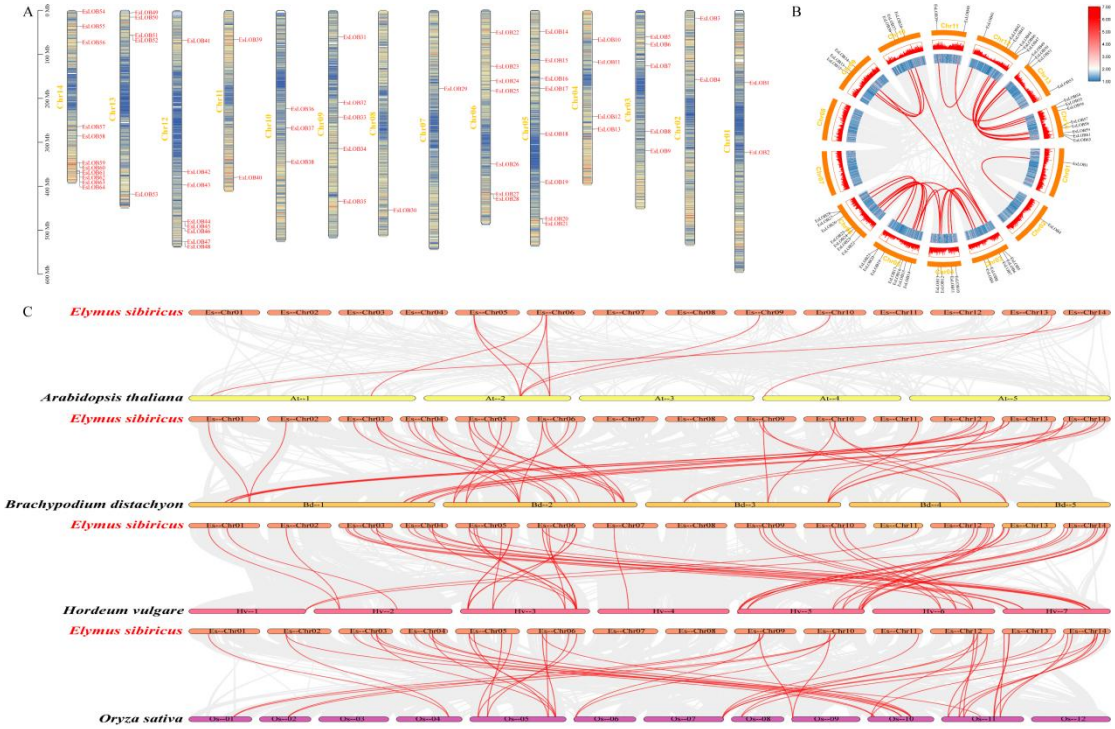



Figure 3. （A）Chromosomal locations of the *As2/LOB* gene family (B) Intra-s

pecies syntenic analysis of *E. sibiricus As2/LOB* family (C) Inter-species synten ic analysis between *E. sibiricus* and four representative plants. Note: Gene dens ity is depicted in a color gradient, with blue indicating regions of lower gene density and red indicating regions of higher gene density. Gray lines indicate c ollinear relationships between *E. sibiricus* and other species; red lines denote h omologous *As2/LOB* gene pairs.

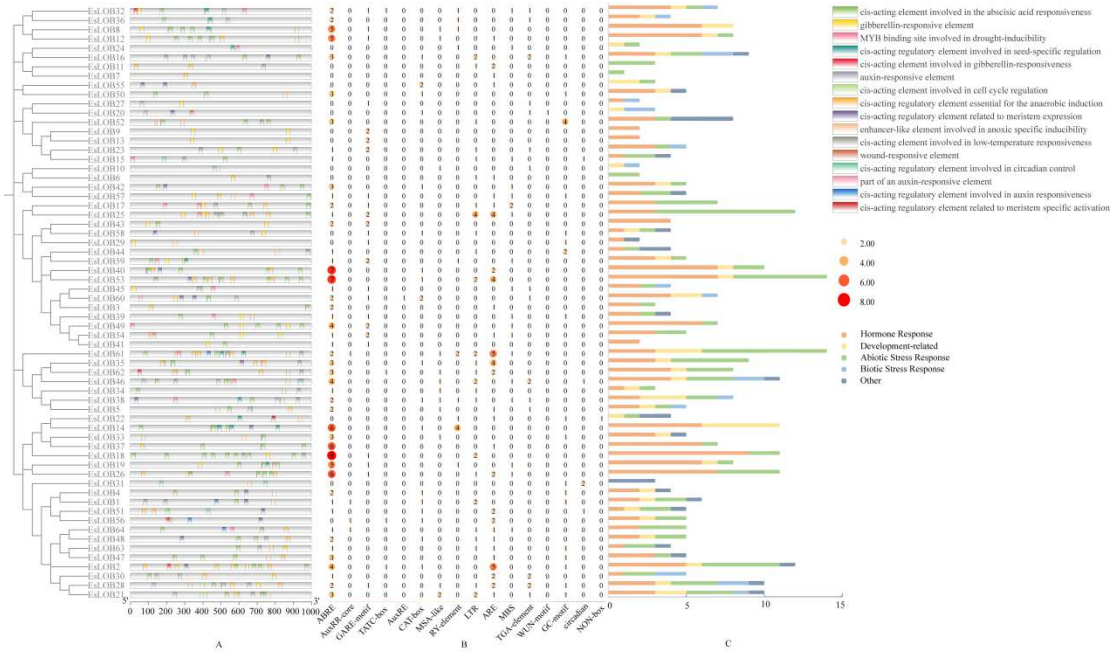
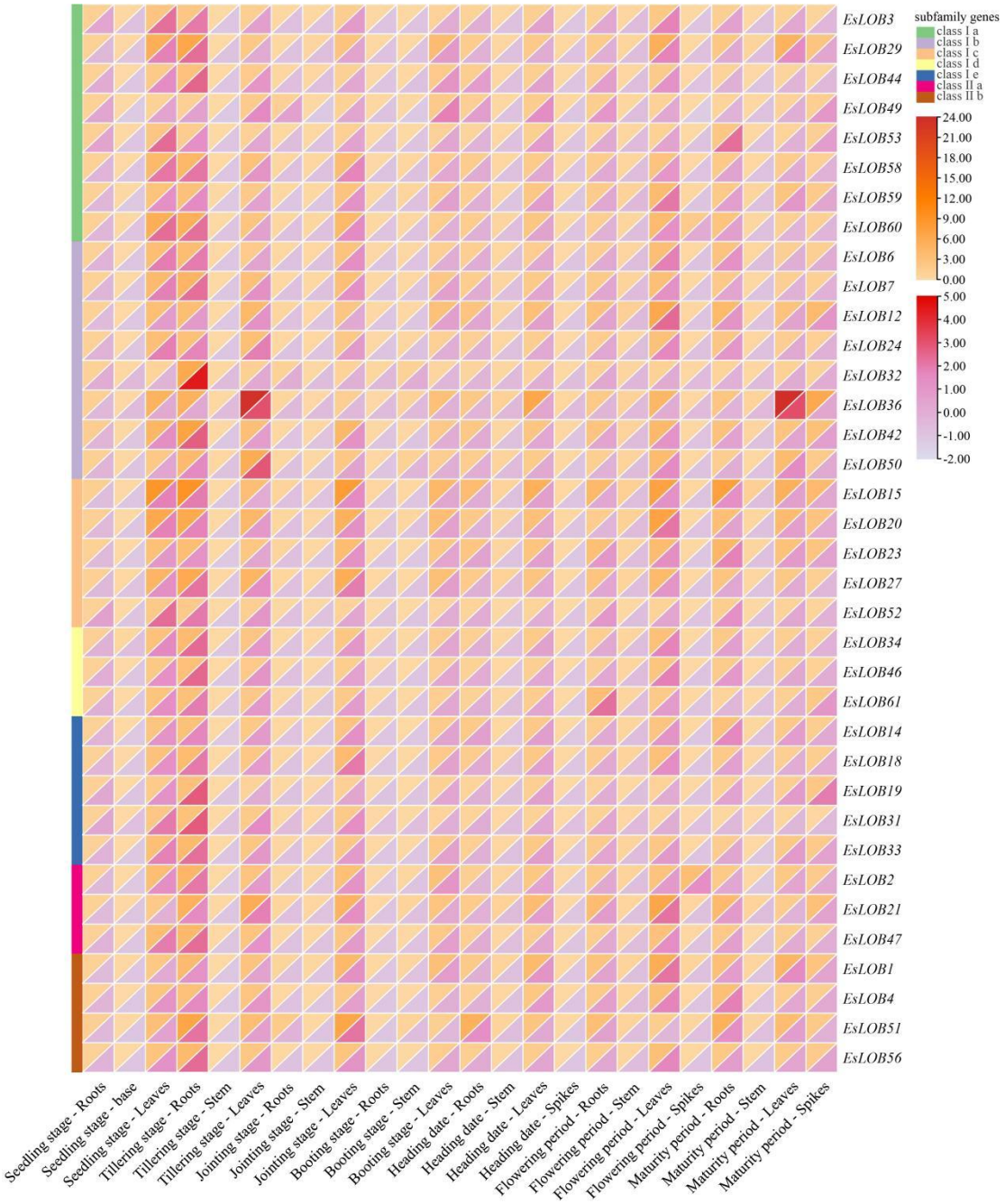



Figure 4. Distribution of cis-acting elements in *As2/LOB* gene family promoter s.




Figure 5. Tertiary structure of *As2/LOB* protein.




930

931 Figure 6. Tissue-specific expression patterns of the *As2/LOB* gene family in *E.*

932 *sibiricus.*

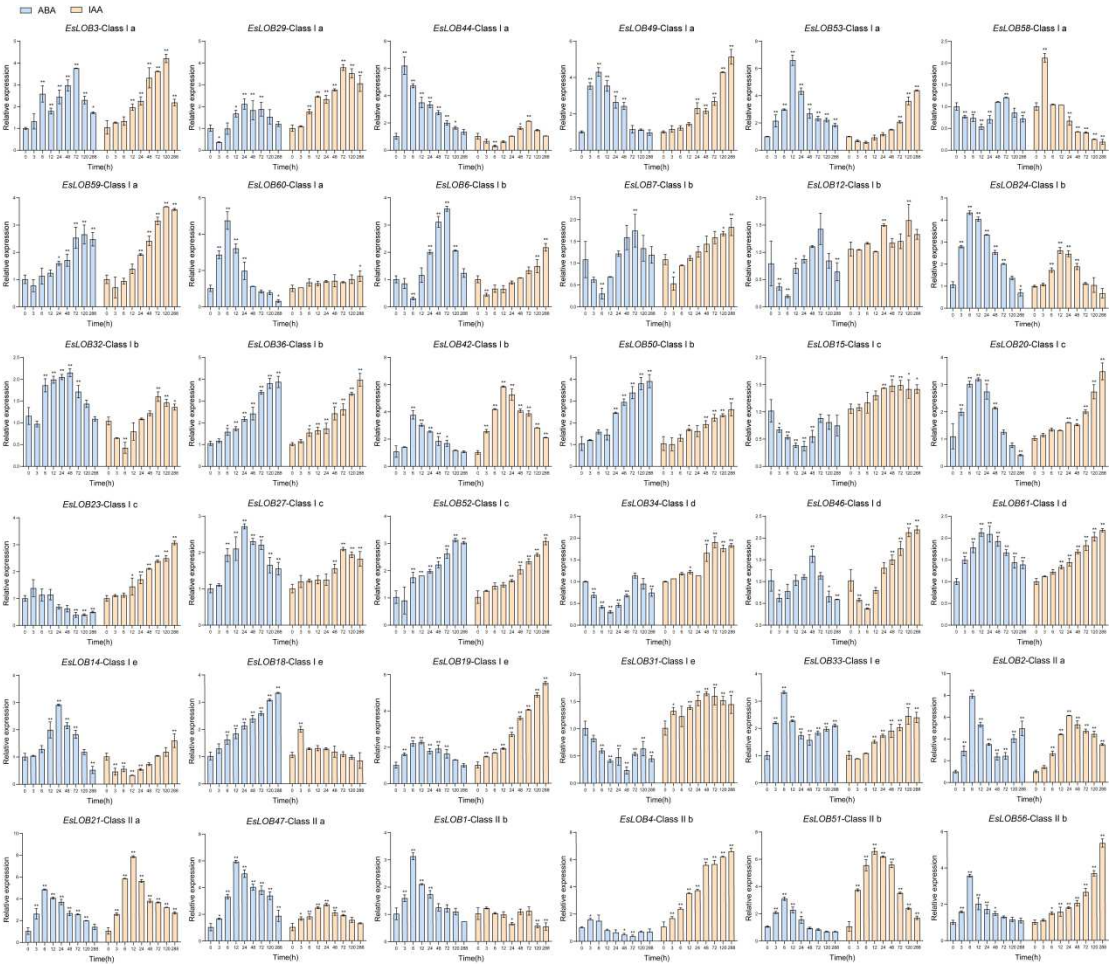



Figure 7. Expression dynamics of *As2/LOB* genes under ABA and IAA treatments in *E. sibiricus*. Time-course analysis (0, 3, 6, 12, 24, 48, 72, 120, and 288 h post-treatment) showing relative expression levels. Values represent mean ± SD of three biological replicates. Statistical significance determined by one-way ANOVA: *P < 0.05; **P < 0.01.

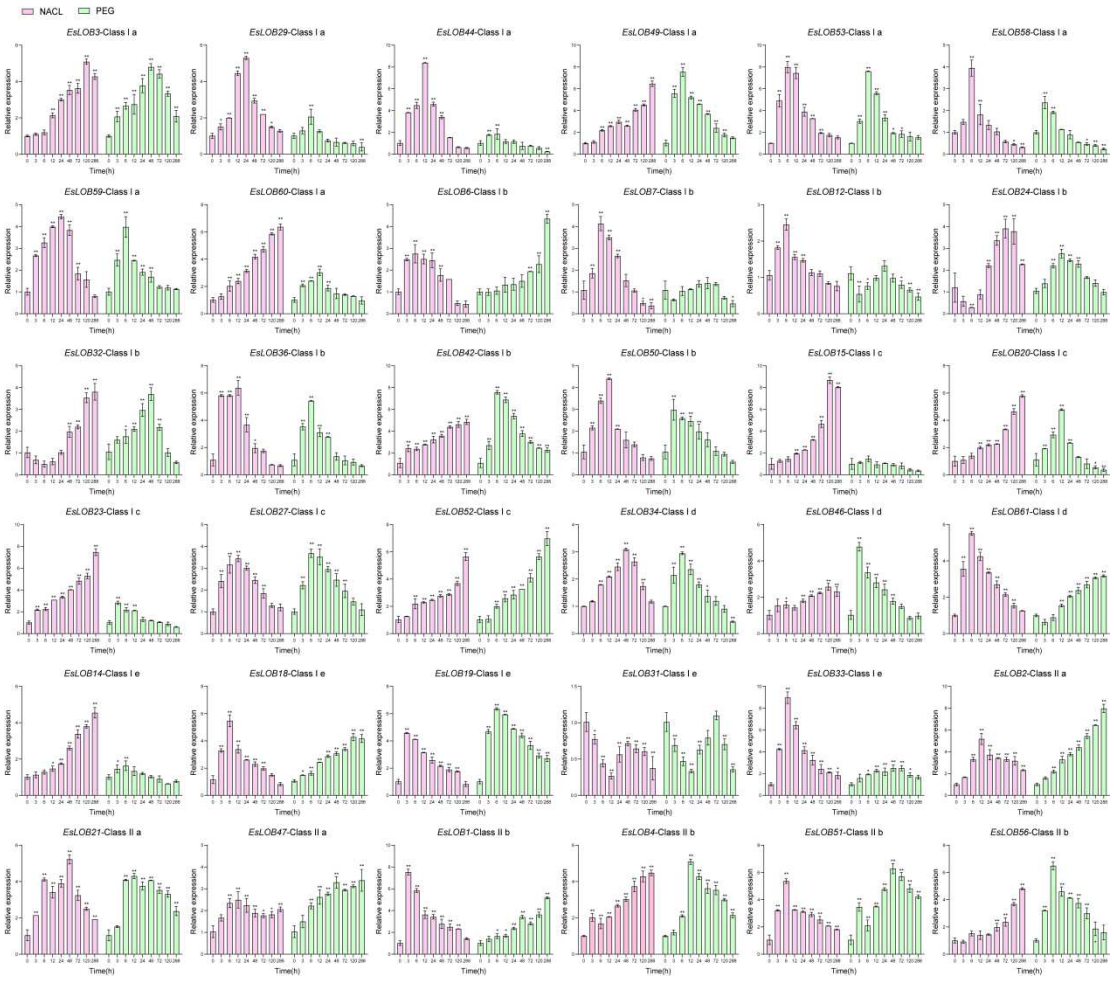



Figure 8. Expression dynamics of *As2/LOB* genes under salt and drought stress in *E. sibiricus*. Time-course analysis (0, 3, 6, 12, 24, 48, 72, 120, and 288 h post-treatment) showing relative expression levels. Values represent mean ± SD of three biological replicates. Statistical significance determined by one-way AN OVA: *P < 0.05; **P < 0.01.

Table 1 Physical and chemical properties of *As2/LOB* gene family.

| Gene Name | Gene ID | Number of Amino Acid | Molecular Weight | Theoretical pI | Instability Index | Aliphatic Index | Grand Average of Hydropathicity | Subcellular localization |
|---|---|---|---|---|---|---|---|---|
| *EsLOB1* | evm.model.Chr01.7111 | 238 | 24592.90 | 8.89 | 55.54 | 74.75 | -0.024 | Nucleus. |
| *EsLOB2* | evm.model.Chr01.13042 | 302 | 32876.50 | 5.61 | 56.53 | 67.02 | -0.618 | Nucleus. |
| *EsLOB3* | evm.model.Chr02.900 | 466 | 51491.35 | 6.01 | 46.18 | 84.25 | -0.190 | Nucleus. |
| *EsLOB4* | evm.model.Chr02.7911 | 234 | 24283.57 | 8.7 | 53.87 | 76.41 | -0.023 | Nucleus. |
| *EsLOB5* | evm.model.Chr03.2653 | 100 | 10699.16 | 4.73 | 54.62 | 91.80 | -0.184 | Nucleus. |
| *EsLOB6* | evm.model.Chr03.3566 | 217 | 22229.19 | 6.13 | 51.72 | 78.02 | 0.042 | Nucleus. |
| *EsLOB7* | evm.model.Chr03.5708 | 210 | 21829.80 | 9.10 | 68.34 | 75.95 | -0.015 | Nucleus. |
| *EsLOB8* | evm.model.Chr03.11309 | 189 | 20571.35 | 6.13 | 57.06 | 73.86 | -0.201 | Nucleus. |
| *EsLOB9* | evm.model.Chr03.13202 | 263 | 27045.36 | 7.61 | 29.72 | 70.57 | -0.043 | Nucleus. |
| *EsLOB10* | evm.model.Chr04.3814 | 216 | 22120.11 | 6.13 | 49.76 | 79.77 | 0.035 | Nucleus. |
| *EsLOB11* | evm.model.Chr04.6181 | 207 | 21546.47 | 9.10 | 67.69 | 75.65 | -0.024 | Nucleus. |
| *EsLOB12* | evm.model.Chr04.12142 | 189 | 20526.32 | 6.35 | 62.00 | 73.86 | -0.199 | Nucleus. |
| *EsLOB13* | evm.model.Chr04.13486 | 263 | 27047.33 | 7.61 | 32.26 | 70.23 | -0.046 | Nucleus. |
| *EsLOB14* | evm.model.Chr05.2395 | 378 | 41207.31 | 4.95 | 53.06 | 69.21 | -0.603 | Nucleus. |
| *EsLOB15* | evm.model.Chr05.5684 | 259 | 26583.95 | 8.21 | 36.48 | 74.32 | -0.041 | Nucleus. |
| *EsLOB16* | evm.model.Chr05.7656 | 267 | 28126.02 | 7.15 | 51.43 | 82.70 | -0.060 | Nucleus. |
| *EsLOB17* | evm.model.Chr05.8797 | 255 | 27392.84 | 8.11 | 58.30 | 73.14 | -0.348 | Nucleus. |
| *EsLOB18* | evm.model.Chr05.13327 | 254 | 27322.88 | 7.13 | 67.50 | 68.54 | -0.426 | Nucleus. |
| *EsLOB19* | evm.model.Chr05.17044 | 245 | 25681.69 | 9.72 | 50.08 | 59.22 | -0.532 | Nucleus. |
| *EsLOB20* | evm.model.Chr05.20489 | 257 | 26499.16 | 7.57 | 52.63 | 76.34 | 0.003 | Nucleus. |
| *EsLOB21* | evm.model.Chr05.20551 | 296 | 31119.89 | 6.18 | 51.19 | 75.27 | -0.258 | Nucleus. |
| *EsLOB22* | evm.model.Chr06.2766 | 379 | 41397.52 | 4.87 | 48.57 | 68.02 | -0.628 | Nucleus. |
| *EsLOB23* | evm.model.Chr06.6817 | 258 | 26450.84 | 8.21 | 33.17 | 74.61 | -0.035 | Nucleus. |
| *EsLOB24* | evm.model.Chr06.8650 | 246 | 25998.40 | 5.71 | 46.59 | 80.20 | -0.090 | Nucleus. |
| *EsLOB25* | evm.model.Chr06.9712 | 260 | 27922.39 | 8.40 | 53.90 | 72.15 | -0.388 | Nucleus. |
| *EsLOB26* | evm.model.Chr06.17228 | 237 | 24628.57 | 9.83 | 51.64 | 59.96 | -0.492 | Nucleus. |
| *EsLOB27* | evm.model.Chr06.20257 | 257 | 26459.12 | 7.55 | 52.06 | 77.86 | 0.030 | Nucleus. |
| *EsLOB28* | evm.model.Chr06.20283 | 295 | 30858.54 | 5.69 | 57.36 | 77.80 | -0.233 | Nucleus. |
| *EsLOB29* | evm.model.Chr07.8841 | 222 | 24094.15 | 5.58 | 52.59 | 78.29 | -0.249 | Nucleus. |
| *EsLOB30* | evm.model.Chr08.21427 | 282 | 30163.73 | 7.11 | 63.05 | 69.33 | -0.488 | Nucleus. |
| *EsLOB31* | evm.model.Chr09.2849 | 248 | 26642.25 | 7.03 | 58.73 | 77.98 | -0.201 | Nucleus. |
| *EsLOB32* | evm.model.Chr09.8915 | 177 | 19686.46 | 6.88 | 65.00 | 79.49 | -0.284 | Nucleus. |
| *EsLOB33* | evm.model.Chr09.10514 | 375 | 39511.81 | 5.16 | 50.78 | 74.96 | -0.288 | Nucleus. |
| *EsLOB34* | evm.model.Chr09.14277 | 190 | 20646.30 | 6.43 | 73.97 | 73.53 | -0.471 | Nucleus. |
| *EsLOB35* | evm.model.Chr09.20390 | 181 | 19981.42 | 5.40 | 66.33 | 76.08 | -0.455 | Nucleus. |
| *EsLOB36* | evm.model.Chr10.8719 | 177 | 19658.40 | 6.88 | 62.49 | 78.42 | -0.293 | Nucleus. |
| *EsLOB37* | evm.model.Chr10.10516 | 373 | 39324.31 | 5.09 | 54.05 | 68.79 | -0.345 | Nucleus. |
| *EsLOB38* | evm.model.Chr10.14210 | 188 | 20424.94 | 5.26 | 78.26 | 74.79 | -0.515 | Nucleus. |
| *EsLOB39* | evm.model.Chr11.3598 | 168 | 18224.75 | 7.02 | 44.56 | 71.01 | -0.167 | Nucleus. |
| *EsLOB40* | evm.model.Chr11.20134 | 206 | 21381.37 | 8.14 | 62.91 | 83.59 | 0.108 | Nucleus. |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *EsLOB41* | evm.model.Chr12.2783 | 157 | 17513.36 | 4.58 | 58.28 | 69.68 | -0.448 | Nucleus. |
| *EsLOB42* | evm.model.Chr12.14148 | 223 | 23849.98 | 5.54 | 64.99 | 75.83 | -0.204 | Nucleus. |
| *EsLOB43* | evm.model.Chr12.15339 | 262 | 27001.53 | 7.69 | 71.56 | 71.60 | -0.218 | Nucleus. |
| *EsLOB44* | evm.model.Chr12.18992 | 187 | 19738.95 | 6.63 | 59.88 | 61.39 | -0.257 | Nucleus. |
| *EsLOB45* | evm.model.Chr12.18993 | 242 | 25263.89 | 5.82 | 44.97 | 63.64 | -0.215 | Nucleus. |
| *EsLOB46* | evm.model.Chr12.19751 | 179 | 19530.85 | 5.04 | 55.07 | 74.80 | -0.407 | Nucleus. |
| *EsLOB47* | evm.model.Chr12.20969 | 264 | 27723.26 | 6.36 | 57.51 | 80.64 | -0.239 | Nucleus. |
| *EsLOB48* | evm.model.Chr12.20970 | 274 | 28778.11 | 6.66 | 54.78 | 73.61 | -0.339 | Nucleus. |
| *EsLOB49* | evm.model.Chr13.71 | 236 | 26146.43 | 5.68 | 44.46 | 67.84 | -0.289 | Nucleus. |
| *EsLOB50* | evm.model.Chr13.590 | 182 | 19361.94 | 8.84 | 52.31 | 70.38 | -0.215 | Nucleus. |
| *EsLOB51* | evm.model.Chr13.2529 | 230 | 23680.74 | 7.50 | 54.10 | 78.65 | -0.030 | Nucleus. |
| *EsLOB52* | evm.model.Chr13.2564 | 297 | 32439.20 | 6.91 | 60.76 | 59.26 | -0.690 | Nucleus. |
| *EsLOB53* | evm.model.Chr13.17259 | 209 | 21503.46 | 8.14 | 63.52 | 81.87 | 0.143 | Nucleus. |
| *EsLOB54* | evm.model.Chr14.108 | 237 | 26267.30 | 5.51 | 56.00 | 57.30 | -0.441 | Nucleus. |
| *EsLOB55* | evm.model.Chr14.1907 | 172 | 18364.90 | 8.59 | 59.15 | 73.26 | -0.103 | Nucleus. |
| *EsLOB56* | evm.model.Chr14.3843 | 231 | 23829.93 | 8.01 | 54.64 | 77.88 | -0.060 | Nucleus. |
| *EsLOB57* | evm.model.Chr14.12497 | 282 | 30621.73 | 7.13 | 61.02 | 73.44 | -0.399 | Nucleus. |
| *EsLOB58* | evm.model.Chr14.13560 | 261 | 26820.38 | 8.20 | 74.46 | 72.26 | -0.186 | Nucleus. |
| *EsLOB59* | evm.model.Chr14.16879 | 162 | 16780.56 | 8.49 | 67.61 | 50.31 | -0.435 | Nucleus. |
| *EsLOB60* | evm.model.Chr14.16880 | 285 | 29805.99 | 6.06 | 52.02 | 64.32 | -0.259 | Nucleus. |
| *EsLOB61* | evm.model.Chr14.18058 | 108 | 11993.45 | 6.64 | 76.59 | 53.43 | -0.611 | Nucleus. |
| *EsLOB62* | evm.model.Chr14.18176 | 181 | 19958.39 | 5.27 | 69.02 | 75.52 | -0.430 | Nucleus. |
| *EsLOB63* | evm.model.Chr14.18455 | 257 | 26824.55 | 8.78 | 68.08 | 79.46 | -0.088 | Nucleus. |
| *EsLOB64* | evm.model.Chr14.18463 | 269 | 28296.63 | 6.71 | 61.23 | 76.02 | -0.339 | Nucleus. |

951

952 Table 2 Secondary structure analysis of *As2/LOB* proteins

| Gene Name | Alpla helix （%） | Beta turn （%） | Random coil （%） | Extended strand （%） |
|---|---|---|---|---|
| *EsLOB1* | 31.09 | 0.84 | 64.71 | 3.36 |
| *EsLOB2* | 24.83 | 1.99 | 67.22 | 5.96 |
| *EsLOB3* | 43.56 | 3.43 | 45.28 | 7.73 |
| *EsLOB4* | 25.21 | 1.28 | 69.23 | 4.27 |
| *EsLOB5* | 42.00 | 0.00 | 57.00 | 1.00 |
| *EsLOB6* | 36.41 | 0.92 | 61.29 | 1.38 |
| *EsLOB7* | 36.19 | 0.48 | 62.38 | 0.95 |
| *EsLOB8* | 46.03 | 0.00 | 52.91 | 1.06 |
| *EsLOB9* | 28.52 | 0.00 | 70.72 | 0.76 |
| *EsLOB10* | 38.89 | 0.00 | 56.02 | 5.09 |
| *EsLOB11* | 35.75 | 0.00 | 61.35 | 2.90 |
| *EsLOB12* | 41.27 | 0.00 | 57.67 | 1.06 |
| *EsLOB13* | 35.36 | 0.00 | 63.88 | 0.76 |
| *EsLOB14* | 30.95 | 0.26 | 67.72 | 1.06 |

| | | | | |
|---|---|---|---|---|
| *EsLOB15* | 35.52 | 0.39 | 60.23 | 3.86 |
| *EsLOB16* | 40.07 | 0.00 | 59.18 | 0.75 |
| *EsLOB17* | 32.16 | 0.00 | 67.06 | 0.78 |
| *EsLOB18* | 28.74 | 0.00 | 70.47 | 0.79 |
| *EsLOB19* | 39.18 | 2.04 | 55.92 | 2.86 |
| *EsLOB20* | 32.68 | 0.78 | 63.04 | 3.50 |
| *EsLOB21* | 22.97 | 3.38 | 66.89 | 6.76 |
| *EsLOB22* | 32.45 | 0.00 | 65.96 | 1.58 |
| *EsLOB23* | 35.27 | 0.00 | 61.63 | 3.10 |
| *EsLOB24* | 34.96 | 0.00 | 63.41 | 1.63 |
| *EsLOB25* | 35.38 | 0.00 | 63.46 | 1.15 |
| *EsLOB26* | 35.86 | 1.27 | 59.92 | 2.95 |
| *EsLOB27* | 35.80 | 1.95 | 58.37 | 3.89 |
| *EsLOB28* | 21.36 | 3.73 | 68.14 | 6.78 |
| *EsLOB29* | 40.09 | 0.45 | 58.56 | 0.90 |
| *EsLOB30* | 26.95 | 4.26 | 62.06 | 6.74 |
| *EsLOB31* | 33.87 | 0.81 | 60.48 | 4.84 |
| *EsLOB32* | 50.28 | 0.00 | 48.59 | 1.13 |
| *EsLOB33* | 27.73 | 0.00 | 71.20 | 1.07 |
| *EsLOB34* | 42.11 | 0.00 | 56.84 | 1.05 |
| *EsLOB35* | 39.23 | 0.00 | 59.67 | 1.10 |
| *EsLOB36* | 45.20 | 0.00 | 53.67 | 1.13 |
| *EsLOB37* | 31.64 | 0.27 | 65.95 | 2.14 |
| *EsLOB38* | 43.09 | 0.00 | 55.85 | 1.06 |
| *EsLOB39* | 52.98 | 1.19 | 44.64 | 1.19 |
| *EsLOB40* | 45.63 | 0.49 | 52.91 | 0.97 |
| *EsLOB41* | 36.94 | 0.00 | 63.06 | 0.00 |
| *EsLOB42* | 36.32 | 0.00 | 62.78 | 0.90 |
| *EsLOB43* | 31.68 | 0.00 | 66.79 | 1.53 |
| *EsLOB44* | 43.32 | 1.07 | 54.01 | 1.60 |
| *EsLOB45* | 44.21 | 0.00 | 54.96 | 0.83 |
| *EsLOB46* | 41.90 | 1.12 | 55.87 | 1.12 |
| *EsLOB47* | 20.45 | 3.79 | 70.08 | 5.68 |
| *EsLOB48* | 21.53 | 0.00 | 70.44 | 8.03 |
| *EsLOB49* | 36.44 | 0.00 | 62.71 | 0.85 |
| *EsLOB50* | 48.90 | 0.00 | 48.35 | 2.75 |
| *EsLOB51* | 29.13 | 0.00 | 66.52 | 4.35 |
| *EsLOB52* | 35.69 | 0.34 | 62.63 | 1.35 |
| *EsLOB53* | 44.02 | 1.44 | 52.63 | 1.91 |
| *EsLOB54* | 31.22 | 0.00 | 67.51 | 1.27 |
| *EsLOB55* | 47.09 | 0.00 | 51.16 | 1.74 |
| *EsLOB56* | 36.80 | 0.87 | 58.87 | 3.46 |
| *EsLOB57* | 30.14 | 0.00 | 65.25 | 4.61 |
| *EsLOB58* | 31.03 | 0.00 | 68.20 | 0.77 |

| | | | | |
|---|---|---|---|---|
| *EsLOB59* | 52.47 | 0.00 | 45.68 | 1.85 |
| *EsLOB60* | 36.49 | 0.00 | 62.81 | 0.70 |
| *EsLOB61* | 46.30 | 0.00 | 49.07 | 4.63 |
| *EsLOB62* | 39.23 | 0.55 | 55.25 | 4.97 |
| *EsLOB63* | 22.57 | 0.00 | 70.43 | 7.00 |
| *EsLOB64* | 25.65 | 1.12 | 65.80 | 7.43 |

953

## Supplementary Files

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

- Supplementarytable5.ListanddetailedinformationofidentifiedLOBgenesinElymussibiricus.xlsx
- Supplementarytable3.PrimerdesignforLOBgenefamily.xlsx
- Supplementarytable4.ConserveddomainoftheLOBgenefamily.xlsx
- Supplementarytable2.Cisactingelementmotif.xlsx
- Supplementarytable5.ListanddetailedinformationofidentifiedLOBgenesinElymussibiricus.xlsx
- Supplementarytable1.ClassificationofLOBcisactingelements.xlsx
- Supplementarytable6.GenesequencesfortheLOBphylogenetictree.xlsx