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Genome-wide identification and characterization of lipoxygenases gene family in *Luffa aegyptiaca* revealed downregulation of LOX genes under heat stress

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Lipoxygenases (LOXs) are key enzymes in plant lipid metabolism and stress responses, yet their genomic organization and functional dynamics in *Luffa aegyptiaca*—a species of culinary, medicinal, and ornamental importance—remain unexplored. Here, we present the first genome-wide identification and characterization of the LOX gene family in *L. aegyptiaca*, revealing 29 LOX genes, including 14 members of 13S-lipoxygenases (13-LOX) and 15 members of 9S-lipoxygenases (9-LOX), respectively. Notably, tandem duplication events shaped the expansion of LOX genes, with 24 genes clustered in two loci, suggesting functional diversification to enhance environmental adaptability. Phylogenetic analysis demonstrated evolutionary conservation of LOX genes across *Cucurbitaceae* species, while collinearity analysis highlighted conserved genomic organization. Promoter cis-element profiling identified stress- and hormone-responsive motifs, implicating LOX genes in developmental and stress regulatory networks. Tissue-specific expression patterns revealed 18 LOX genes predominantly expressed in tendril, fruit, root, and male flower, linking them to organ-specific physiological roles. Crucially, under heat stress, 9 out of 11 expressed LOX genes were significantly downregulated, indicating their potential role in thermal stress adaptation through metabolic reconfiguration. This study provides foundational insights into the LOX family's contribution to *L. aegyptiaca*'s resilience and offers genetic targets for breeding strategies to improve stress tolerance in cucurbit crops.

Keywords Lipoxygenases, *Luffa aegyptiaca*, Tandem duplication, RNA-seq, Heat stress

Lipoxygenases (LOXs) are a family of non-heme iron-containing enzymes that play a crucial role in the metabolism of polyunsaturated fatty acids (PUFAs) in plants. These enzymes catalyze the oxidation of PUFAs, leading to the formation of hydroperoxides, which are precursors for a variety of bioactive molecules with diverse functions in plant physiology and defense mechanisms[1,2]. The LOX gene family is evolutionarily conserved across plants and is known for its involvement in the production of signaling molecules such as jasmonic acid (JA) and other oxylipins. These molecules regulate various aspects of plant growth, development, and responses to biotic and abiotic stresses. The LOX-mediated oxygenation of PUFAs is a critical step in the biosynthesis of these signaling molecules, highlighting the significance of LOX enzymes in plant biology[3,4].

The lipoxygenase (LOX) gene family has been a focal point of research across various plant species due to its significant role in the biosynthesis of oxylipins, which are involved in plant growth, development, and stress responses. Studies have identified and characterized numerous LOX genes in different crops, such as radish, passion fruit, sweet cherry, chickpea, and turnip, among others[5–10]. Research on the LOX gene family in plants has expanded rapidly in recent years, with a focus on understanding their molecular mechanisms, gene regulation, and functional diversity[11]. Previous studies on LOX gene family has advanced our understanding

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of their diverse roles in plant physiology and their potential applications in improving crop resistance to stresses and enhancing the quality of agricultural products. Studies have identified different LOX isoforms in various plant species, each with distinct substrate specificities and expression patterns[5,8–10,12]. The functional characterization of these LOX genes has provided insights into their roles in plant defense against pathogens, herbivores, and environmental stresses, as well as their involvement in developmental processes[5–10,12–16]. For example, functional characterization of CsLOX13 in *Cannabis sativa* has demonstrated their involvement in the biosynthesis of jasmonates and other oxylipins, which are essential for plant defense mechanisms and secondary metabolism[15].

Luffa aegyptiaca, commonly known as Egyptian loofah or simply luffa, is a versatile plant species belonging to the gourd family, Cucurbitaceae. Indigenous to tropical and subtropical regions, it is cultivated for its fruit which serves both culinary and medicinal purposes. The luffa fruit is edible when young and tender, and its fibrous mature fruit is used as a sponge for bathing and cleaning. Additionally, *L. aegyptiaca* possesses ornamental value and plays a role in trellising, providing shade and support for other climbing plants. However, the growth and survival of *L. aegyptiaca* can be challenged by various environmental stresses, such as fluctuations in water quality, temperature extremes, and pathogen attacks. Therefore, studying the LOX gene family in *L. aegyptiaca* is of particular interest due to the potential involvement of these genes in the plant's stress response and adaptation mechanisms. By understanding the structure, expression patterns, and functional roles of the LOX genes in *L. aegyptiaca*, researchers can gain insights into the molecular basis of the plant's resilience and ability to thrive in specific environments. Moreover, the knowledge of LOX genes and their regulatory mechanisms can inform strategies for the conservation and management of *L. aegyptiaca* populations in the face of environmental changes. It may also contribute to the development of breeding programs aimed at enhancing the stress tolerance of this species, ensuring its continued availability for ecological and decorative purposes. In aggregate, the study of the LOX gene family in *L. aegyptiaca* is crucial for unraveling the complex interplay between genetic factors and environmental conditions that shape the adaptability and survival of this plant.

Materials and methods

Identification of LOX genes in *L. aegyptiaca* genome

The protein sequences of LOX genes of *Arabidopsis thaliana* were downloaded from NCBI database, which was used for BLASTP (v. 2.7.1) to identify homologous LOX genes in *L. aegyptiaca* genome. Then, the seed file of lipoxygenase was obtained from Pfam database with accession number of Pfam00305, which was exported to HMM (v3.3.2) searches with with E-value = 10^{-5}[17,18]. Finally, the prediction results based on BLASTP and HMM searches were merged, and totally 29 LOX genes (Laeg_LOXs) were obtained. These LOX genes were named through comparative analysis with *Arabidopsis thaliana* LOX genes. All the putative LOX genes were further validated to possessed lipoxygenase domains using Pfam database, InterPro4 and NCBI Conserved Domain Database (CDD)[18–20]. This procedure was also used for prediction of LOX genes in the genomes of *Luffa acutangula*, *Cucurbita pepo*, *Cucurbita argyrosperma*, *Cucumis sativus*, *Cucumis melo* and *Benincasa hispida*. The molecular weight and theoretical isoelectric point (pI) of the Laeg_LOXs, which are key physical and chemical properties, were calculated using the ExPASy6 (v6) tool[21]. All associated bioinformatics tools used in this study were shown in the Supplementary Table 4.

Phylogenetic and colinear analysis

Mafft (v7.505) software was used for multiple sequences alignment with default parameter, and then Gblocks (v0.91b) was exploited to obtain the conserved regions[22,23]. The multiple sequences alignment was visualized using Jalview (v2.11.4.0) software[24]. The phylogenetic tree was built using RAxML (v8.2.12) software, which leveraged both Maximum Likelihood and Neighbor-Joining methods for its construction. The synteny of LOX genes among *L. aegyptiaca, L. acutangula, C. pepo,* and *B. hispida* genomes were analyzed and visualized using JCVI (v1.4.23) software[25].

Gene structures, conserved motifs and promoter analysis

The lipoxygenase conserved domain was obtained using Pfam and CDD. The conserved motifs were analyzed using MEME (v5.5.7) software[26]. The results of exon–intron structures, LOX domains and conserved motifs were visualized using TBtools (v1.6)[27]. The upstream 2 kb sequences of Laeg_LOXs were extracted using in-house Perl scripts, which were considered as putative promoters of Laeg_LOXs and used for prediction of cis-elements by using PlantCARE[28].

Gene expression analysis of Laeg_LOXs in various tissues and heat stress

Raw RNA-seq reads of eight *L. aegyptiaca* tissues were downloaded from NCBI with accession number of 'PRJNA732226'. The raw reads were processed with the fastp (v0.23.4) quality control tool[29]. Subsequently, cleaned reads were aligned to the *L. aegyptiaca* genome using Hisat2 (v2.1.0) and Samtools (v1.17)[30,31]. Gene expression levels were quantified with Stringtie (v2.2.1) and Featurecount (v2.0.1)[32,33]. The expression patterns of Laeg_LOXs were visualized using Pheatmap (v1.0.12) R package[34]. The analysis procedure was applied to the RNA-seq of *L. aegyptiaca* under heat stress. DESeq2 (v1.44.0) was then applied for the analysis of differential gene expression[35]. We defined differentially expressed genes (DEGs) based on a false discovery rate (FDR) below 0.05 and an absolute log2 fold change (log2FC) exceeding 1.

Seed material, germination and cultivation

The experimental sponge gourd seeds were sourced from the Fujian Provincial Key Laboratory of Vegetable Genetics and Breeding. The seeds intended for the experiment were germinated at 26 °C. Once germinated, the seeds were sown in 20-cell plug trays with one seed per cell, and two trays were used per variety, totaling 40

seedlings per variety. The seedlings were then placed in a controlled environment chamber with a photoperiod of 14 h (day) / 10 h (night), temperatures set at 25 °C (day) / 15 °C (night), a light intensity of 400 μmol/m^2·s, and a relative humidity of 75%.

The seedlings were grown in a specialized vegetable substrate, and the substrate moisture was regularly replenished. When the sponge gourd seedlings reached the two-leaf and one-heart stage, uniform seedlings were selected. Half of the seedlings were then transferred to another artificial climate chamber for high-temperature treatment, with conditions set at 42 °C (day) / 32 °C (night), while the other half remained in the control chamber with temperatures of 25 °C (day) / 15 °C (night). Both chambers maintained a light intensity of 400 μmol/m^2·s and a relative humidity of 75%.

For the preservation of samples, the leaf samples were immediately placed in liquid nitrogen upon collection to prevent degradation. They were then transferred to an ultra-low temperature freezer for long-term storage, ensuring the integrity of the samples for future DNA and RNA extraction and analysis.

High-temperature treatment

After four days of high-temperature treatment, leaf samples were collected from the seedlings and quickly preserved in liquid nitrogen. The samples were then stored in an ultra-low temperature freezer for subsequent DNA/RNA extraction.

RNA extraction, library preparation and qRT-PCR verification

Total RNA was extracted from selected tissues using a plant-specific RNA extraction kit, ensuring minimal degradation and contamination. We then performed quality assessment of the isolated RNA with a bioanalyzer to confirm integrity and purity. Following DNase treatment to eliminate any genomic DNA contamination, we synthesized cDNA using a high-fidelity reverse transcriptase and carried out second-strand synthesis to obtain double-stranded cDNA. The cDNA was processed through end repair, A-tailing, and adapter ligation to prepare it for size selection. We selected cDNA fragments of an optimal size range and amplified them using PCR to create a library suitable for next-generation sequencing. The library was quantified and validated for quality and size distribution before being sequenced on an Illumina platform.

Ten differentially expressed Laeg_LOXs were selected to validate expression patterns under heat stress. PCR primers were designed using the Primer3plus (Supplementary Table 3). We employed the SYBR Green dye method for quantitative real-time PCR (RT-qPCR), which was conducted following the manufacturer's instructions for the SYBR Premix Ex Taq II kit from TaKaRa. The RT-qPCR was performed on a 7500 Real-Time System instrument [ABI (USA) Company], with each sample run in triplicate. The reaction system of 10 μL consisted of 5 μL of 2×SYBR Premix Ex Taq II, 0.4 μL of each Primer F (10 μmol/L), 0.4 μL of each Primer R (10 μmol/L), 0.2 μL of ROX Reference Dye (10 μmol/L), 2 μL of cDNA, and 2 μL of ddH2O. The PCR amplification program was as follows: initial denaturation at 95 °C for 30 s; followed by 40 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 34 s. After amplification, a melting curve analysis was conducted using the following program: 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. The relative gene expression levels were calculated using $2^{-\Delta\Delta CT}$ method.

Result

Genome-wide identification of lipoxygenase gene family in *L. aegyptiaca* genome

In total, 29 LOX genes (Laeg_LOXs) were identified using BlastP and HMM Search methods, including 14 13S-lipoxygenases (13-LOX) and 15 9S-lipoxygenases (9-LOX) (Table 1). We further performed comparative analysis with the LOX genes in *Arabidopsis thaliana* to determine the gene name of Laeg_LOX genes (Supplementary Table 1). The protein length of Laeg_LOXs ranged from 718 aa (*Laeg_LOX4*) to 926 aa (*Laeg_LOX6*), with an average length of 858 aa. Furthermore, physiochemical properties showed the molecular weight ranged from 82 kDa (*Laeg_LOX4*) to 104 kDa (*Laeg_LOX6*), and theoretical pI values ranged from 5.25 (*Laeg_LOX1-14*) to 8.47 (*Laeg_LOX6*).

In our study, we conducted a comparative analysis of LOX genes between *L. aegyptiaca* and *A. thaliana*, with a particular focus on the Ka/Ks ratio to gain insights into the selective pressures acting on these genes (Supplementary Table 1). For the majority of the LOX gene pairs, the Ka/Ks values were found to be less than 1. This suggested that these genes have predominantly experienced purifying selection throughout their evolutionary history. Purifying selection typically acts to maintain the functional integrity of genes by removing deleterious mutations. In the context of LOX genes, which are often involved in key biological processes such as lipid metabolism and signaling pathways, this finding is consistent with the expectation that their core functions are highly conserved. However, it is worth noting that several LOX gene pairs exhibited Ka/Ks values close to or slightly above 1, particularly some of the LOX2 gene pairs and the LOX6 gene pair. These values may indicate regions of the genes where selective pressures are more relaxed, or potentially, instances of weak positive selection. For example, the LOX2-4 pair had a Ka/Ks value of 1.99292, suggesting that this specific region might have undergone positive selection. Similarly, the LOX6 pair had a Ka/Ks value of 1.66343, which could hint at adaptive evolution in this particular gene segment. These higher Ka/Ks values may reflect functional divergence or adaptation to specific environmental conditions or biological challenges in the respective species.

The Laeg_LOXs were distributed on 6 chromosomes of *L. aegyptiaca* genome. Interestingly, we discovered that majority of the LOX genes (24 out of 29) were gathered in two genomic loci, suggesting the amplification of LOX genes in the *L. aegyptiaca* genome through tandem duplication (Fig. 1). Furthermore, the two gene clusters were corresponding to LOX2 (13-LOX) and LOX1 (9-LOX) separately, implying the potentially functional divergence, where the two types of LOX enzymes have evolved to serve distinct roles in the biosynthesis of lipid mediators. Tandem duplication of lipoxygenase genes can lead to functional diversification, allowing the enzymes to specialize in different substrate specificities or regulatory responses. This genetic redundancy

Gene ID	Chr	Start	End	CDS Len (bp)	Protein Len (aa)	Class	Molecular Weight (Da)	Theoretical pI
Laeg_LOX1-1	CM029402.1	45,189,897	45,194,466	2685	894	9-LOX	101,648.5	6.43
Laeg_LOX1-2	CM029402.1	45,241,185	45,246,031	2682	893	9-LOX	101,211.9	6.04
Laeg_LOX1-3	CM029402.1	45,269,844	45,276,510	2682	893	9-LOX	101,197.9	5.93
Laeg_LOX1-4	CM029402.1	45,296,209	45,300,377	2628	877	9-LOX	99,263.9	6.19
Laeg_LOX1-5	CM029402.1	45,308,713	45,313,217	2634	877	9-LOX	99,469.85	5.94
Laeg_LOX1-6	CM029402.1	45,330,863	45,334,546	2385	794	9-LOX	91,375.53	7.94
Laeg_LOX1-7	CM029402.1	45,345,641	45,349,818	2640	879	9-LOX	99,271.49	5.62
Laeg_LOX1-8	CM029402.1	45,364,060	45,367,663	2642	879	9-LOX	99,521.09	5.94
Laeg_LOX1-9	CM029402.1	45,370,818	45,375,270	2424	807	9-LOX	91,514.91	5.52
Laeg_LOX1-10	CM029402.1	45,387,068	45,392,704	2322	773	9-LOX	89,236.51	5.8
Laeg_LOX1-11	CM029402.1	45,396,294	45,387,067	2580	859	9-LOX	99,118.83	6.13
Laeg_LOX1-12	CM029402.1	45,410,672	45,425,965	2253	750	9-LOX	86,615.91	7.06
Laeg_LOX1-13	CM029402.1	45,449,715	45,458,520	2589	862	9-LOX	97,413.62	5.77
Laeg_LOX1-14	CM029402.1	45,479,439	45,486,075	2565	854	9-LOX	97,405.55	5.25
Laeg_LOX2-1	CM029401.1	47,274,814	47,279,686	2706	901	13-LOX	102,565	6
Laeg_LOX2-2	CM029401.1	47,286,522	47,291,318	2655	884	13-LOX	100,297.9	5.55
Laeg_LOX2-3	CM029401.1	47,291,253	47,286,589	2712	903	13-LOX	102,614.6	5.9
Laeg_LOX2-4	CM029401.1	47,301,939	47,296,952	2709	902	13-LOX	102,601.6	5.84
Laeg_LOX2-5	CM029401.1	47,325,966	47,331,303	2565	854	13-LOX	98,139.77	7.35
Laeg_LOX2-6	CM029401.1	47,347,872	47,352,856	2496	831	13-LOX	95,251.63	6.19
Laeg_LOX2-7	CM029401.1	47,370,452	47,375,260	2706	901	13-LOX	101,834	5.52
Laeg_LOX2-8	CM029401.1	47,383,481	47,388,858	2703	900	13-LOX	102,001.9	5.74
Laeg_LOX2-9	CM029401.1	47,395,313	47,399,524	2556	851	13-LOX	96,925.02	5.45
Laeg_LOX2-10	CM029401.1	47,407,256	47,411,845	2490	829	13-LOX	94,786.88	6.05
Laeg_LOX2-11	CM029404.1	30,665,256	30,669,148	2454	817	13-LOX	92,503.57	5.94
Laeg_LOX3	CM029399.1	843,374	847,799	2736	911	13-LOX	103,028.7	6.69
Laeg_LOX4	CM029399.1	8,172,267	8,164,437	2157	718	13-LOX	82,676.81	6.13
Laeg_LOX5	CM029395.1	2,640,524	2,644,825	2655	884	9-LOX	100,588	5.83
Laeg_LOX6	CM029407.1	738,276	743,183	2781	926	13-LOX	104,688.3	8.72

Table 1. Characteristics of lipoxygenase genes in *L. aegyptiaca* genome.

may enhance *L. aegyptiaca* to respond to various environmental stresses and contribute to the complexity of metabolic pathways, ultimately providing a broader range of bioactive molecules for immune and inflammatory responses. The expansion of LOX genes via tandem duplication in *L. aegyptiaca* mirrors evolutionary trends observed in other *Cucurbitaceae* species, such as cucumber (*Cucumis sativus*) and melon (*Cucumis melo*), where LOX gene clusters are linked to environmental adaptability[36,37].

Conserved domain analysis of Laeg_LOXs

According to the results of Conserved Domain Database (CDD) and Pfam database, all Laeg_LOXs possessed conserved plant lipoxygenase domain (Pfam00305) (Fig. 2, Supplementary Table 2). Further investigation of the lipoxygenase domains showed high sequence conservation among the Laeg_LOX genes, with an average protein sequence identity of more than 60% (Fig. 3C). The amino acid length of the lipoxygenase domain ranged from 593 to 676 aa, covering 72.5% to 92.2% of the protein sequences, demonstrating the lipoxygenase domain as the major element of the Laeg_LOXs. Noticeably, we discovered several protein sequence loci that are distinguishable between 9-LOX and 13-LOX genes, suggesting the potential differences in the three-dimensional structure that could affect the way they interact with their substrates or other proteins.

We exploited both the Maximum Likelihood (ML) and the Neighbor-Joining (NJ) methods to construct the phylogenetic tree for the Laeg_LOXs. As expected, the Laeg_LOXs were grouped into two major clades that were corresponding to 13-LOX and 9-LOX subfamilies, respectively (Fig. 3A). Majority of the Laeg_LOXs showed similar gene structure and exon numbers (8 or 9 exons), except for *Laeg_LOX3* and *Laeg_LOX2-10* that contain 7 and 10 exons, respectively (Fig. 3B). Noticeably, several Laeg_LOXs (e.g. Laeg_LOX1-11/12/13) displayed larger introns possibly due to the accumulation of repetitive elements or regulatory mutations, which may offer opportunities for alternative splicing and novel regulatory roles that can drive functional diversification and evolutionary innovation.

We identified six conserved motifs across the 29 Laeg_LOXs and all of them contained at least one copy of the six motifs (Fig. 3D). Among these motifs, Motif1, known as 38-residue motif, had been reported as a specifically conserved sequence of 38 amino acids within the protein encoded by the LOX gene. Consistent with the previous studies[5,10,12,38], the Motif1 was characterized by five conserved histidine (His-$(X)_4$-His-$(X)_4$-His-$(X)_{17}$-His-$(X)_8$-

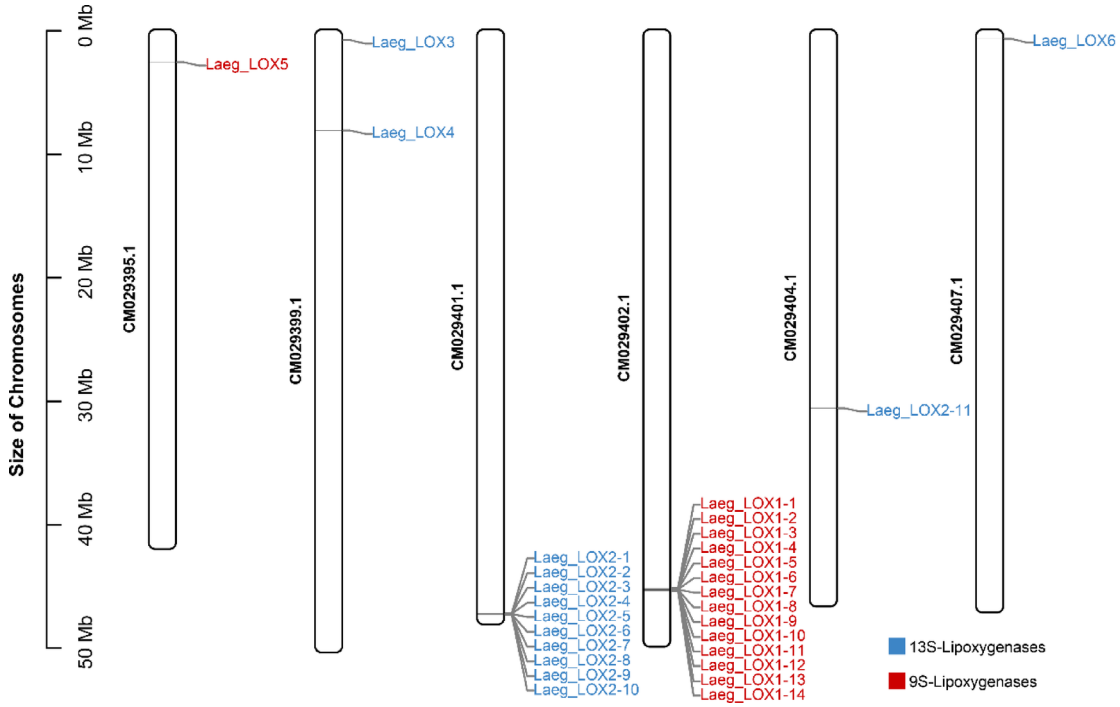

Fig. 1. Distribution of Laeg_LOXs on *L. aegyptiaca* chromosomes. The blue and red colors represent 13-LOX and 9-LOX subfamilies, respectively.

His, where X represented any other kinds of amino acid) (Fig. 3E). Further investigation of the Motif1 revealed 12 additional conserved amino acids, including one glutamine, tryptophan, isoleucine, alanine, arginine, serine at position 2, 7, 19, 20, 23, 26 respectively, two proline at positions 30 and 37, and four leucine at positions 8, 25, 34, 35. These conserved amino acids within the motif are crucial for maintaining enzyme structure and function, potentially participating in substrate binding, catalytic activity, and regulatory interactions, with variations at these positions possibly impacting disease susceptibility.

Analysis of cis-acting elements in Laeg_LOXs

Cis-acting elements in the promoter are crucial for initiating transcription and regulating gene expression. To analyze cis-acting elements in promoter of the Laeg_LOXs, the up-stream 2 kb sequences from the initiation codon was extracted for prediction of cis-acting elements using PlantCARE database. The identifed cis-acting elements were related to development, environment stress, hormone responsive and light responsive, and most of them were hormone responsive and light responsive elements (Fig. 4). Among these cis-acting elements, anaerobic response elements (AREs) were significantly enriched in the promoters of several Laeg_LOXs, including *Laeg_LOX5*, *Laeg_LOX1-7/8/9/10/12/14* and *Laeg_LOX2-11*, suggesting that these genes could be essential for the coordinated response to low-oxygen conditions, enabling *L. aegyptiaca* to adapt and survive under oxygen-limited environments. Additionally, presence of abundant light responsive cis-elements indicated the vital roles of Laeg_LOXs in an array of biological functions that are sensitive to light exposure, such as photomorphogenesis, circadian rhythm regulation, and plant defense mechanisms. They may also participate in processes like floral development, chlorophyll biosynthesis, and stomatal patterning, enabling *L. aegyptiaca* to adapt and respond to different light conditions for optimal growth and defense.

Phylogenetic and collinear analysis of Laeg_LOXs

To determine the phylogenetic relationship of Laeg_LOXs, we included 125 additional LOX genes belonging to six species, including 28 LOX genes from *Luffa acutangula* (Lacu_LOXs, same for other species), 23 from *Cucurbita pepo*, 5 from *Cucurbita argyrosperma,* 24 from *Cucumis sativus*, 26 from *Cucumis melo*, 19 from *Benincasa hispida* (Fig. 5). Consistently, all LOX genes were classified into two subfamilies, 9-LOX and 13-LOX. Laeg_LOXs were phylogenetically closest to Lacu_LOXs, followed by Cpep_LOXs. According the phylogenetic tree, the Laeg_LOXs could be emerged through multiple rounds of tandem duplication events that happened on different time. For example, Laeg_LOX1-4/5/6/7/8 formed after the divergence of *L. aegyptiaca* with *Cucumis* and *Cucurbita* while prior to the divergence between *L. aegyptiaca* and *L. acutangula*. In addition, Laeg_LOX1-9/10/11/12/13/14 were tandemly duplicated before the divergence of *Luffa*, *Cucumis*, *Cucurbita* and *Benincasa* from their common ancestors. Furthermore, we performed synteny analysis of LOX genes among four species, including *L. aegyptiaca*, *L. acutangula*, *C. pepo*, and *B. hispida* (Fig. 6). Noticeably, all the identified LOX genes showed excellent collinearity, and all of them were distributed on seven genomic loci and six chromosomes, suggesting a conserved genomic organization that has been maintained throughout evolution.

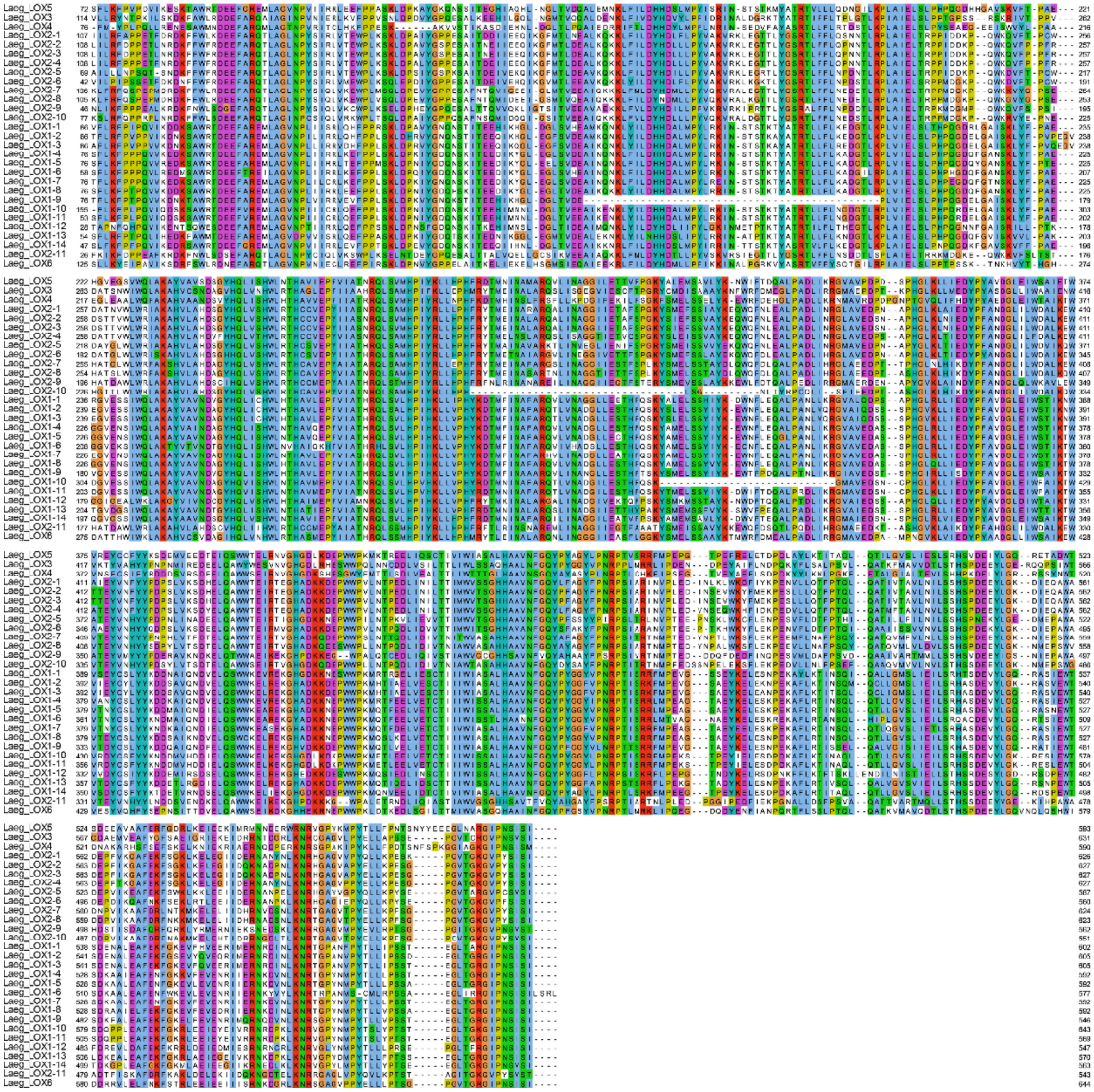

Fig. 2. The lipoxygenase domains of 29 Laeg_LOXs were extracted to perform multiple sequences alignment. The blue and red colors of gene name represent 13-LOX and 9-LOX subfamilies, respectively.

Gene expression patterns of Laeg_LOXs across various tissues

To validate the expression profiles and explore the possible roles of LOX genes in the growth and development of *L. aegyptiaca*, we examined the expression levels across eight different tissues—tendril, root, stem, fruit, male flower, female flower, shoot apex, and leaf—utilizing the RNA-sequencing data that has been previously reported (under NCBI with accession number of 'PRJNA732226'). According to the expression matrix, 18 out of 29 Laeg_LOXs were expressed in at least one of the eight tissues (Fig. 7). Several Laeg_LOXs showed tissue-specific expression. For example, *Laeg_LOX1-5* was specifically expressed in leaf and nearly silenced in other tissues, and *Laeg_LOX2-8* and Laeg_*LOX1-2* were predominantly expressed in fruit and shoot apex, respectively. *Laeg_LOX1-2*, *Laeg_LOX1-14*, *Laeg_LOX3* and *Laeg_LOX6* were expressed across all tissues, suggesting that these genes may play crucial roles in fundamental biological processes such as inflammation, redox balance, and tissue homeostasis, potentially influencing the organism's response to various stimuli and maintaining overall health. Most of the Laeg_LOXs showed high expression levels in tendril, fruit and root, implying the vital roles of Laeg_LOXs in the specialized physiological and developmental processes unique to these organs, such as the synthesis of bioactive compounds important for tendril curling, fruit development, ripening, or root growth and nutrient absorption, as well as in defense mechanisms against environmental stressors or pathogens specific to these tissues. The tissue-specific expression of Laeg_LOXs aligns with functional studies in passion fruit (*Passiflora edulis*), where LOX genes (e.g., PeLOX4) were highly expressed in fruits and linked to ripening and aroma synthesis[5]. Conversely, in banana (*Musa acuminata*), LOX genes exhibited dominant expression in leaves and roots, indicating divergent roles in vegetative growth versus reproductive development. Such comparisons emphasize that while LOX genes are broadly conserved, their expression patterns and functional specialization are shaped by species-specific ecological and developmental demands.

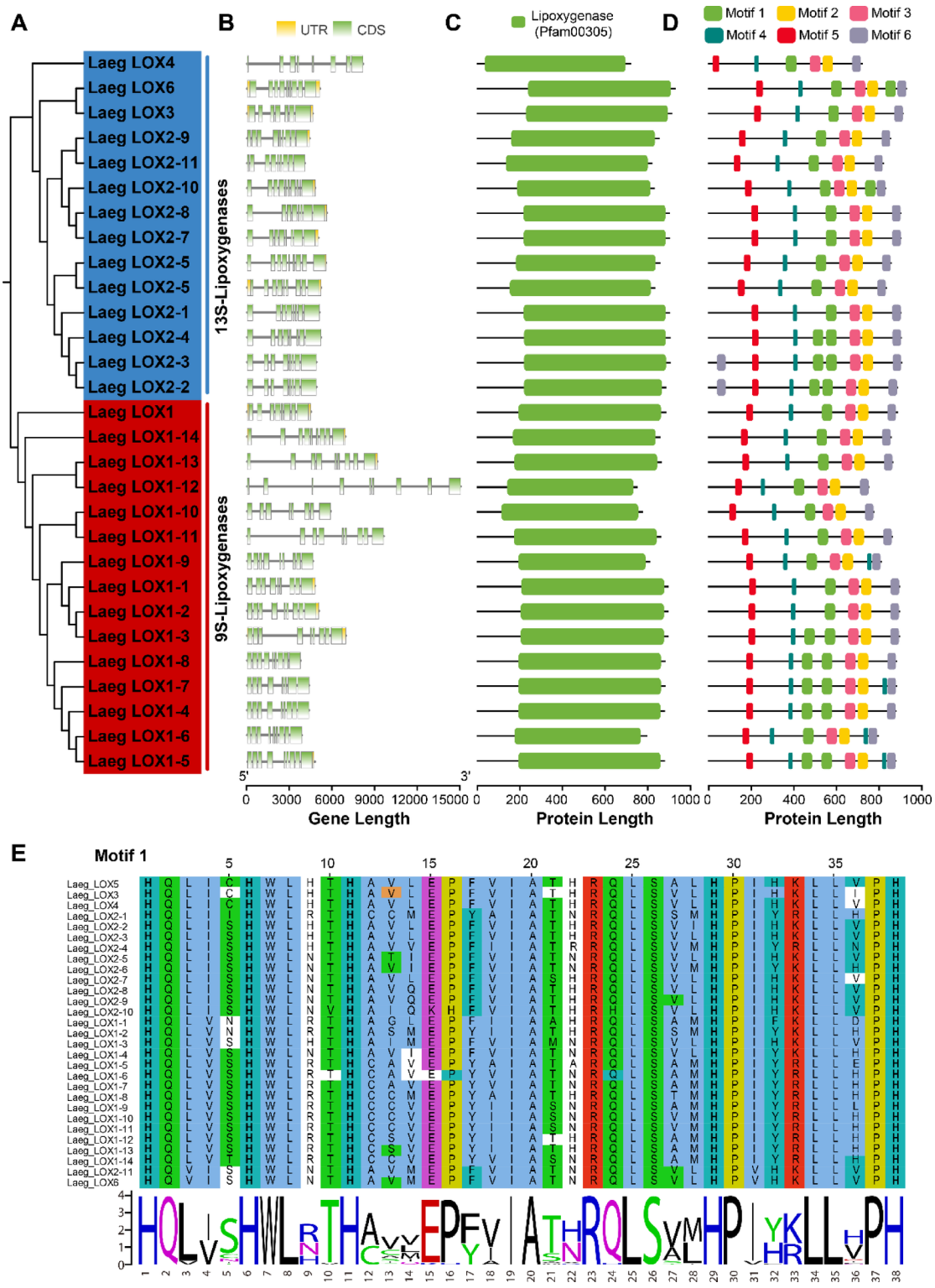

Fig. 3. (**A**) The phylogenetic tree of 29 Laeg_LOXs, and the tree was divided into two clades that were corresponding to 13-LOX and 9-LOX subfamilies, respectively. (**B**) Exon–intron structures of 29 Laeg_LOXs were visualized. (**C**) The lipoxygenase domains of 29 Laeg_LOXs were visualized. (**D**) Six conserved motifs were identified across 29 Laeg_LOXs and the relative position of these motifs were visualized. (**E**) The 38-residue motif was present and conserved across 29 Laeg_LOXs. Their sequences were extracted and aligned, and the consensus of this motif was marked below.

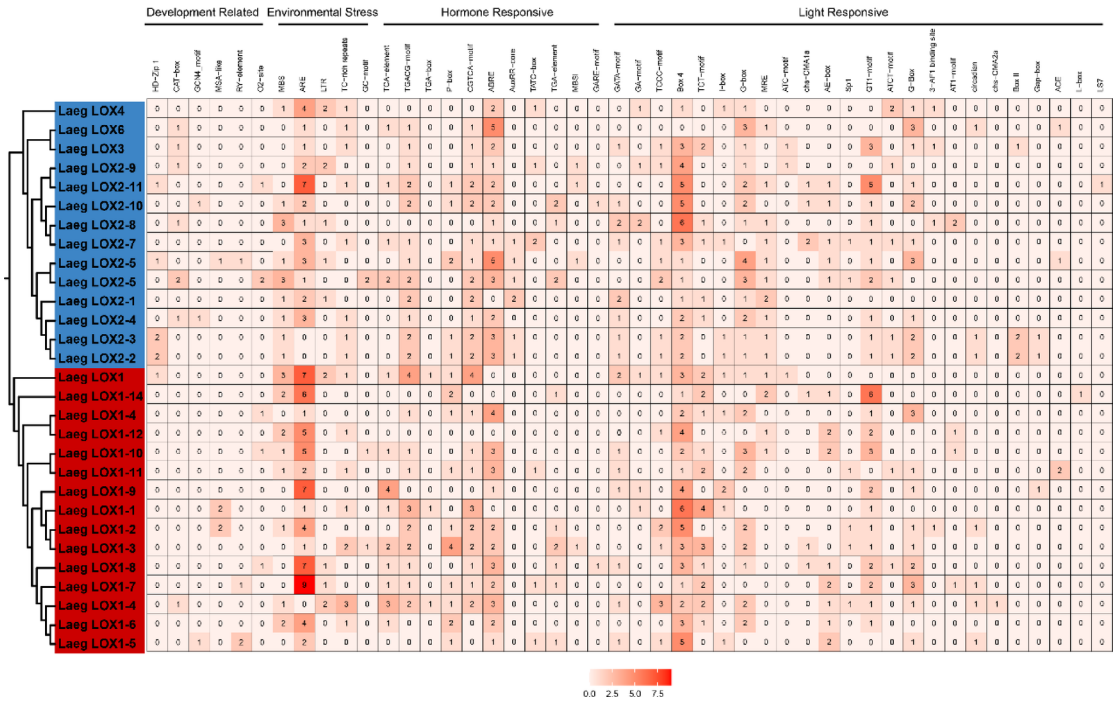

Fig. 4. The upstream 2 kb sequences of 29 Laeg_LOXs were extracted for prediction of cis-elements. The discovered cis-elements were classified based on their potential functions. The exact numbers of cis-elements were counted for each Laeg_LOX and each subfamily.

Analysis of Laeg_LOXs expression in response to heat stress

Previous research has demonstrated that LOX genes are crucial for plants' protective mechanisms against various forms of stress, both from non-living (abiotic) and living (biotic) factors[5,7,8,10,16]. In this study, we exposed *L. aegyptiaca* to heat stress, and then we examined the expressional tendency of Laeg_LOXs using RNA-seq analysis. In total, we discovered 11 expressed Laeg_LOXs. Interestingly, 9 out of 11 expressed Laeg_LOXs were significantly downregulated under heat stress (Fig. 8), and the other two LOX genes were slightly upregulated (*Laeg_LOX1-14*) and remained unchanged (*Laeg_LOX6*). The expressional change of these genes was further validated using qRT-PCR, demonstrating the accuracy of RNA-seq analysis. The significant downregulation of these genes suggests that they may play important roles in the plant's thermal protection and metabolic readjustment under heat stress. This response could be part of a broader heat shock reaction, aiming to maintain cellular integrity, alter lipid mediator profiles, and prioritize the production of heat shock proteins over other cellular processes[39–42].

Discussion

In this study, we performed a comprehensive analysis of lipoxygenase (LOX) gene family in *Luffa aegyptiaca*, revealing a total of 29 LOX genes with distinct physiochemical properties and chromosomal distribution. The identification and characterization of these genes contributed significantly to our understanding of the molecular mechanisms underlying the plant's response to environmental stresses and its developmental processes. One of the most intriguing findings is the clustering of a majority of LOX genes in two genomic loci, suggesting a history of gene amplification through tandem duplication. This observation implied that *L. aegyptiaca* may have evolved a strategy to enhance its adaptability to various environmental stresses by diversifying the functions of its LOX enzymes. The functional redundancy resulting from gene duplication events could have allowed for specialization in substrate specificity and regulatory responses, thereby enriching the plant's metabolic pathways and its capacity to produce a diverse array of bioactive molecules for immune and stress responses. Additionally, the tandem duplication of LOX genes in *L. aegyptiaca* can enrich the genetic diversity and functional complexity, potentially enhancing its adaptability to environmental challenges and contributing to the sophistication of its developmental programs.

The phylogenetic and collinear analysis further supported the notion of functional divergence among the LOX genes. The distinct grouping of 13-LOX and 9-LOX subfamilies and the identification of conserved motifs within the LOX domains highlighted the evolutionary conservation and potential differences in the structural and functional aspects of these enzymes. Consistent with the previous researches[5,10,12,38], we discovered the presence of conserved histidine residues within the 38-residue motif. These residues play a crucial role in the enzyme's catalytic mechanism in LOX genes. Particularly, they are involved in the coordination of the non-heme iron, which is essential for the enzyme's activity. The iron ion is a critical component of the active site, where it participates in the abstraction of a hydrogen atom from the polyunsaturated fatty acid (PUFA) substrate, initiating the oxidation process that leads to the formation of hydroperoxides. Moreover, the conserved histidine residues

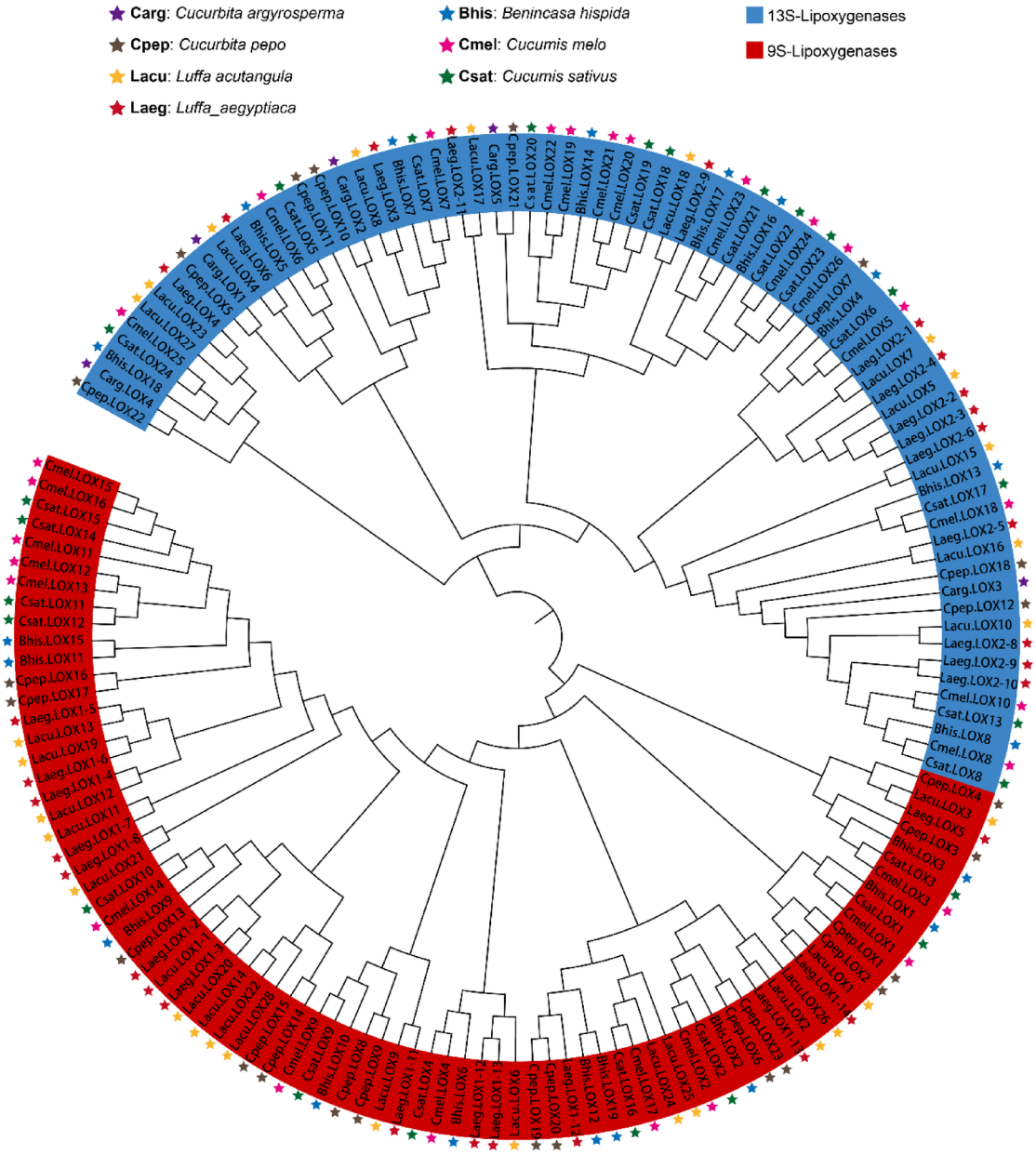

Fig. 5. The phylogenetic tree was built based on the LOX genes of *L. aegyptiaca* and six related species, including *Luffa acutangula* (Lacu), *Cucurbita pepo* (Cpep), *Cucurbita argyrosperma* (Carg), *Cucumis sativus* (Csat), *Cucumis melo* (Cmel), and *Benincasa hispida* (Bhis). The different colors of pentagrams represented different species of LOX genes. Consistently, the tree was divided into two clades that were corresponding to 13-LOX and 9-LOX subfamilies, respectively.

also contribute to the overall structure and stability of the enzyme. They help maintain the three-dimensional structure necessary for substrate binding and catalysis. In some cases, these residues have been shown to be involved in the fine-tuning of the enzyme's specificity and regioselectivity, ensuring that the oxygenation of the PUFA substrate occurs at the correct position[38,43–45]. Furthermore, mutations of these histidine residues can lead to significant changes in the enzyme's activity, as they can affect the iron coordination and the enzyme's ability to bind and oxidize the substrate, highlighting the importance of these residues for the enzyme's function[38,46–48].

Moreover, the analysis of cis-acting elements in the promoter regions of the LOX genes points to the complex regulation of these genes in response to developmental cues, environmental stresses, and light exposure. The enrichment of anaerobic response elements (ARE) and light-responsive elements in particular, suggested that the LOX genes may play a crucial role in the plant's adaptation to low-oxygen conditions and light-mediated processes, such as photomorphogenesis and circadian rhythm regulation.

Finally, the examination of gene expression patterns across various tissues and under heat stress conditions shed light on the potential roles of LOX genes in the growth, development, and stress response mechanisms of *L. aegyptiaca*. The tissue-specific expression of certain LOX genes, along with the downregulation of several

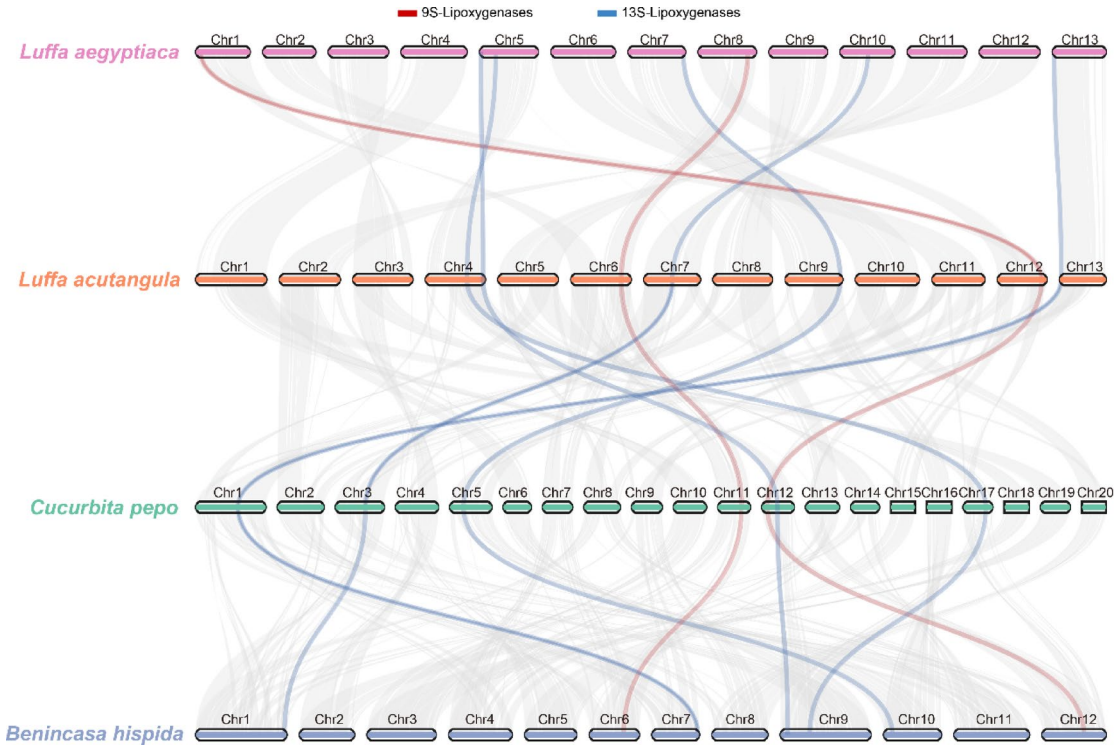

Fig. 6. The synteny of LOX genes among *L. aegyptiaca*, *L. acutangula*, *C. pepo*, and *B. hispida* was visualized.

genes under heat stress, indicated a sophisticated regulatory network that modulates the plant's physiological responses to different stimuli. The expression patterns of LOX genes in different plant species under heat stress conditions have been studied extensively, revealing a complex regulatory network that contributes to plant stress tolerance.

Usually, the expression of LOX genes is often upregulated under heat stress, indicating their involvement in the plant's defense mechanisms against thermal damage. For instance, in cotton, the expression analysis of LOX genes revealed that most of the GhLOX genes were induced in at least two tissues, and the majority of them were up-regulated in response to heat and salinity stress[49]. This suggests that LOX genes might be part of a general stress response mechanism in plants. However, several studies have also the downregulation of LOX under heat stress conditions. For example, in a study on the influence of temperature conditions on lipoxygenase activity in seedlings of rape (B*rassica napus* var. Oleifera), it was observed that low temperature was responsible for reduced LOX activity by 34% in heat-resistant cultivar, while subsequent heat stress gave no change to LOX activity. In contrast, the LOX activity in heat-stressed cold-resistant cultivar was reduced two-fold[39]. Another study on tomato (*Solanum lycopersicum* cv. Ailsa Craig) LOX gene family members in response to heat stress showed that some LOX genes were downregulated at early time points of heat exposure and remained downregulated throughout the exposure period[50]. This indicates that not all LOX genes are upregulated under heat stress and that the response can be complex and gene-specific. This suggests that the response of LOX genes to heat stress can differ between cultivars and may involve both upregulation and downregulation depending on the specific conditions and the plant's inherent stress tolerance. Together, these findings highlight the complexity of LOX gene regulation under heat stress and suggest that the role of LOX genes in plant stress responses may be more diverse than previously thought. The downregulation of certain LOX genes under heat stress could be part of a broader regulatory response aimed at maintaining cellular homeostasis and protecting the plant from damage caused by excessive reactive oxygen species (ROS) production under thermal stress.

The observed downregulation of LOX genes in *L. aegyptiaca* under heat stress may reflect a trade-off between oxylipin biosynthesis and energy conservation. Similar patterns were reported in *Arabidopsis*, where heat stress suppressed LOX-mediated jasmonate production to prioritize heat shock protein synthesis[42]. Additionally, in chickpea (*Cicer arietinum*), LOX genes were downregulated under accelerated aging (a stress-related condition), further supporting the hypothesis that LOX suppression is a conserved strategy to mitigate oxidative damage under abiotic stress[16]. However, the functional consequences of LOX downregulation may vary in *L. aegyptiaca*, this could reduce lipid peroxidation and stabilize membrane integrity, whereas in *Cannabis sativa*, LOX inhibition disrupted defense signaling against pathogens[15]. These comparisons underscore the context-dependent roles of LOX genes in stress adaptation.

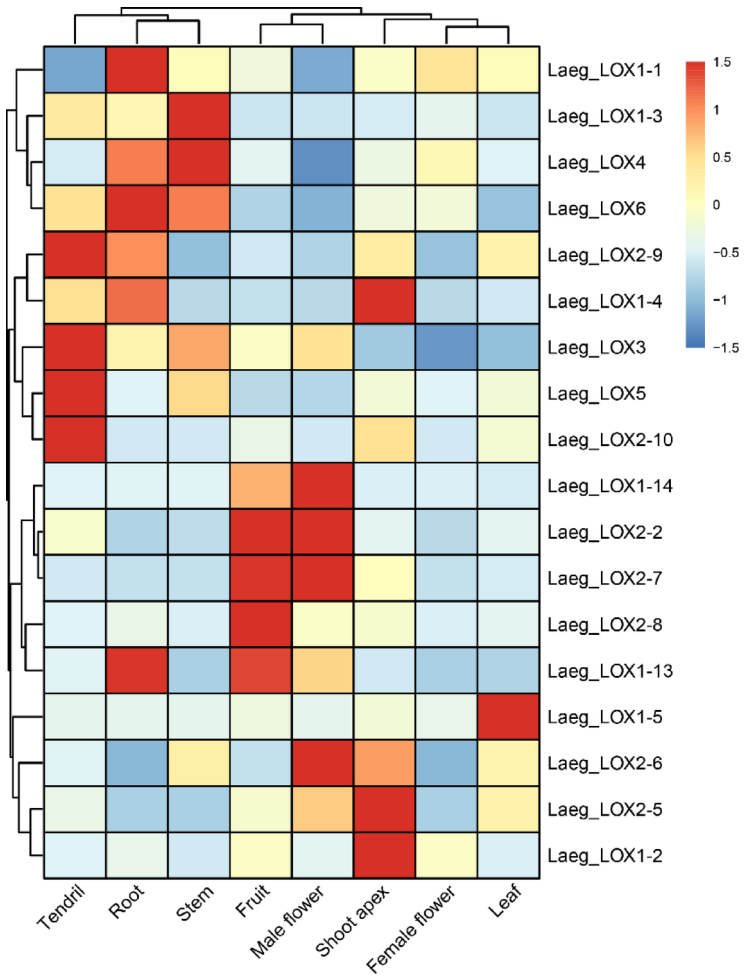

Fig. 7. Gene expression levels of Laeg_LOXs were quantified across eight tissues of *L. aegyptiaca*. The relative expression of the expressed Laeg_LOXs were visualized in heatmap. The scale bar represented the relative expression levels of Laeg_LOX genes.

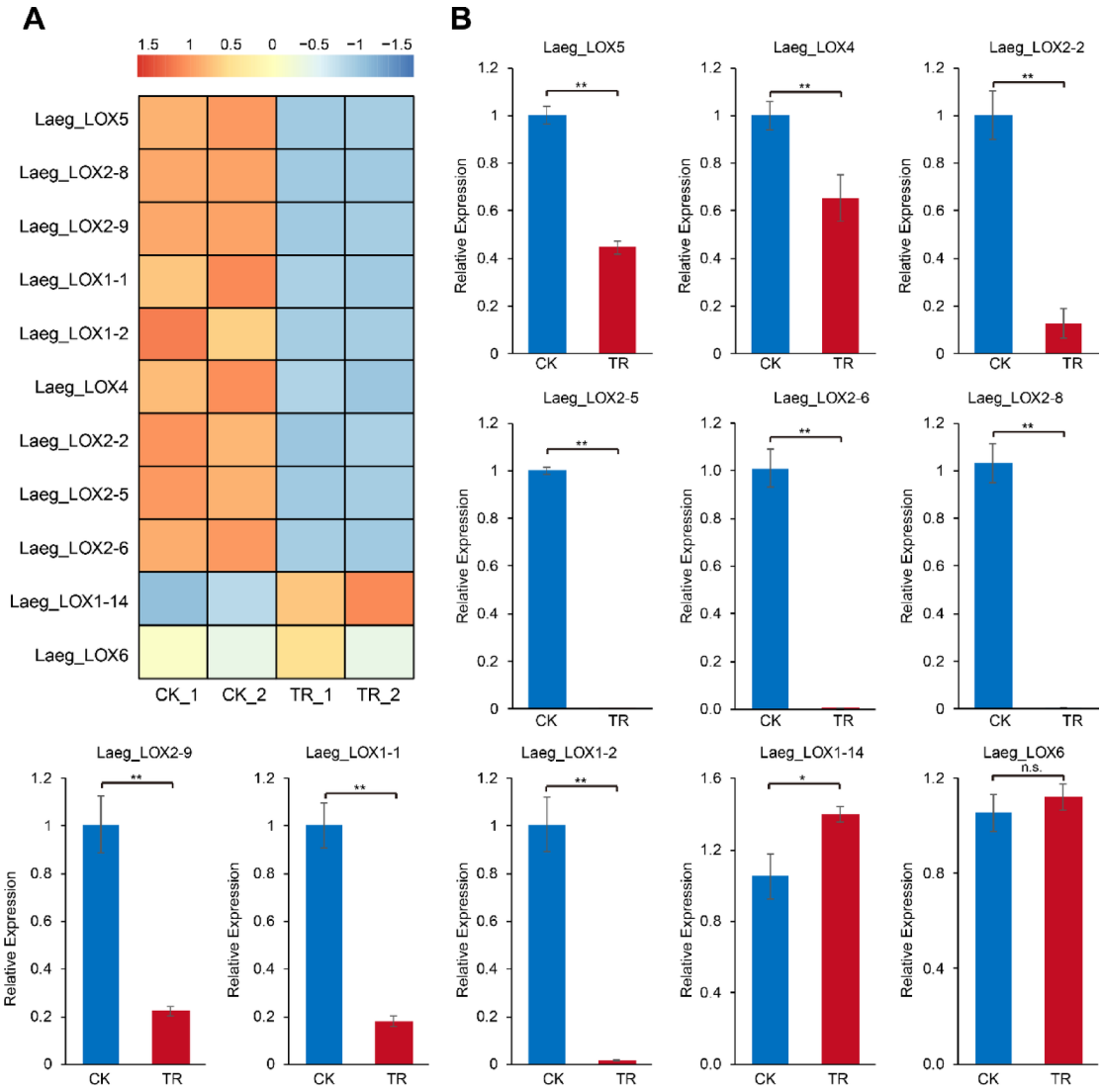

Fig. 8. (**A**) Gene expression levels of Laeg_LOXs were quantified under heat stress. Nine LOX genes were significantly downregulated and one gene was slightly upregulated. The scale bar represented the relative expression levels of Laeg_LOX genes. (**B**) The expression levels of Laeg_LOXs under heat stress were validated using qRT-PCR. The y-axis represented the relative expression levels of Laeg_LOX genes. The pairwise t-test was used to estimate the significance of expressional change. Two asterisks (**) represented that q-values less than 1e-10, and one asterisk represented that q-values less than 0.01, and 'n.s.' represented no significance with p-values larger than 0.01.

Data availability

Raw RNA-seq data of *L. aegyptiaca* under heat stress were deposited at NCBI with accession number of GSE275983. The RNA-seq data of different tissues of *L. aegyptiaca* were retrieved from NCBI under project PRJNA732226.

Code availability

No specific script was used in this work. The codes and pipelines used for data processing were all executed according to the manual and protocols of the corresponding bioinformatics software.

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Author contributions

Hao Huang and Haisheng Zhu conceived the idea, analyzed the data, wrote the original draft, revised the manuscript, and got the funding; Hao Huang and Yongping Li analyzed the data; Qingfang Wen and Huifei Ma prepared the materials and performed experiments for verification; Haisheng Zhu supervised the project; All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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