horticulturae

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Molecular Characterization and Expression of *CmobHLH* Genes in Pumpkin

Wenhong Ao [1,2], Weirong Luo [1,2], Wenchen Xu [1,2], Xudong Wang [1,2], Junjun Liu [1,2] and Yongdong Sun [1,2,*]

[1] School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang 453003, China; whao202304@126.com (W.A.); wrluo2008@126.com (W.L.); xuwenchen2015@163.com (W.X.); wangxudong@stu.hist.edu.cn (X.W.); jjliu2018@163.com (J.L.)
[2] Henan Province Engineering Research Center of Horticultural Plant Resource Utilization and Germplasm Enhancement, Xinxiang 453003, China
* Correspondence: ydsun2019@126.com

Abstract: The transcription factor *bHLH* gene family plays fundamental roles in plant development and mitigating diverse biotic and abiotic stresses. However, the information of *bHLH* genes in pumpkin (*Cucurbita moschata*) is still unknown. In this current study, 222 *CmobHLH* genes were identified and mapped onto different chromosomes through bioinformatics analysis in pumpkin. CmobHLH and AtbHLH proteins could be classified into 19 subfamilies according to the phylogenetic tree. CmobHLH proteins within the same subfamily had similar motif composition and gene structures. Gene ontology (GO), *cis*-regulatory elements (CREs) and protein–protein interaction analyses suggested the potential regulatory roles of *CmobHLH* genes during the plant development process and abiotic stresses response in pumpkin. Tissue expression patterns based on transcriptome data demonstrated that *CmobHLH* genes were involved in pumpkin development process, and they had unique functions in different tissues. The expression patterns of five selected *CmobHLH* genes after exposure to abiotic stresses showed that the *CmobHLH* genes played varied roles in the stress responses of pumpkin to NaCl, waterlogging, cold, ABA and drought. In brief, these findings offer important information for further functional research of *CmobHLH* genes and resistance breeding in pumpkin.

Keywords: abiotic stresses; *bHLH* genes; bioinformatics analysis; *Cucurbita moschata*; expression analysis

Citation: Ao, W.; Luo, W.; Xu, W.; Wang, X.; Liu, J.; Sun, Y. Molecular Characterization and Expression of *CmobHLH* Genes in Pumpkin. *Horticulturae* **2023**, *9*, 648. https://doi.org/10.3390/horticulturae9060648

Academic Editor: Liwang Liu

Received: 24 April 2023
Revised: 23 May 2023
Accepted: 29 May 2023
Published: 31 May 2023

1. Introduction

The *bHLH* gene family is one of the most extensive transcription factor (TF) families found in plants, usually classified into 15–26 subfamilies [1]. *bHLH* family genes play crucial roles in controlling plant development and tolerating diverse environmental stresses through regulating the expression of downstream target genes [2–4]. *bHLH* family proteins are composed of two functional regions: the basic region located in the N-terminus and the HLH region in the C-terminus [5,6]. The basic region, which has 15–20 amino acids (aa), is primarily involved in binding of TFs through binding to the *cis*-regulatory elements (CREs) [1,7]. The HLH domain has 40–50 aa and is composed of two α-helices connected by a loop, which can promote protein interactions and form homodimers or heterodimers to induce a range of diverse activities [8–10]. Identification of *bHLH* family genes was initially recorded in maize (*Zea mays* L.) [11]. Thereafter, an increasing number of *bHLH* genes were widely characterized in numerous plant species, such as *Arabidopsis* [12], rice [13], tomato [14], poplar [1], apple [15], eggplant [16], grass pea [17], persimmon [18], sweet wormwood [19] and *Ipomoea aquatica* [20].

A number of evidence has shown that *bHLH* family genes are involved in plant development processes [21,22]. For example, the *bHLH* TF SPATULA (*SPT*) inhibited root growth and size through limiting cell proliferation and expansion in *Arabidopsis* [23]. EAT1,

a rice *bHLH* TF regulated programmed cell death (PCD) in tapetal cells [24]. Overexpression of *AtbHLH68* and *AtbHLH112* suppressed lateral root development in *Arabidopsis* seedlings [25,26]. Some *bHLH* genes related to fruit development have also been found. Eleven *SlbHLH* genes have been reported to participate in young fruit development and ripening in tomato [27]. In melon, *CmbHLH32* exhibited higher expression in the process of early fruit development, and overexpression of *CmbHLH32* led to early fruit ripening [28]. The MYB-bHLH-WD40 complex was positively associated with anthocyanin accumulation and blueberry fruit color development [29]. Furthermore, *bHLH* family genes significantly strengthened salinity [30], drought [31,32] and cold [33] tolerance. In grapes, expression of the *VvICE1a* and *VvICE1b* genes, two *bHLH* family genes, improved the stresses tolerance of salinity, drought and cold in transgenic *Arabidopsis* [34]. In addition, *bHLH* family genes were involved in the regulation of iron homeostasis. In maize, *ZmbHLH105* significantly decreased Mn buildup through suppressing the expression of Mn/Fe-regulated transporter genes [35]. In rice, *OsbHLH133* was an important regulator of Fe distribution in the roots and shoots [36]. According to the above reports, *bHLH* family genes would be great candidate genes for improving stress tolerance and resistance breeding in plants.

Pumpkin (*Cucurbita moschata*) is cultivated and consumed around the world with a very high nutritional and health care value [37]. However, diverse environmental stresses, such as salinity, cold and drought severely affect the processes of growth and development in pumpkin. Although some *bHLH* genes have been elaborated in plants, and overexpression of *CmbHLH87* gene in tobacco increased the resistance to powdery mildew [38], there is no report of *CmobHLH* genes in resistance to abiotic stresses in pumpkin. In this current study, the *CmobHLH* gene family was identified and characterized in pumpkin through bioinformatics analysis. Furthermore, we investigated the gene transcription patterns under different tissues and different abiotic stresses, which gave crucial insights for further understanding of the functions of *CmobHLH* genes and resistance breeding in pumpkin.

2. Materials and Methods

2.1. Identification of CmobHLH Genes in Pumpkin

The *AtbHLH* genes were found through calling up their gene IDs from TAIR database (https://www.arabidopsis.org/, accessed on 12 February 2023) [39], and 165 *Arabidopsis* bHLH protein sequences were used as reference sequences to search the pumpkin bHLH proteins from the Cucurbit Genomics Database (CuGenDB) (http://www.cucurbitgenomics.org/, accessed on 12 February 2023) [40]. To search for pumpkin *bHLH* genes, the Pfam online database (http://pfam-legacy.xfam.org/, accessed on 12 February 2023) [41] was used to obtain the hidden Markov model (HMM) file of the bHLH domain (PF00010). Subsequently, Pfam and Conserved Domain Database (CDD) (https://www.ncbi.nlm.nih.gov/guide/domains-structures/, accessed on 13 February 2023) [42] were used to search the bHLH-conserved domain. Finally, CmobHLH proteins with bHLH-conserved domain were identified in pumpkin. Various physical and chemical properties of the CmobHLH proteins were analyzed using the online ProtParam tool (https://web.expasy.org/protparam/, accessed on 13 February 2023) [43]. Subcellular localization of CmobHLH proteins was predicted using the online tool Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/#, accessed on 13 February 2023) [44].

2.2. Phylogenetic Relationship, Gene Structure and Protein–Protein Interaction Networks of CmobHLH Proteins

Multiple sequence alignments of CmobHLH proteins were conducted using ClustalW software. The phylogenetic tree was constructed using the neighbor-joining (NJ) method with MEGA 7.0 (the Bootstrap = 1000 and other parameters were set to default values) [45]. The online tool Interactive Tree Of Life (iTOL) (https://itol.embl.de/, accessed on 14 February 2023) [46] was used to modify and annotate the generated phylogenetic tree. The syntenic analysis of *bHLH* genes in pumpkin and *Arabidopsis* was performed using TBtools [47]. The MEME online tool (https://meme-suite.org/meme/tools/meme,

accessed on 14 February 2023) [48] was used for the identification of the conserved motifs, with the default parameters and a maximum number of motifs to 10. The gene structures of the *CmobHLH* genes were exhibited with TBtools. GO analysis was performed using the GO enrichment tool of CuGenDB, and Gene Ontology [49] was used as an additional validation tool. The 2000 bp upstream promoter sequences of 222 *CmobHLH* genes were retrieved using TBtools and submitted to the online PlantCARE database (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 14 February 2023) [50] to predict the CREs. Protein–protein interaction networks among CmobHLH proteins were predicted with STRING tool (https://cn.string-db.org/, accessed on 14 February 2023) [51].

2.3. Plant Materials, Growth Conditions and Treatments

A six-generation-inbred line of 'Hantailang' was used as the pumpkin material in this study. Pumpkin seeds were germinated at 37 °C for two days, and the germinated seeds were sown in plug trays and grown in the growth chamber under 28 °C with 12 h light and 12 h dark. Pumpkin seedlings at two-leaf stage were treated with different abiotic stresses. For waterlogging, water 2 cm was maintained above the soil surface. For cold, pumpkin seedlings were cultivated at 15 °C day/5 °C night. For ABA and drought stresses, pumpkin seedings were dipped in Hoagland nutrient solution containing 100 µmol ABA and 10% PEG 6000, respectively. Pumpkin leaves were collected on the 10th day after treatment for gene expression analysis.

2.4. Gene Expression Analysis

The transcriptional profiles of *CmobHLH* genes in different tissues were obtained from the RNA-seq data (PRJNA385310) of interspecific F1 hybrid 'Shintosa'. The transcriptional profiles of *CmobHLH* genes under 75 mmol/L NaCl stress for 24 h were obtained from the RNA-seq data (PRJNA437579) of the root apexes of (*Cucurbita maxima* × *Cucurbita moschata*) cv. 'Chaojiquanwang'. In this study, the count of reads was normalized to fragments per kilobase of transcript per million fragments (FPKM) of *CmobHLH* genes. An expression heatmap was generated with $\log_2$FPKM values. Expression patterns of 5 *CmobHLH* genes under waterlogging, cold, ABA and drought stresses were performed using qRT-PCR. For this purpose, total RNA from pumpkin leaves of various treatments was extracted using OminiPlant RNA Kit (CWBIO, Suzhou, China), and PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Dalian, China) was used to synthesize the first chain cDNA. To demonstrate the expression patterns under diverse stresses, qRT-PCR was performed using TB Green® Premix Ex Taq™ II (TaKaRa, Dalian, China). Each experiment included three biological replicates, following the manufacturer's protocol. Supplementary Table S1 contains the primer sequences of *CmobHLH* genes and reference gene (*CmoActin*) used in this investigation. The relative expression levels of *CmobHLH* genes were quantified using the $2^{-(\Delta\Delta Ct)}$ method [52].

3. Results

3.1. Identification and Characterization of CmobHLH Family Genes

In total, 222 *bHLH* genes were recognized in pumpkin and renamed as *CmobHLH1* to *CmobHLH222* based on their chromosomal locations. Based on pumpkin genomic information, they were unevenly placed onto the twenty chromosomes of pumpkin (Figure 1). Chromosome 11 contained the maximum number of *CmobHLH* genes (19), while chromosome 20 had the minimum number of *CmobHLH* genes (4). In general, 6–14 *CmobHLH* genes were present on each chromosome. The characterization of these CmobHLH proteins was presented in Supplementary Table S2. The coding sequence (CDS) lengths of *CmobHLH* genes varied from 228 to 5469 bp, with 75 to 1822 aa, predicted molecular weight (MW) from 9.32 to 202.73 kDa, isoelectric point (pI) from 4.50 to 9.98 and an instability index (II) from 32.25 to 99.87. The majority of CmobHLH proteins were hydrophilic based on their Grand average of hydropathicity (GRAVY) values. The results of subcellular local-

ization revealed that 221 of CmobHLH proteins were located in the nucleus, while only CmobHLH136 was in the cell membrane.

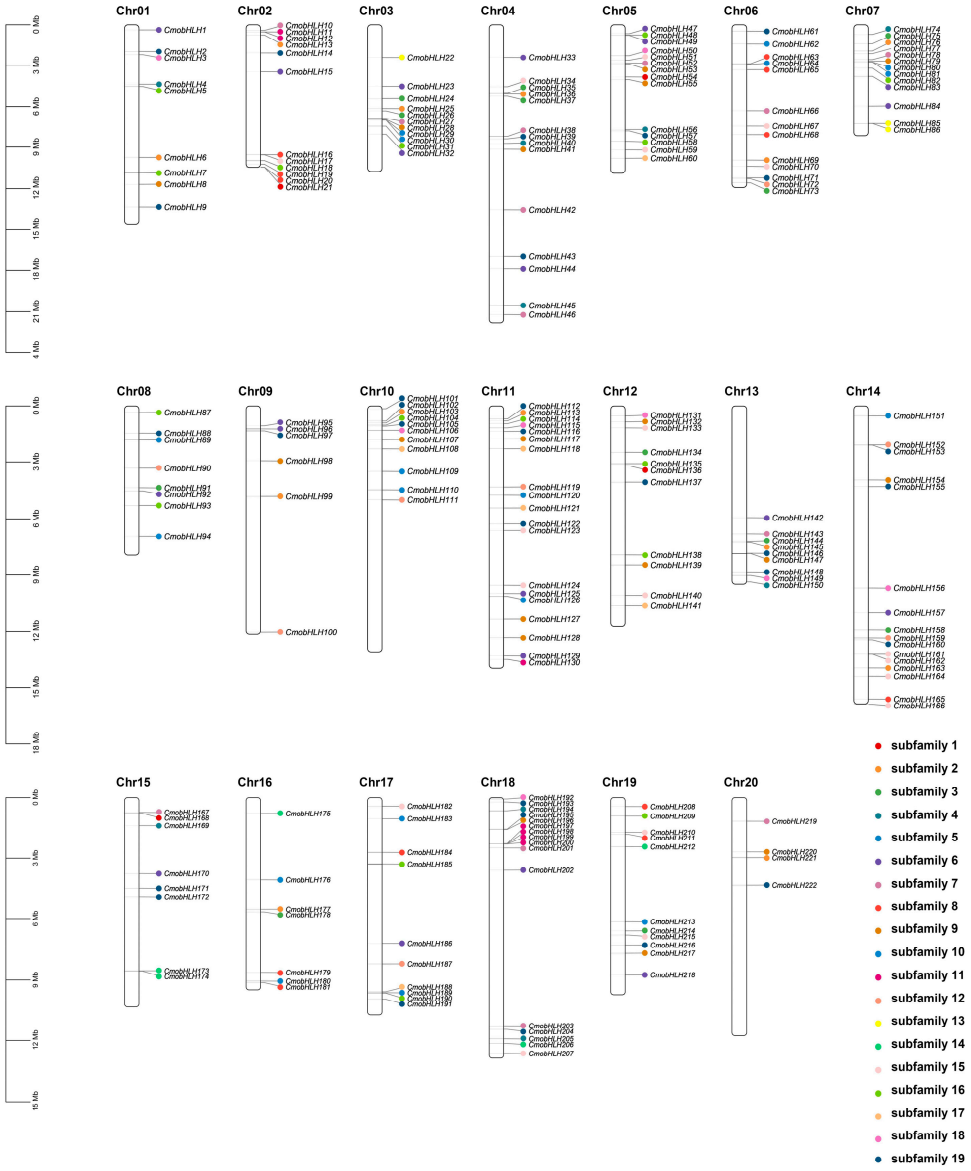

Figure 1. Chromosomal localization of 222 *CmobHLH* genes.

3.2. Phylogenetic Tree and Synteny Analysis of the CmobHLH Proteins

To classify the evolutionary relationships of CmobHLH proteins, the phylogenetic tree was constructed based on 165 AtbHLH and 222 CmobHLH protein sequences. As shown in Figure 2, 387 bHLH proteins were classified into 19 subfamilies. Among them, subfamily 19 comprised the maximum number of CmobHLH proteins (30), followed the subfamily 6 (21), while subfamily 13 contained the minimum number (3). Moreover, CmobHLH proteins and AtbHLH proteins were present in every subfamily; however, the number varied in every subfamily. To further elucidate the synteny of *bHLH* genes, the synteny map between pumpkin and *Arabidopsis* was generated. The result showed that 138 *CmobHLH* genes were determined to be collinear with 103 *AtbHLH* genes, and 174 orthologous pairs were discovered (Figure 3).

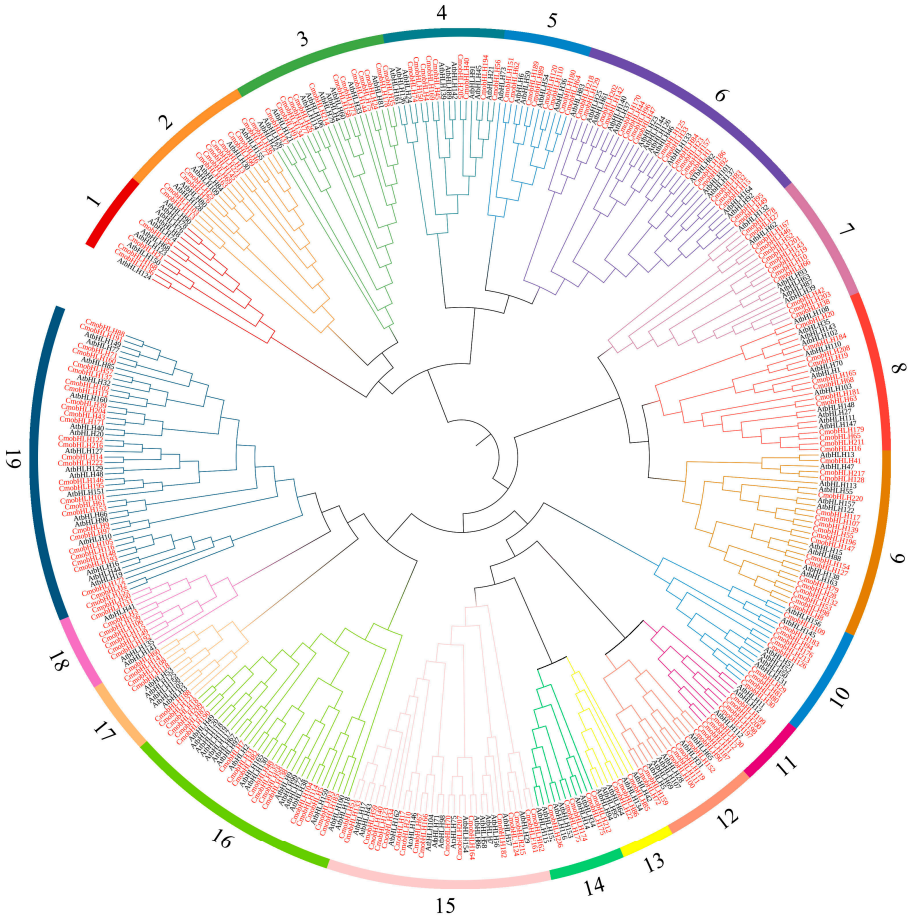

Figure 2. The phylogenetic tree of pumpkin and *Arabidopsis* bHLH proteins. Cmo: pumpkin, At: *Arabidopsis*. The number 1–19 indicates the different subfamily.

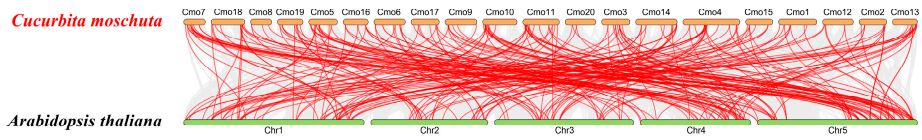

Figure 3. Synteny analysis of pumpkin and *Arabidopsis bHLH* genes. Identified collinear genes are lined by red lines.

3.3. Conserved Motifs and Gene Structures of CmobHLH Genes

The MEME online program was employed to indicate the conserved motifs in CmobHLH proteins. CmobHLH proteins possessed different number of conserved motifs, ranging from 1–9. CmobHLH proteins from the same subfamily had a common motif composition (Figure 4B). For instance, the CmobHLH proteins in subfamilies 7, 8, 9, 10, 11 and 18 shared motifs 1, 2, 3 and 4, the CmobHLH proteins in subfamily 4 contained motifs 1 and 2, and the CmobHLH proteins in subfamilies 12, 17 and 19 involved motifs 1, 2, 3 and 5. Furthermore, we found that some of the motifs were only distributed in the specific position of the protein sequences. For example, motif 3 was always distributed at the start of the protein sequences in subfamilies 2, 6, 7, 9, 10, 11, 13, 15, 16 and 18, and motif 5 was almost always distributed at the start of the protein sequences in subfamilies 12, 17 and 19. While motif 4 was almost always distributed at the end of the protein sequences in subfamilies 7, 8, 9, 10, 11, 13, 14, 15 and 18, motif 7 was distributed at the end of the protein sequences in the subfamily 2. In addition, the exon–intron structures of *CmobHLH* genes were also investigated to obtain their gene structure characteristics using TBtools software (Figure 4C). The *CmobHLH* family members had a varied number of exons from 1 to 34. Among them, 18 *CmobHLH*

family members only contained 1 exon, whereas *CmobHLH147* had the large number of exons with 34. However, the *CmobHLH* genes in the same subfamily had a comparable number of exons and introns.

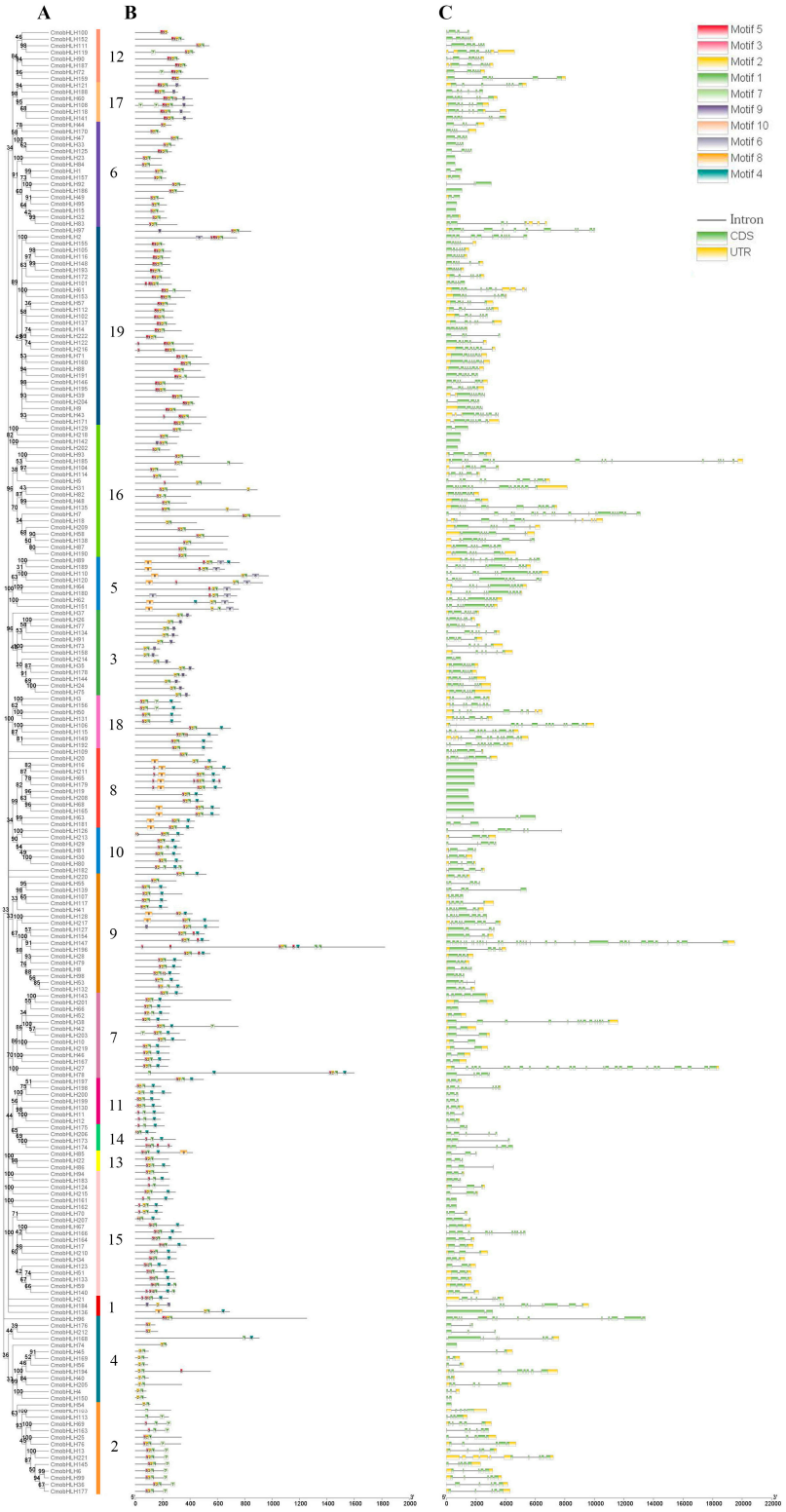

Figure 4. Conserved motifs and gene structures of CmobHLH proteins. (**A**) Phylogenetic tree. Different number indicates the different subfamily. (**B**) Conserved motifs. (**C**) Gene structures.

3.4. GO Enrichment and CREs Analysis

To determine the specific functions of *CmobHLH* genes, 222 *CmobHLH* genes were annotated with GO enrichment. The findings revealed that the 222 *CmobHLH* genes were mainly enriched in three categories (Supplementary Table S3). According to the biological process, they were predicted to be involved in the metabolic process, biosynthetic process, gene expression and other processes. Additionally, the majority of *CmobHLH* genes were annotated to biosynthetic process (146/222). In the cellular component, most of the *CmobHLH* genes were assigned to the cellular component (174/222). *CmobHLH* genes were also assigned to the protein complex (40/222), transcription factor complex (39/222) and nucleus (38/222). In the molecular function, most of *CmobHLH* genes were involved in binding (222/222), protein binding (221/222) and protein dimerization activity (220/222). A part of *CmobHLH* genes were involved in nucleic acid binding transcription factor activity (39/222), transcription factor activity, sequence-specific DNA binding (39/222) and DNA binding (35/222). A few *CmobHLH* genes were involved in cytoplasm amino acid binding, carboxylic acid binding and organic acid binding.

To further investigate the potential functions of *CmobHLH* genes, the CREs in the promoter sequence of each *CmobHLH* gene were predicted. As shown in Supplementary Table S4, it was discovered that 222 *CmobHLH* genes contained a plethora of light response elements, such as Box 4, G-box, GT1-motif, I-box and MRE. Plant growth and development CREs were also identified, including CAT-box, CCGTCC-box, GCN4_motif, O2-site and circadian, etc. CREs related to biotic and abiotic stress elements, including ARE, WUN-motif, LTR, STRE and MBS were widely present in the promoter region. Furthermore, phytohormone response elements, such as ABRE, TGA-element, P-box, CGTCA-motif and ERE were found. The different types of CREs presenting in *CmobHLH* genes indicated functional diversity and complexity.

3.5. Protein–Protein Interaction Networks of CmobHLH Proteins

In this study, most CmobHLH proteins were predicted to have significant interactions (Figure 5). For instance, SPCH (ortholog of CmobHLH207), MUTE (ortholog of CmobHLH70) and FMA (ortholog of CmobHLH164) in coordination with ICE1 (ortholog of CmobHLH127/154/196) could be associated with stomatal differentiation [53]. FBH4 (ortholog of CmobHLH90/111/152/187) and AT5G50915 (ortholog of CmobHLH101/146/195) were involved in the regulation of flowering periods [54]. HEC1 (ortholog of CmobHLH15), HEC3 (ortholog of CmobHLH32/83) and HEC2 (ortholog of CmobHLH49/95) interacted with SPT (ortholog of CmobHLH48) to jointly regulate pistil development [55]. BEE1 (ortholog of CmobHLH148) and CES (ortholog of CmobHLH155) regulated the biosynthesis of BR [56,57]. TT8 (ortholog of CmobHLH20) interacted with TTG1 and TT2 (MYB) to regulate plant growth and development [58,59]. LRL1 (ortholog of CmobHLH60/108/118/141), RSL2 (ortholog of CmobHLH33/47/125), GL3 (ortholog of CmobHLH184) and EGL3 had antagonistic functions in controlling root hair development. ILR3 (ortholog of CmobHLH6/13/36/99/145/117/221) and PYE (ortholog of CmobHLH103/113) were tightly connected to plant response to Fe deficiency [60]. PIF7 (ortholog of CmobHLH5/104/114) and PIF3 (ortholog of CmobHLH58/138) were phytochrome interacting factors [57]. AMS (ortholog of CmobHLH41/128/217) and DYT1 could regulate the development of tapetum [57]. Therefore, *CmobHLH* genes might be indispensable to regulate the development and stress response in pumpkin, and different genes have different functions.

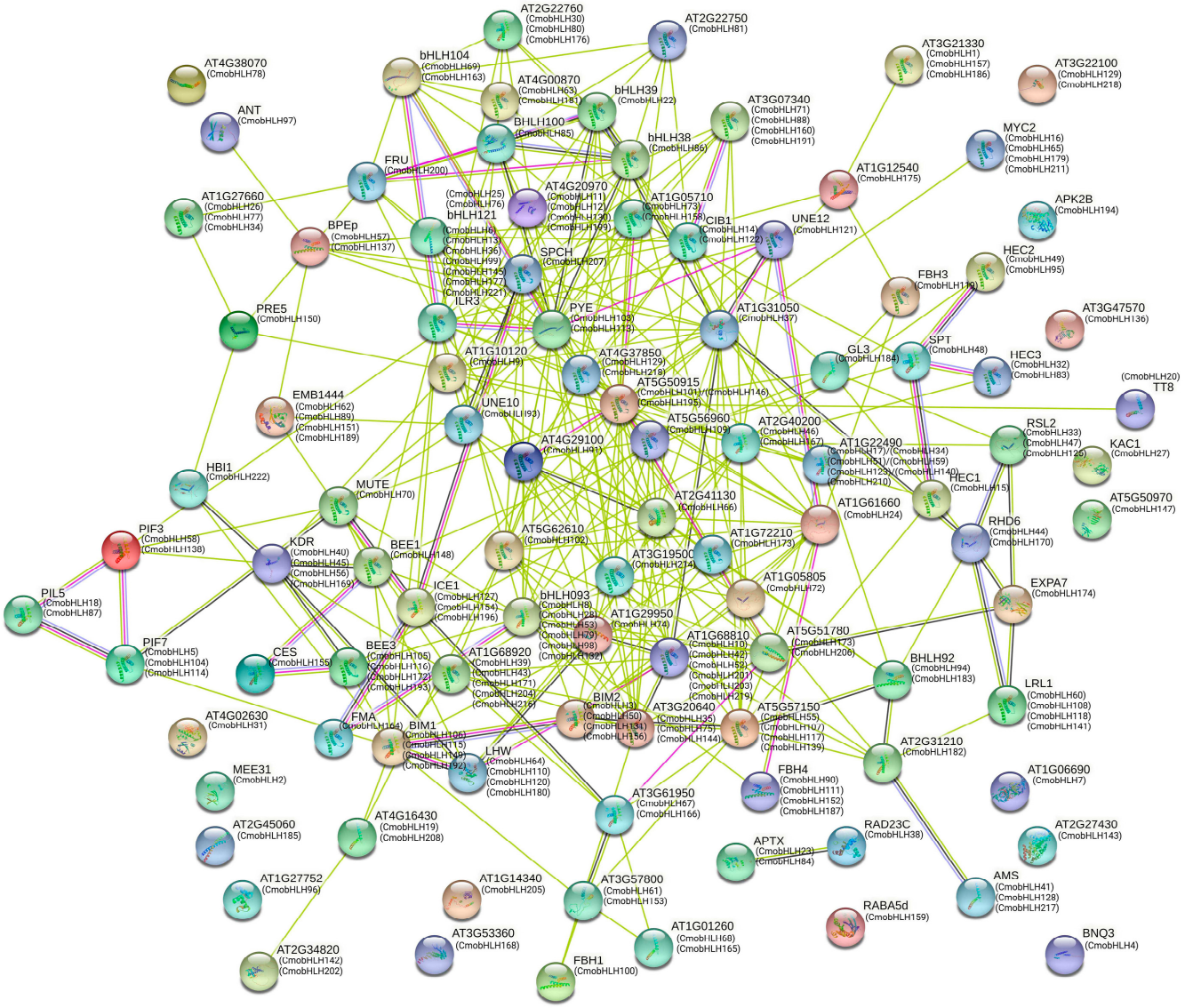

Figure 5. Interaction networks of CmobHLH proteins.

3.6. Tissue Expression of CmobHLH Genes

We used RNA-seq data to examine the transcriptional levels of *CmobHLH* genes in the roots, stems, leaves and fruits to ascertain their roles (Figure 6). In the four tissues studied, 222 *CmobHLH* genes were expressed at varying abundance levels. Three obvious transcriptional clusters were shown, with the higher abundance in cluster I and the lower in cluster III. Furthermore, we found that the expression levels of *CmobHLH* genes in cluster I were generally high in the four tissues, while *CmobHLH55* and *CmobHLH179* were the highest in the fruits. A majority of *CmobHLH* genes in cluster II exhibited higher expression in the stems and roots. However, the expression levels of *CmobHLHs* in cluster III were generally lower in all four tissues. The various expression profiles of *CmobHLH* genes in different tissues suggested the different functions in the growth and development of pumpkin.

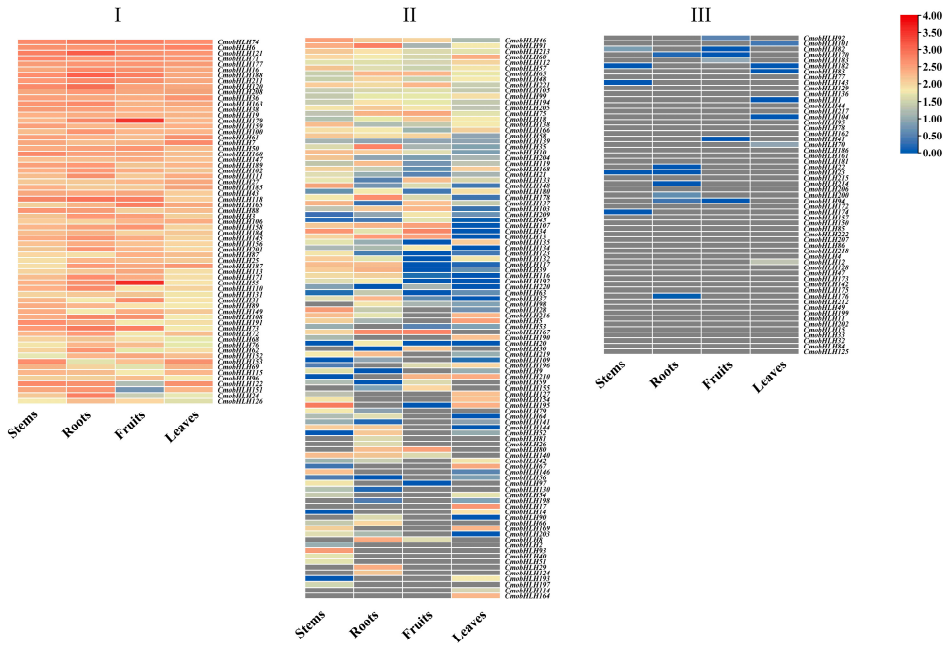

Figure 6. Transcriptional levels of *CmobHLH* genes in the different tissues. I, higher expression abundance; II, lower expression abundance; III, almost no expression.

3.7. Expression Analysis of CmobHLH Genes under Abiotic Stresses

CREs analysis indicated that *CmobHLH* genes could be associated with stress response. To further explore possible functions under abiotic stresses, expression profiles of five selected *CmobHLH* genes were detected with the treatments of salinity, waterlogging, cold, ABA and drought. As shown in Figure 7, transcriptional levels of all five *CmobHLH* genes were up-regulated after 24 h of 75 mmol/L NaCl stress compared with the normal condition. Furthermore, the expression profiles of *CmobHLH* genes under waterlogging, cold, ABA and drought stresses were analyzed using qRT-PCR. The results demonstrated that five *CmobHLH* genes exhibited a significantly up-regulated expression under cold, ABA and drought stresses after 10 days, in contrast with the normal condition (Figure 8). However, *CmobHLH25*, *CmobHLH46*, *CmobHLH81* and *CmobHLH167* showed higher expression levels by 1.64-, 1.82-, 1.35-, 1.17-fold under waterlogging stress, respectively, while the expression of *CmobHLH98* was down-regulated. From the above results, we learned that the five selected *CmobHLH* genes could respond to NaCl, waterlogging, cold, ABA and drought stress in pumpkin.

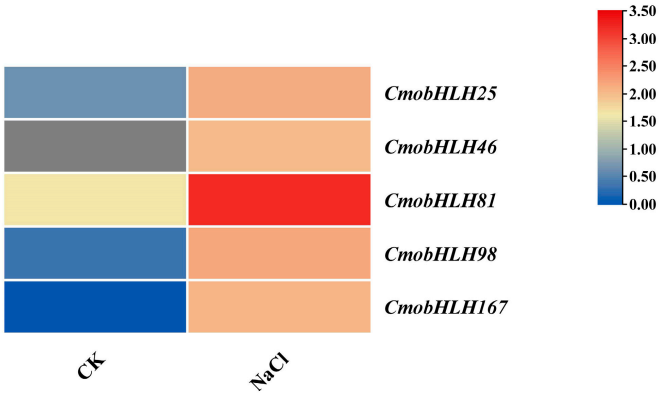

Figure 7. Transcriptional profiles of *CmobHLH* genes under NaCl stress. The red color indicates higher expression; the blue color indicates lower expression.

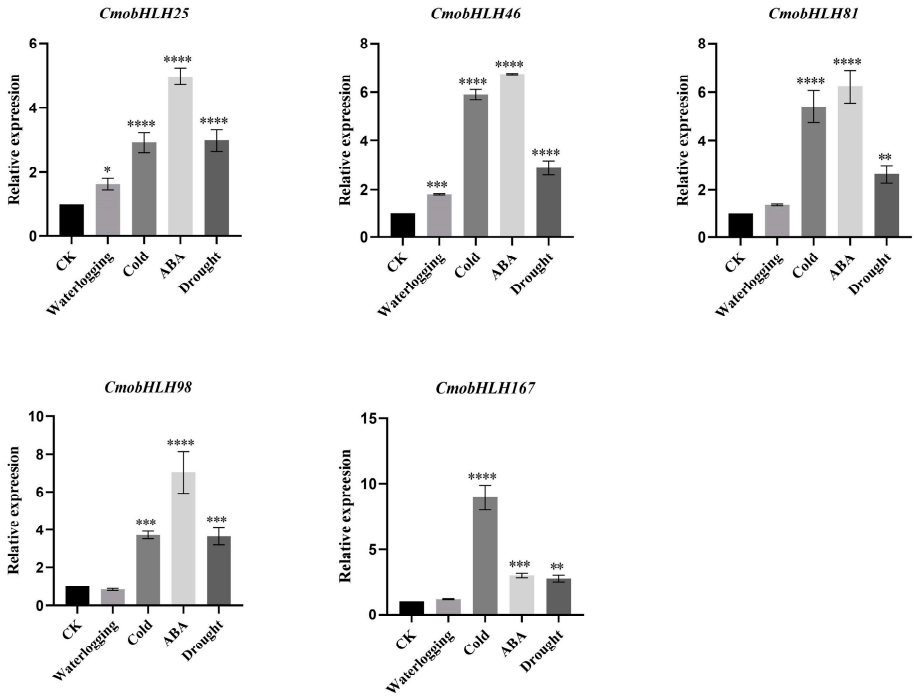

Figure 8. Expression levels of *CmobHLH* genes in response to waterlogging, cold, ABA and drought stresses after 10 days. The error bars are denoted as the means ± SEs. Asterisks indicate significant differences at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

4. Discussion

bHLH TFs have been identified and characterized in many plants. However, information about *bHLH* genes in resistance to abiotic stresses in pumpkin is missing. In this study, we identified and characterized 222 *CmobHLH* genes in pumpkin, which were further divided into 19 subfamilies based on their alignment with AtbHLH proteins. Furthermore, CmobHLH proteins within the same subfamily were discovered to have comparable motif composition and gene structures, highlighting the closer evolutionary relationship of CmobHLH proteins in the same subfamily. Ke et al. [61] reported that genes from the same subfamily shared the common evolutionary origin and similar physiological functions.

The CREs analysis in the promoter regions offers a theoretical foundation for investigating the physiological functions of *CmobHLH* genes. *bHLH* genes have been reported to participate in phytohormone signal crosstalk, which plays crucial roles in plant development and response to various abiotic stresses [62–67]. Previous studies have also shown the expression profiles of *bHLH* genes in response to salicylic acid (SA), 6-benzylaminopurine (6-BA) [68] and jasmonic acid (JA) [69]. In this study, there were a series of CREs in the promoters of *CmobHLH* genes, including light, plant growth and development, abiotic stresses and plant phytohormones, suggesting that these genes serve as regulators that are widely involved in growth and development, as well as various stress responses in pumpkin. In addition, GO annotation and protein–protein interaction networks also indicated the important roles of *CmobHLH* genes in pumpkin development and stress response.

Transcriptome data in the different tissues, as well as protein–protein interaction networks analysis, might aid in predicting the potential roles of the studied genes. In *Prunus mume*, many *PmbHLH* genes were specifically expressed in the roots, implying that they might be connected to root development [57]. In fig, a large number of *FcbHLH* genes exhibited widespread expression in the fruits, indicating that *FcbHLH* genes play a significant role in fig fruit development [70]. In this current study, 222 *CmobHLH* genes were divided into three clusters according to their abundance levels, and the expression profiles of *CmobHLH* genes varied in different tissues. For example, *CmobHLH55* and *CmobHLH179* were the highest in the fruits, and the majority of *CmobHLH* genes in cluster

II exhibited higher expression in the stems and roots, which suggested the unique functions of *CmobHLH* genes in pumpkin development. Additionally, *bHLH* TFs have also been discovered to play an essential role in the process of plant response to abiotic stresses [71]. For instance, in grass pea, most of *LsbHLH* genes showed higher expression levels under 75 mM NaCl treatment [17]. In cucumber, the expression profile of *CsbHLH032* was down-regulated after 4 °C treatment [72]. In *Hibiscus hamabo*, *HhbHLH20* displayed significantly lower expression in response to ABA stress [73]. In Mongolian oak, the expression levels of *QmbHLH81* and *QmbHLH30* first increased significantly and then decreased remarkably with PEG 6000 treatment [74]. In sorghum, *SbbHLH045* showed significant up-regulation under flooding stress [75]. This current study showed that *CmobHLH25*, *CmobHLH46*, *CmobHLH81*, *CmobHLH98* and *CmobHLH167* exhibited an increased expression after 24 h of NaCl stress and 10 days of cold, ABA and drought, indicating that *CmobHLH* genes might play the vital roles in NaCl, cold, ABA and drought stresses. Additionally, *CmobHLH25* and *CmobHLH46* were significantly expressed after 10 days of waterlogging stress, indicating an opposite pattern for *CmobHLH25* and *CmobHLH46* in response to waterlogging stress. Our results were aligned with previous reports on *Andrographis paniculate* [68], banana [76] and foxtail millet [77]. In summary, *CmobHLH* genes might mediate the stress response of pumpkin to NaCl, cold, ABA, drought and waterlogging, but the activities of individual *CmobHLH* genes were different with the different stress. Further research is required to comprehend the functions of *CmobHLH* genes through performing a gain-of-function or loss-of-function assay.

5. Conclusions

In general, we identified 222 *CmobHLH* genes in pumpkin. Furthermore, a systematic investigation was carried out, including chromosomal localization, protein properties, phylogenetic tree, synteny analysis, conserved motifs, gene structures, GO enrichment, CREs and protein–protein interaction networks. Gene expression patterns in different tissues and response to abiotic stresses were examined in pumpkin. Our results present a systematic understanding of the characterization and demonstrated the potential roles in development and stress response in pumpkin, which would provide foundation for further studies on the functions and regulatory mechanisms of *CmobHLH* genes in specific environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9060648/s1, Table S1: Primer sequences for *CmobHLHs*; Table S2: Gene ID, and physical and chemical properties of CmobHLHs; Table S3: GO annotation of *CmobHLH* genes; Table S4: The distribution of *cis*-regulatory elements in *CmobHLH* promoter sequences.

Author Contributions: Y.S. conceived and designed the experiments. W.A. carried out the experiments. W.A., W.L., W.X., X.W. and J.L. analyzed the data, prepared figures and tables. W.A. wrote the manuscript. Y.S. reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Research and Development Program of Henan Province (No. 232102110177).

Data Availability Statement: The data are available by contacting the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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