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Genome-Wide Identification of the *SmHD-zip* Genes That Respond to Multiple Ripening-Related Signals in Eggplant Fruit

Caiqian Jiang †, Yunrong Mo †, Haoran Zhang, Kaiyun Chen, Ying Zhou, Zushuai Ma, Yuhao Jing, Yu Liu, Yanyan Wang * and Kai Zhao *

Key Laboratory of Vegetable Biology of Yunnan Province, College of Landscape and Horticulture, Yunnan Agricultural University, Kunming 650201, China; jcq0320@163.com (C.J.); moyunrong@126.com (Y.M.); zhr3081@163.com (H.Z.); chenkaiyun2025@163.com (K.C.); zhouyingg2025@163.com (Y.Z.); mazushuai@126.com (Z.M.); jyhjyhjyh2022@163.com (Y.J.); liuyu19387074749@163.com (Y.L.)

* Correspondence: yabofei1212@163.com (Y.W.); 2013035@ynau.edu.cn (K.Z.)

† These authors contributed equally to this work.

Abstract: The homeodomain–leucine zipper (*HD-zip*) gene family plays a crucial role in plant development and stress responses. However, systematic identification studies of this gene family in eggplant are still lacking. In this study, we systematically identified 44 *HD-zip* genes in the eggplant genome database using bioinformatics methods and analyzed their expression levels under light and multiple hormones by RT-qPCR. The results show that members of the *SmHD-zip* gene family were classified into four groups (HD-zip I, II, III, and IV) based on the phylogenetic relationship. *Cis*-acting elements related to plant development, hormones, and stress were identified in the promoter regions of the *SmHD-zip* gene family. Furthermore, the expression of the *SmHDZ2* gene was upregulated during the fruit development stage, while nine *SmHD-zip* genes exhibited downregulated expression patterns. Notably, some *SmHD-zip* genes were identified as key regulators of eggplant responses to light and multiple hormone signals. Overall, these findings not only provide valuable insights into the evolutionary and functional characteristics of eggplant HD-Zips but also suggest that *HD-zip* genes likely play a significant role in regulating fruit development and ripening by integrating light and multiple hormone signaling pathways. Therefore, this study laid the foundation for further research on eggplant quality.

Keywords: *Solanum melongena* L.; HD-zip; fruit ripening; light; hormones

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1. Introduction

Transcription factors (TFs) generally play an important role in the regulation of gene expression in plants. They are a class of proteins that interact with specific DNA sequences in the promoter region of target genes to regulate mRNA expression and subsequent protein synthesis in eukaryotic organisms [1–3]. The homeodomain–leucine zipper (HD-zip) gene family is plant-specific and contains a homeodomain (HD) and a leucine zipper (LZ) domain; HD is responsible for specific binding to target DNA, while LZ mediates the formation of functional protein dimers [4–7]. The *HD-zip* gene family can be divided into four subfamilies based on their structural characteristics and functional properties (HD-zip I, II, III, and IV) [8]. HD-zip subfamilies I and II have a simple structure, containing only HD and LZ domains, and are involved in abiotic stress responses [9,10]. Compared with subfamily I, HD-zip subfamily II also has an additional N-terminal conserved region and a unique CPSCE (Cys-Pro-Ser-Cys-Glu) motif, which is mainly involved in the regulation of light signals [11]. In contrast, HD-zip subfamily III contains more SAD, START, and

MEKHLA domains than subfamily I and is involved in the regulation of vascular bundle and meristem development [8,12]. Additionally, the role of HD-zip subfamily III including leaf polarity and meristem size is crucial, which contributes to the interdependence between meristem and developing leaves [13]. The structure of HD-zip subfamily IV is similar to that of subfamily III but lacks a MEKHLA domain, which is involved in the regulation of plant epidermal differentiation [6]. The class III HD-ZIP protein directly binds the consensus sequence GTAAT [G/C] ATTAC, while members of the HD-zip IV family recognize the sequence TAAATG [C/T] A [14,15].

HD-zip genes have been identified in *Arabidopsis thaliana* (48 members) and rice (*Oryza sativa* L., 49 members) [8]. Furthermore, members of the *HD-zip* gene family have been identified in other plants through genome-wide analysis, including grape (*Vitis vinifera* L.) [16], potato (*Solanum tuberosum* L.) [1], and sesame (*Sesamum indicum* L.) [17]. Previous studies have reported that HD-zip proteins regulate plant growth and development, including fruit development, maturation, anthocyanin accumulation, flowering, vascular tissue development, and epidermal cell development [18]. Recent reports reveal that HD-ZIP proteins regulate fruit ripening by regulating cell wall degradation and ethylene biosynthesis. For example, in banana (*Musa acuminata* L.), the *HD-ZIP I* genes *MaHDZI.19* and *MaHDZI.26* and the *HD-ZIP II* genes *MaHDZII.4* and *MaHDZII.7* are significantly upregulated during fruit ripening [19]. Additionally, these four genes can also activate several maturation-related genes, including *MaACO5* (ethylene biosynthesis) and *MaEXP2*, *MaEXPA10*, *MaPG4*, and *MaPL4* (cell wall degradation) [19]. Similarly, in litchi (*Litchi chinensis* L.), *LcHB2* (HD-ZIP I subfamily) directly activates cell wall degradation-related genes (*LcCEL2* and *LcCEL8*) to regulate fruit drop, while *LcHB3* regulates fruit drop by promoting ethylene biosynthesis [20]. In addition to the HD-I family, the HD-II family gene *PpHB.G7* promotes fruit ripening by increasing ethylene content in peach (*Prunus persica* L.) [21]. Although HD-zip is involved in regulating fruit ripening in a variety of crops, it has rarely been reported in eggplant.

Eggplant (*Solanum melongena* L.), an important economic crop of solanaceous fruit, is widely cultivated because of its nutrient-rich fruits. As a member of the Solanaceae family, eggplant quality significantly impacts human health [22]. So far, the rapid development of high-throughput sequencing technology for Solanaceae crops has led to the publication of various eggplant genome data [23], facilitating bioinformatics analysis to explore the whole-genome function of eggplant. Increasingly, transcription factor families, including WRKY [24], GATA [25], and AP2-ERF [26], have been identified in eggplant. However, no systematic studies about the HD-zip TF family have been attempted on eggplant.

Our study aims to analyze the basic information and screen the key *SmHD-Zip* genes involved in the regulation of fruit ripening in eggplant. Therefore, we comprehensively identified the *HD-zip* gene family in the eggplant genome and systematically analyzed their gene structures, motif compositions, chromosomal distributions, phylogenetic relationships, and evolution patterns. Additionally, we conducted cis-element analysis and examined the expression patterns of *HD-zip* genes in various tissues and under multiple ripening-related signals. Our findings provide valuable insights into the potential functions of *HD-zip* genes in eggplant.

2. Results

2.1. Identification of SmHD-zip Genes in Solanum melongena

After removing redundant sequences, 44 putative *SmHD-zip* genes were identified in eggplant and named SmHDZ1-SmHDZ44 according to their locations in the reference genome (Figure S1). Detailed information of the *SmHD-zip* genes is listed in Table 1, including gene name, protein length, chromosome location, molecular weight, theoretical isoelectric point, aliphatic index, and the Grand Average of Hydropathicity (GRAVY) value.

The 44 SmHD-zip proteins varied in molecular length and weight, ranging from 118 amino acids (SmHDZ5) to 1484 amino acids (SmHDZ34). SmHDZ5 had the lowest molecular weight (13.89 kDa), while SmHDZ34 had the highest (168.34 kDa), indicating a large range. Except for SmeHDZ4, SmeHDZ5, SmeHDZ8, SmeHDZ10, SmeHDZ12, and SmeHDZ16, the other proteins had isoelectric points between 4.72 and 6.87, indicating that most eggplant HD-zip proteins are acidic.

Table 1. The physiochemical characteristics of the *SmHD-zip* gene family in *Solanum melongena* L.

Name	Genome ID	Protein Length/aa	Chrom	Chr Start	Chr End	MW (Da)	pI	Aliphatic Index	GRAVY
SmHDZ1	Smechr0101299.1	257	Chr1	12,588,851	12,597,004	29,162.42	5.52	63.42	−0.833
SmHDZ2	Smechr0101591.1	826	Chr1	15,597,095	15,603,032	90,131.25	5.97	79.58	−0.322
SmHDZ3	Smechr0101697.1	282	Chr1	16,935,525	16,938,582	31,495.45	5.97	72.98	−0.743
SmHDZ4	Smechr0101915.1	380	Chr1	19,575,004	19,577,744	41,258.04	8.08	62.42	−0.637
SmHDZ5	Smechr0103648.1	118	Chr1	96,300,845	96,306,567	13,893.92	8.93	79.41	−0.887
SmHDZ6	Smechr0200129.1	907	Chr2	11,092,838	11,098,401	100,094.30	6.58	85.71	−0.226
SmHDZ7	Smechr0200761.1	201	Chr2	47,977,301	47,979,883	23,518.64	6.87	64.03	−0.989
SmHDZ8	Smechr0200863.1	241	Chr2	50,396,475	50,397,965	27,163.64	8.98	70.41	−0.820
SmHDZ9	Smechr0201080.1	255	Chr2	54,789,348	54,797,075	29,281.66	5.76	63.49	−0.922
SmHDZ10	Smechr0201659.1	177	Chr2	61,727,689	61,728,610	20,516.98	7.74	77.68	−0.876
SmHDZ11	Smechr0201902.1	726	Chr2	64,140,549	64,145,567	79,843.27	5.68	78.97	−0.363
SmHDZ12	Smechr0202551.1	261	Chr2	69,757,778	69,759,330	29,121.72	7.60	69.16	−0.731
SmHDZ13	Smechr0202951.1	305	Chr2	73,206,063	73,208,102	34,869.77	5.95	56.59	−0.970
SmHDZ14	Smechr0203037.1	776	Chr2	73,893,789	73,897,785	85,826.59	5.10	80.04	−0.459
SmHDZ15	Smechr0300196.1	728	Chr3	2,373,704	2,381,403	79,776.37	5.55	83.83	−0.305
SmHDZ16	Smechr0300320.1	167	Chr3	3,712,936	3,714,206	19,768.71	9.58	80.00	−0.854
SmHDZ17	Smechr0300325.1	224	Chr3	3,794,580	3,796,394	25,965.22	6.62	75.71	−0.776
SmHDZ18	Smechr0302263.1	216	Chr3	82,786,950	82,787,919	25,224.17	5.17	67.27	−0.992
SmHDZ19	Smechr0302477.1	666	Chr3	85,078,273	85,081,875	72,747.09	5.93	83.30	−0.316
SmHDZ20	Smechr0303487.1	773	Chr3	94,511,653	94,520,019	86,185.61	6.27	76.08	−0.475
SmHDZ21	Smechr0303520.1	782	Chr3	94,710,348	94,717,438	86,430.14	6.14	87.20	−0.120
SmHDZ22	Smechr0400235.1	544	Chr4	2,479,449	2,480,634	60,623.15	6.03	116.95	0.398
SmHDZ23	Smechr0400520.1	615	Chr4	6,580,098	6,588,250	68,910.76	6.13	77.54	−0.514
SmHDZ24	Smechr0401924.1	287	Chr4	72,800,591	72,802,605	32,958.41	5.16	67.25	−0.956
SmHDZ25	Smechr0402063.1	259	Chr4	74,835,105	74,836,211	29,803.59	8.89	79.42	−0.839
SmHDZ26	Smechr0402065.1	259	Chr4	74,847,989	74,849,667	29,636.33	8.76	79.42	−0.815
SmHDZ27	Smechr0500181.1	348	Chr5	2,131,446	2,134,561	39,782.86	5.00	62.79	−0.851
SmHDZ28	Smechr0501670.1	955	Chr5	58,518,203	58,527,247	104,964.19	6.56	89.76	−0.145
SmHDZ29	Smechr0601407.1	217	Chr6	68,139,079	68,140,392	25,398.24	5.25	62.90	−1.134
SmHDZ30	Smechr0601803.1	278	Chr6	76,285,853	76,288,445	31,697.12	8.49	65.61	−0.826
SmHDZ31	Smechr0602556.1	708	Chr6	84,297,656	84,301,539	78,026.79	6.27	85.49	−0.261
SmHDZ32	Smechr0700782.1	613	Chr7	33,240,754	33,245,920	67,709.46	4.90	87.93	−0.247
SmHDZ33	Smechr0702033.1	212	Chr7	98,824,686	98,825,578	24,263.76	9.28	69.01	−0.867
SmHDZ34	Smechr0800044.1	1484	Chr8	687,257	690,287	168,338.26	5.88	91.15	−0.313
SmHDZ35	Smechr0801392.1	789	Chr8	66,884,601	66,891,197	87,156.14	5.99	89.26	−0.109
SmHDZ36	Smechr0801916.1	819	Chr8	79,167,138	79,174,098	91,110.10	5.14	75.91	−0.403
SmHDZ37	Smechr0802130.1	277	Chr8	81,852,289	81,854,237	31,346.17	7.58	62.67	−0.909
SmHDZ38	Smechr0802554.1	210	Chr8	86,509,323	86,510,568	24,514.54	5.31	64.57	−0.951
SmHDZ39	Smechr0900151.1	490	Chr9	2,257,356	2,275,167	56,179.77	8.03	75.98	−0.483
SmHDZ40	Smechr1000249.1	754	Chr10	2,991,779	2,998,093	83,615.53	5.70	79.11	−0.383
SmHDZ41	Smechr1001559.1	257	Chr10	63,092,964	63,095,845	30,004.23	4.72	68.64	−0.972
SmHDZ42	Smechr1001580.1	123	Chr10	63,609,600	63,614,425	14,318.16	8.55	63.58	−1.166
SmHDZ43	Smechr1100234.1	394	Chr11	2,684,707	2,687,766	44,368.20	4.89	66.80	−0.722
SmHDZ44	Smechr1201882.1	835	Chr12	73,207,126	73,214,030	91,373.12	6.07	86.24	−0.120

The aliphatic index was above 56.59 (highest, 116.95), indicating low polar amino acid content and good solubility. Except for SmeHDZ22, the other proteins had negative hydrophilicity and hydrophobicity values, indicating that most eggplant HD-zip proteins are hydrophobic and highly stable (Table 1).

2.2. Characterization and Phylogenetic Reconstruction of the SmHD-zip Gene Family

Similar to findings in other plants, all 44 SmHD-zip proteins contain the homeodomain, which means that the conserved domain of HD-zip proteins in eggplant is the homeodomain (Figure S2). Notably, in addition to the highly conserved homeodomain, many other highly conserved domains exist, which may relate to the functions of HD-zip proteins

in eggplant. Phylogenetic tree analysis showed that eggplant HD-zip proteins were similar to those in *Arabidopsis thaliana* and tomato and were assigned to four subfamilies: I, II, III, and IV (Figure 1). Subfamily I has the most members (19), followed by subfamilies II and IV (10 each), while subfamily III has the fewest, including SmHDZ6, SmHDZ21, SmHDZ28, SmHDZ35, and SmHDZ44. Based on the phylogenetic tree branches, we identified orthologous genes among eggplant, Arabidopsis thaliana, and tomato. Since tomato and eggplant are both Solanum crops, almost all SmHD-zips have counterparts in tomato. Based on the functions of these homologous genes in *Arabidopsis thaliana* and tomato, we can provide a scientific basis for predicting the functions of corresponding genes in eggplant.

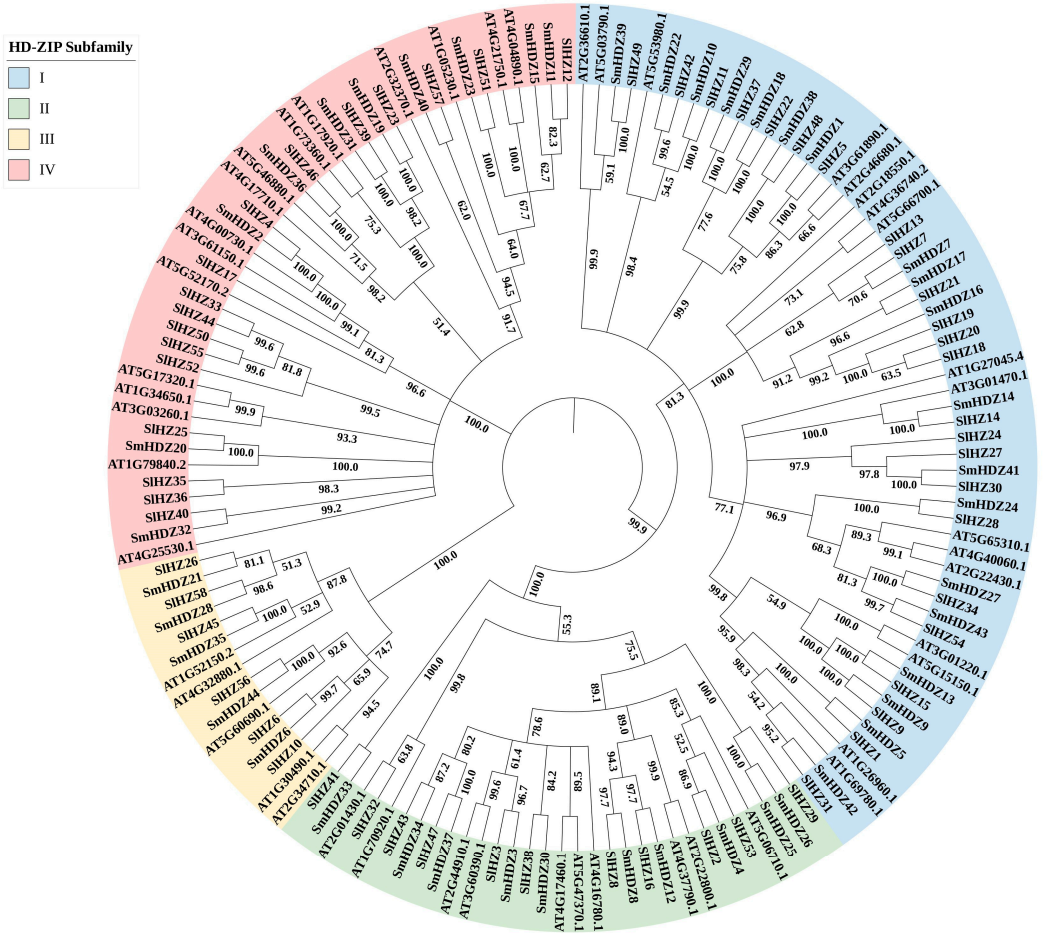

Figure 1. Phylogenetic tree of HD-zip proteins in eggplant, tomato, and *Arabidopsis*.

2.3. Gene Structure and Conserved Motif Composition Analysis of SmHD-zip Family

A total of 10 motifs were identified and visualized using the MEME suite website (Figure S3), of which motifs 1 and 2 were predicted by the website to correspond to the HD domain, and motif 5 to the LZ domain. All SmHD-zips contain the above three motifs (Figure 2), but for subfamilies I and II, subfamilies III and IV contain more motifs and their member proteins are significantly longer. Genetic structure analysis showed that the number of exons of 44 *SmHD-zip* genes ranged from 1 to 18, and there were great differences among the four subfamilies. The genetic structure of subfamilies I and II is relatively simple. In subfamily I, except for *SmHDZ10*, which has only 1 exon, *SmHDZ39* and *SmHDZ1* have 6 and 7 exons, respectively, and most of the exons are 2–4. The exons of 10 members of subfamily II are 3–4. The number of exons in subfamilies III and IV was more than 10, and the number of exons in subfamily III was the most, except for *SmHDZ21*, which had 17 exons, the other four subfamily members had 18 exons.

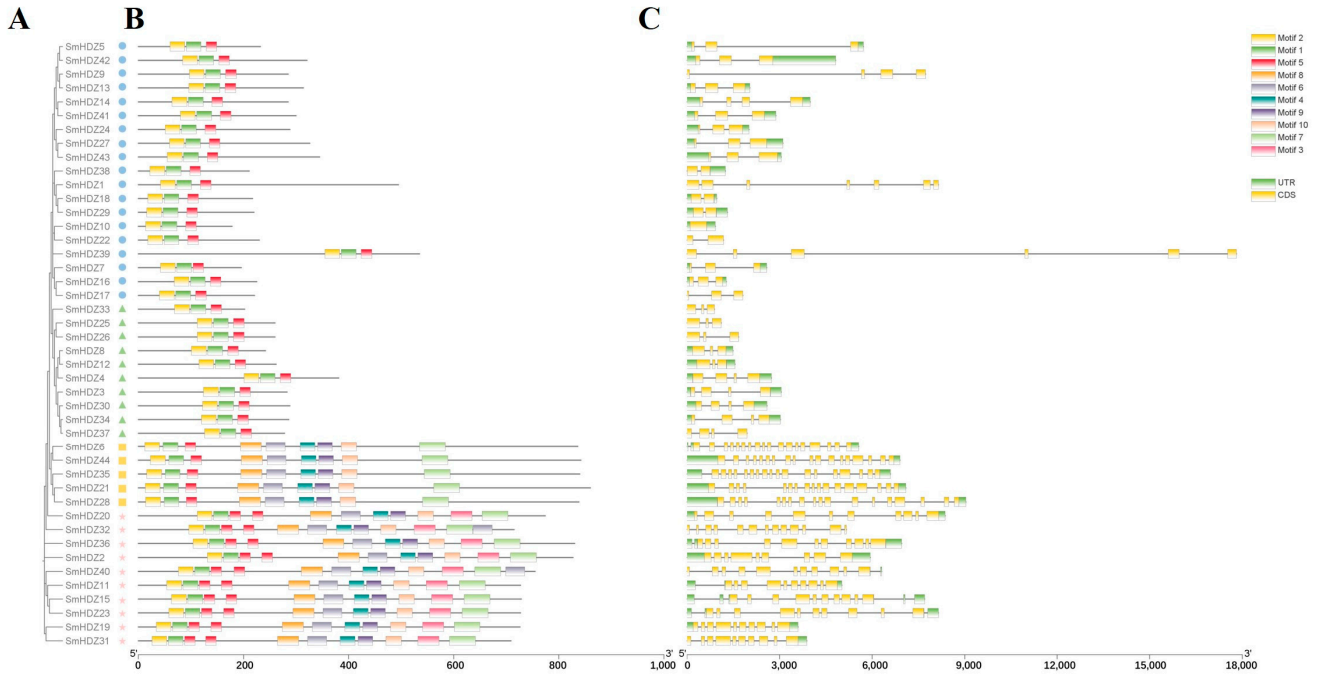

Figure 2. Motif architectures and UTR (untranslated region)-CDS (coding sequence or exons) structures of HD-zip genes in eggplant: (**A**) The phylogenetic relationships containing SmHD-zip proteins. The blue circle represents I, the green triangle represents II, the yellow rectangle represents III, and the pink five-pointed star represents IV. (**B**) The amino acid motifs architectures of SmHD-zips are displayed in ten colored boxes. (**C**) UTR-CDS structures of SmHD-zip genes. The green rectangle shows UTRs, and the yellow rectangle shows CDSs.

2.4. Cis-Acting Regulatory Elements Analysis in the Promoter Region of SmHD-zips

In our study, 26 elements were found of *SmHD-zip* genes in the promoter region, in addition to common elements such as the ATAT box, CAAT box, and protein binding sites, we selected 14 main *cis*-acting elements (Figure 3), including those responsive to light, hormones (MeJA (Methyl jasmonate), SA (Salicylic acid), ABA (Abscisic acid), GA (Gibberellic acid), IAA (Indole acetic acid)), tissue expression (meristem, endosperm), and abiotic stresses (drought, anaerobic conditions, low temperature, mechanical injury, defense, and stress). Among these, light-responsive elements were the most widely distributed and abundant, present in all members; abiotic stress-responsive elements were next, absent only in *SmHDZ40*; hormone-responsive elements were found in all 44 family members, while tissue-expressed elements, the least common, were found in only 19 members. In conclusion, *SmHD-zips* may be involved in eggplant growth and development, light signal regulation, abiotic stress responses, and hormone signal transduction.

2.5. Gene Duplication Events Analysis of SmHD-zips

In this study, we identified 23 eggplant *HD-zip* genes with syntenic genes, forming 17 homologous gene pairs, which may have similar functions (Figure 4). Additionally, seven eggplant *HD-zip* genes (*SmHDZ6*, *7*, *11*, *16*, *22*, *39*, and *41*) showed collinearity with genes outside the family. *SmHD-zips* were unevenly distributed across the 12 linked regions of the eggplant genome (EG), with the highest number in EG2, followed by EG3; EG12 had no duplicated genes.

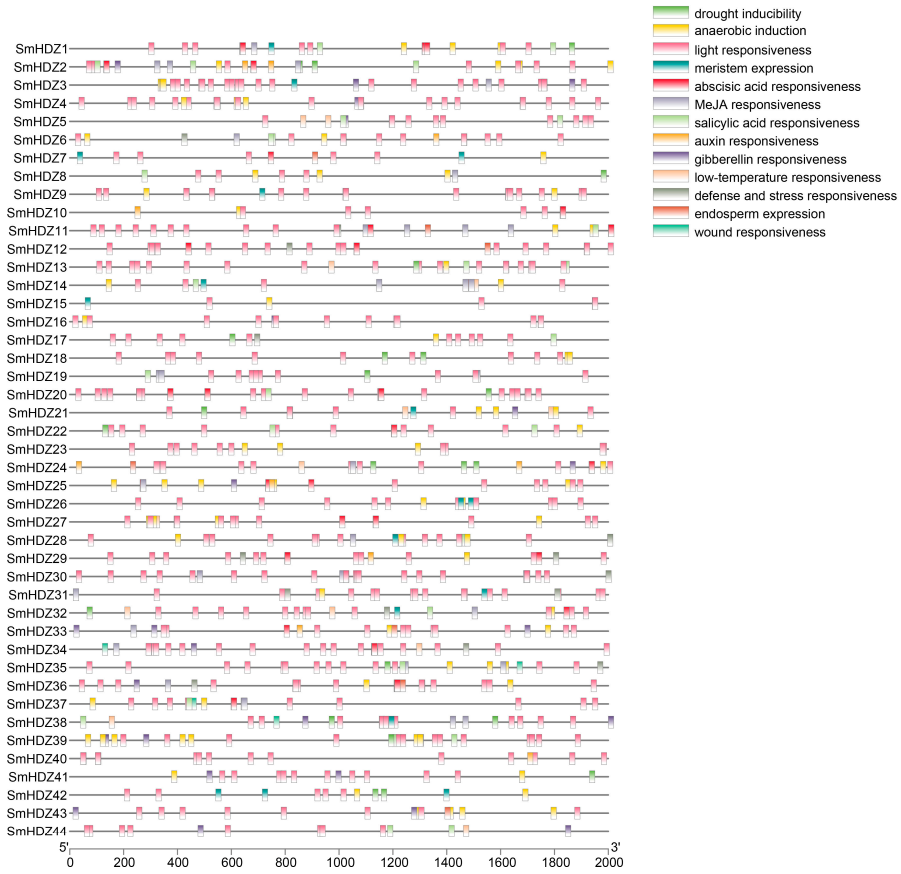

Figure 3. *Cis*-acting elements in the promoter regions of the *SmHD-zip* family.

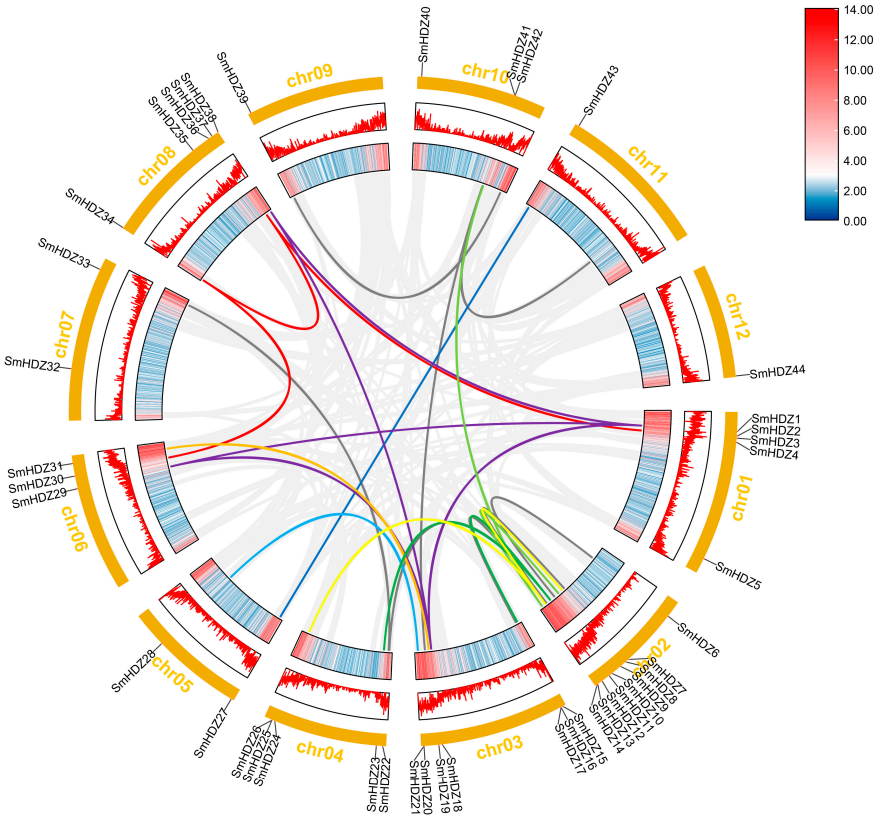

Figure 4. The collinearity of HD-zip genes in eggplant, with colored lines indicating duplicated HD-zip gene pairs. Chromosome numbers are displayed within each chromosome.

2.6. Expression Analysis of SmHD-zip Genes in Different Tissues and Fruit Ripening

Expression analysis via RT-qPCR was conducted in roots, stems, leaves, flowers, and fruits. As shown in Figure 5A, all 44 *SmHD-zip* genes were detected across all tested tissues (five-month-old eggplant), suggesting their universal role. Moreover, in fruit, 11 *SmHD-zip* genes (*SmHDZ1*, *7*, *9*, *32*, *33*, *37*, *38*, *39*, *41*, and *43*) showed low expression in other tissues. Furthermore, some *SmHD-zip* genes exhibited tissue-specific expression (Figure 5A). For example, *SmHDZ6*, *21*, *26*, *28*, *35*, and *44* displayed relatively high expression in roots and stems, but low expression in leaves and flowers. *SmHDZ11*, *15*, *19*, *24*, *29*, and *34* exhibited high expression in flowers but relatively low expression in other tissues. These findings indicate that *SmHD-zip* genes play differential roles in tissue development.

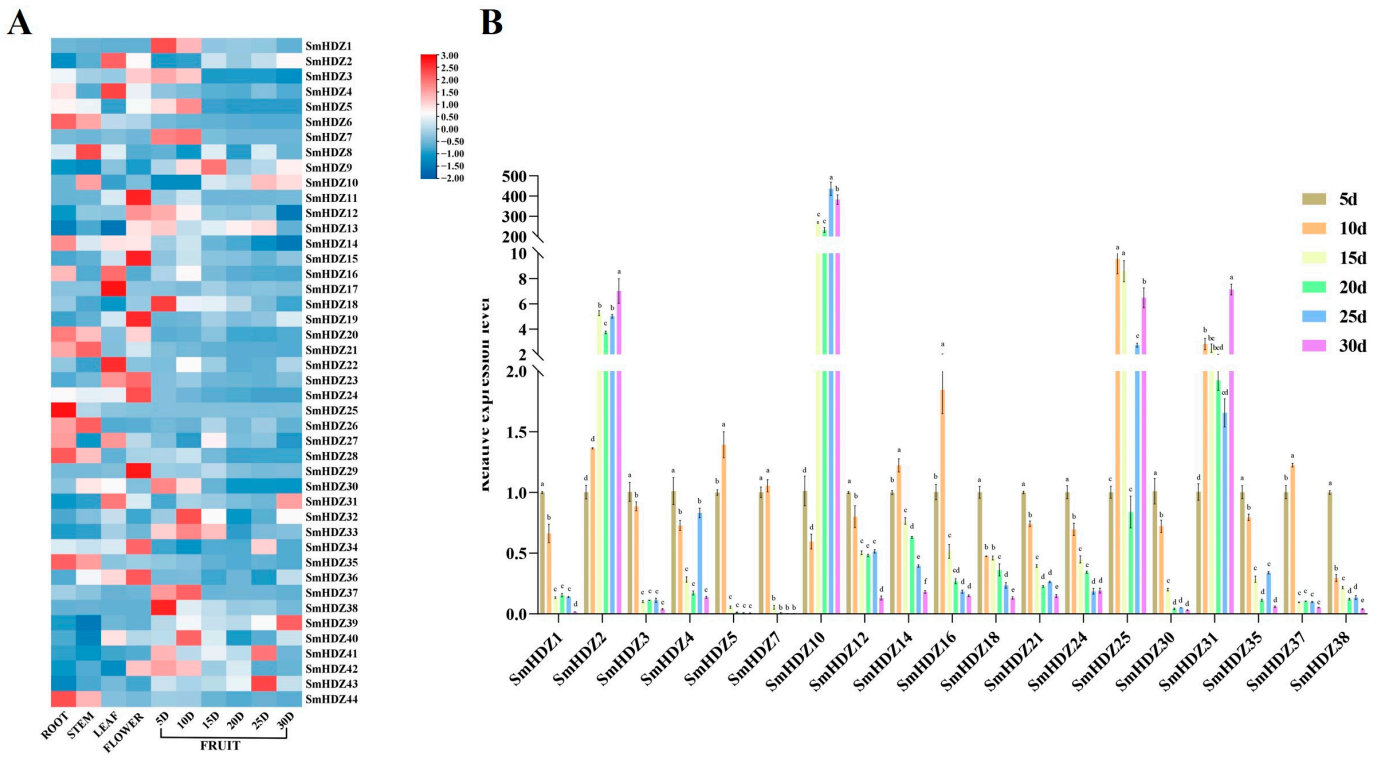

Figure 5. Expression profiles of the eggplant *SmHD-zip* genes across various eggplant tissues and fruit developmental: (**A**) Hierarchical clustering of the expression profiles of 44 *SmHD-zip* genes in different tissues and fruit developmental. (**B**) RT-qPCR analysis of 19 selected *SmHD-zip* genes during fruit development. *SmActin* was used for delta CT analysis to normalize template levels. The relative *SmHD-zip* family genes mRNA level is presented as the mean ± SD (n = 3). Different letters indicate significant differences between means as determined using ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

HD-zip proteins are transcription factors that play a crucial role in regulating growth and development. To clarify their roles in fruit development and maturation, we analyzed the expression patterns of 44 *SmHD-zip* genes during five distinct developmental stages of eggplant fruit (Figure 5). Different *SmHD-zip* genes exhibited unique expression patterns at various stages of eggplant fruit development. The expression of four genes (*SmHDZ5*, *14*, *16*, and *37*) first increased and then decreased, while SmHDZ2 was upregulated during fruit development (Figure 5B). Nine *SmHD-zip* genes (*SmHDZ1*, *3*, *7*, *18*, *21*, *24*, *30*, *37*, and *38*) showed downregulated expression patterns. These findings suggest that certain *SmHD-zip* genes may have multiple critical roles in the development of eggplant fruit.

2.7. Regulation of SmHD-zip Gene Expression Under Light Induction

Based on the differences in anthocyanin accumulation in response to light during eggplant fruit growth, fruits can be classified as photosensitive or non-photosensitive [27,28]. The signaling pathway regulating anthocyanin synthesis remains unclear. The HD-zip family promoter region contains numerous light-responsive elements (Figure 3). To analyze the potential role of *SmHD-zip* genes in anthocyanin biosynthesis in photosensitive eggplants, RT-qPCR was performed to assess their expression levels after bag removal. The expression levels of different genes varied significantly (Figure 6A). Figure 6B shows that 20 *SmHD-zip* genes were upregulated more than two-fold under light treatment. Notably, *SmHDZ15*, *SmHDZ16*, *SmHDZ21*, and *SmHDZ31* were upregulated nearly 20-fold after 6 days of light induction. These findings indicate that these *HD-zip* genes play crucial roles in light-induced anthocyanin biosynthesis in eggplants.

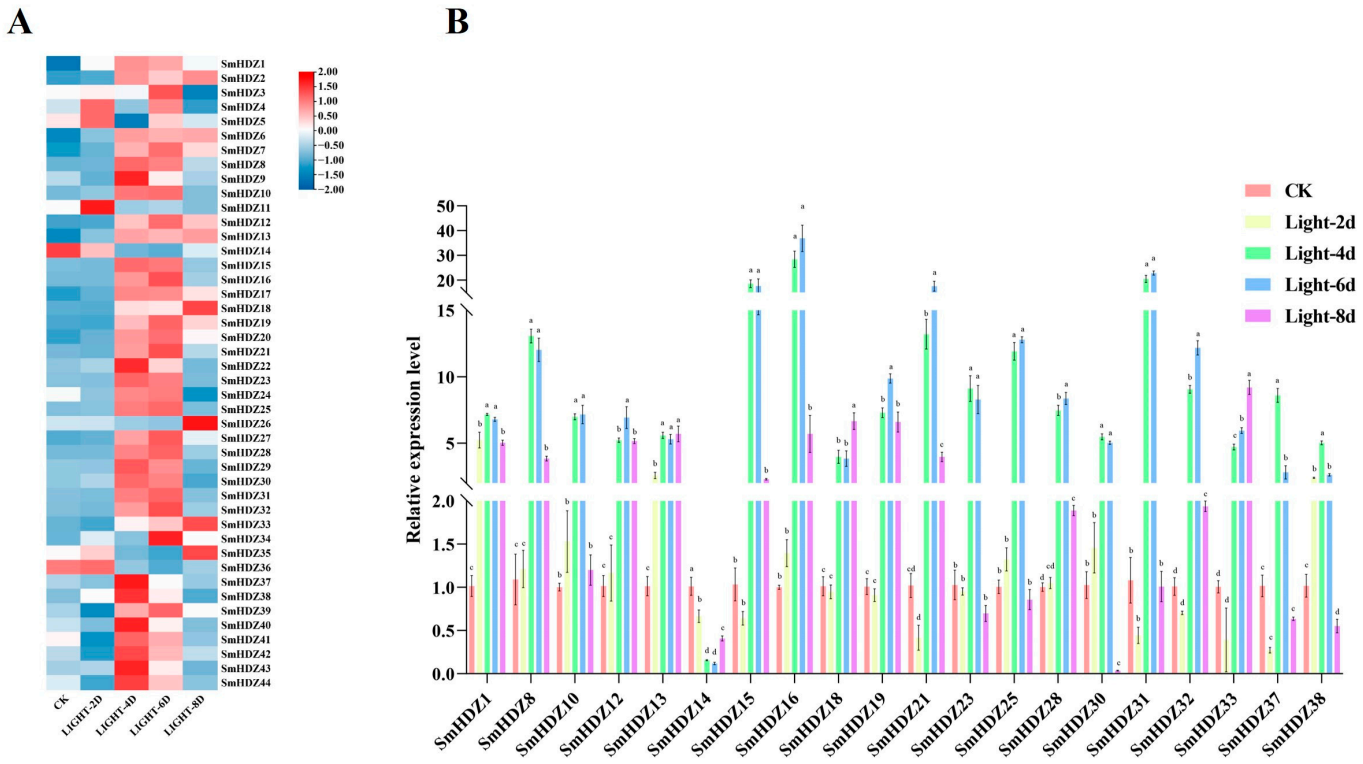

Figure 6. The expression levels of SmHD-zip genes in 'Zaohongqie 2' fruit under light treatment: (**A**) Hierarchical clustering of the expression levels of 44 SmHD-zip genes under light treatment. (**B**) RT-qPCR analysis of 20 selected *SmHD-zip* genes under light treatment. *SmActin* was used for delta CT analysis to normalize template levels. The relative *SmHD-zip* family genes mRNA level is presented as the mean ± SD (n = 3). Different letters indicate significant differences between means, determined by ANOVA followed by Tukey's multiple comparison test (p <0.05).

2.8. Expression Analysis of SmHD-zip Genes in Response to Hormone Treatments

Hormonal signals and environmental changes play vital roles in fruit development and ripening. To analyze the potential roles of the HDZ family in hormone signaling pathways, the expression levels of *SmHD-zip* genes in response to ABA, GA, and MeJA treatments were measured using RT-qPCR (Figure 7). As anticipated, the *SmHD-zip* genes exhibited varied expression patterns in response to different hormonal treatments. Most *SmHD-zip* genes were significantly downregulated under GA treatment. During MeJA treatment, 11 genes were significantly upregulated, indicating positive responsiveness, while 16 genes were significantly downregulated, indicating negative responsiveness to MeJA. The 44 *SmHD-zip* genes responded to ABA to various extents. Notably, *SmHDZ32* was significantly downreg-

ulated under GA treatment but significantly upregulated under MeJA and ABA treatments, suggesting it may play an active role in ABA- and MeJA-mediated fruit ripening. Overall, most *SmHD-zip* genes were sensitive to various hormone treatments.

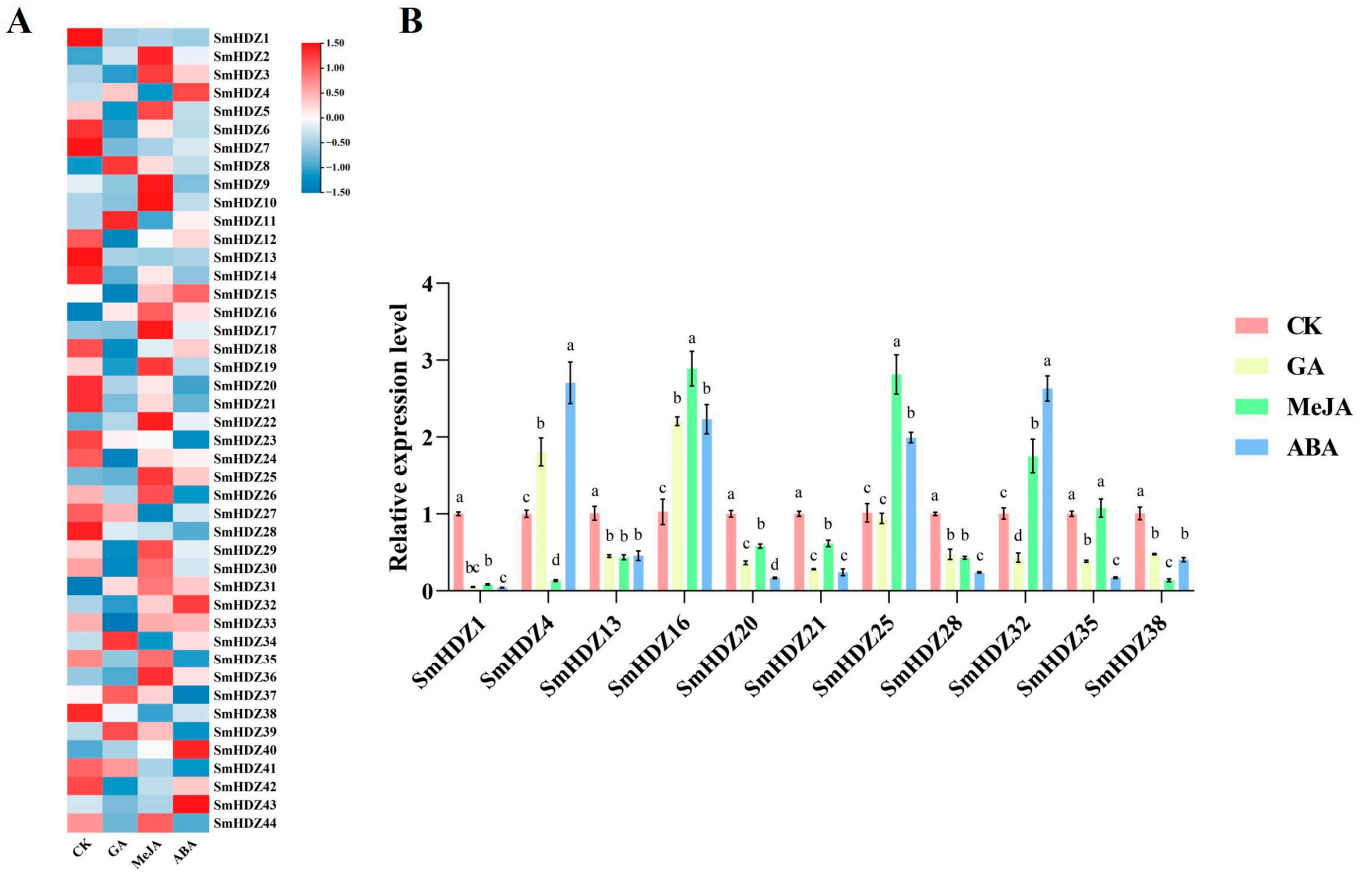

Figure 7. Expression patterns of eggplant *SmHD-zip* genes under ABA, GA, and MeJA treatments: (**A**) Hierarchical clustering of the expression patterns of 44 *SmHD-zip* genes following ABA, GA, and MeJA treatments. (**B**) RT-qPCR analysis of 11 selected *SmHD-zip* genes under hormone treatments. *SmActin* was used for delta CT analysis to normalize template levels. Relative SmHD-zip family genes mRNA level is presented as the mean ± SD (n = 3). Different letters indicate significant differences between means, determined by ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

3. Discussion

3.1. Evolutionary Analysis of Eggplant HD-zip Genes

HD-zip transcription factors, specific to plants, play a crucial role in regulating plant growth, development, light signaling, abiotic stress responses, and hormone signal transduction [17]. Previous studies have shown that HD-zip transcription factors are widely present in various plants. For example, there are 48 HD-zip family members in *Arabidopsis* [8], 40 in cucumber (*Cucumis sativus* L.) [29], 32 in barley (*Hordeum vulgare* L.) [30], and 58 in tomato (*Solanum lycopersicum*) [31] and poplar (*Populus trichocarpa*) [32]. In this study, 44 *SmHD-zip* genes were investigated through bioinformatics analyses. These discrepancies may result from differences in genome data size and complexity across different species. The 44 *HD-zip* genes were grouped into four subfamilies (I, II, III, and IV) (Figure 1), aligning with findings from most HD-zip family studies [8,31], which suggests the evolutionary stability of the HD-zip family. Among the four subfamilies, subfamily I includes 19 members, subfamily II has 10, subfamily III has 5, and subfamily IV has 10 members (Figure 1). The distribution of HD-zip subfamily III was similar to that in tomatoes and Arabidopsis, indicating its high conservation across

different species [8,31]. Based on exon and intron distribution (Figure 2), the genetic structure of the same subfamily in the eggplant HD-zip gene family was similar, with fewer members in subfamilies I and II and the most in subfamily III, consistent with findings in tomatoes [31]. According to the motif analysis, subfamilies I and II only contain motifs 1, 2, and 5, corresponding to the conserved HD and ZIP motifs, while subfamilies III and IV have more complex motif structures, consistent with previous studies [33,34]. Promoter function predictions revealed the presence of light response, hormone response, tissue expression, and abiotic stress response elements in the eggplant *HD-zip* gene family, consistent with previous studies, indicating the family's roles in plant growth, development, light signaling, abiotic stress responses, and hormone signal transduction. Through synteny analysis (Figure 4), we found that chromosome duplication fragments containing SmHD-zips were detectable on all chromosomes except chromosome 12, indicating that fragment duplication is the main driver of HDZ gene family amplification in eggplants, consistent with findings in melons and peppers [35,36].

3.2. Potential Role of SmHD-zip Genes in Fruit Development and Ripening of Eggplant

The development and maturation of eggplant is a complex physiological and biochemical process, affected by a variety of transcription factors and regulatory proteins, thereby affecting fruit quality [37]. We analyzed the expression profiles of *SmHD-zips* in various tissues, revealing their potential roles in regulating eggplant growth and development (Figure 5). Furthermore, our study showed that certain *SmHD-zip* genes exhibited high expression levels during the late stage of fruit development (Figure 5), suggesting potential roles in fruit ripening. The involvement of HD genes in fruit ripening regulation has been extensively studied in other species [38,39]. *AtANL2* is the first HD-zip IV gene found to be involved in tissue-specific anthocyanin accumulation [40]. In a recent study, *LeHB1*, a tomato HD-ZIP I gene, was shown to play key roles in carpel development and fruit maturation [38]. Similarly, *MdHB1*, the homolog of LeHB1, has been reported to be involved in regulating anthocyanin accumulation in apples [39]. Four HD-ZIPs (MaHDZI.19, MaHDZI.26, MaHDZII.4, and MaHDZII.7) are involved in banana fruit ripening by activating the transcription of genes related to ethylene biosynthesis and cell wall degradation [24]. Additionally, the tomato HD-zip I transcription factor VAHOX1 acts as a negative regulator of fruit ripening [41]. Here, the expression of *SmHDZ2* is significantly upregulated during fruit development (Figure 5), indicating its involvement in the ripening process in eggplants. Finally, nine *SmHD-zip* genes (*SmHDZ1*, *3*, *7*, *18*, *21*, *24*, *30*, *37*, and *38*) were downregulated during the late stage of fruit development, implying their potential roles as negative regulators of fruit ripening in eggplants.

3.3. Potential Role of SmHD-zip Genes in Mediating Response to Light and Multiple Hormones

Light significantly influences plant growth and development [42,43] (Jaakola, 2013), and HD-zip transcription factors may regulate photomorphogenesis [44]. For example, the HD-ZIP II transcription factor HOMEODOMAIN ARABIDOPSIS THALIANA1 (HAT1) modulates hypocotyl growth during photomorphogenesis and serves as a key node. Additionally, COP1 interacts with HAT1 and facilitates its degradation via ubiquitination in darkness [44]. Additionally, HAT1 restrains anthocyanin accumulation by inhibiting the MBW protein complex's activity [45]. *Arabidopsis thaliana* HomeoBox 1 (AtHB1), a homeodomain–leucine zipper I (HD-zip I) transcription factor, is regulated by phytochrome-interacting factors. Its apple homolog, MdHB1, has been shown to significantly inhibit anthocyanin accumulation in apple fruit [39]. Anthocyanin accumulation in photosensitive eggplants is strongly influenced by light. Our findings reveal that *SmHDZ2* is upregulated, whereas

nine *SmHD-zip* genes are downregulated, during light exposure, suggesting that these genes may contribute to light-induced anthocyanin biosynthesis in eggplants (Figure 6).

Eggplant, a non-climacteric fruit, relies on multiple hormones, including MeJA, for its development [46]. However, the precise functions of SmHD-zip proteins in hormone signaling pathways have yet to be fully elucidated. Recent data indicate that HD-zip family members directly regulate multiple hormone-signaling pathways in other plants [6]. For example, compared to wild-type plants, exogenous ABA treatment upregulated *ZmHDZ1* expression, increasing ABA sensitivity in overexpressing transgenic seedlings [47]. ABI1, a protein phosphatase, is a key component of ABA signal transduction, and ATHB6, an HD-ZIP I family gene that negatively regulates ABA signaling, is a target of the protein phosphatase ABA-insensitive 1 (ABI1) and acts downstream of ABI1 [48]. In loquat fruit, the HD-ZIP transcription factor EjHB1 is repressed by MeJA pretreatment [49]. JASMONATE ZIM-DOMAIN (JAZ) proteins, critical repressors of jasmonate signaling, interact with the HD-ZIP IV subfamily member CsGL2-LIKE to function [50]. In *Arabidopsis*, DELLA proteins, which negatively regulate GA signaling, directly interact with two HD-ZIP transcription factors (ATML1 and PDF2) to regulate GA signaling in the epidermis via the L1 Box cis-element [51]. RT-qPCR analysis demonstrated that *SmHD-zip* genes responded to various hormonal treatments (Figure 7). More than half of these genes were regulated by multiple hormones, suggesting their potential involvement in coordinating interactions among diverse hormonal signaling pathways at the physiological level.

4. Materials and Methods

4.1. Plant Growth Conditions, Light, and Multiple Hormone Treatments

The purple eggplant (cultivar 'Zaohongqie 2') was grown in an artificial climate chamber at 22–25 °C with a 16 h photoperiod. Fruit samples were collected at 5, 10, 15, 20, 25, and 30 days after flowering to study the expression characteristics of the *SmHD-zip* gene. Before flowering, fruit bagging was performed. At 25 days after flowering (25 DAF), bags were removed from the eggplant fruits, and samples were collected at 0, 2, 4, 6, and 8 days post-removal. For hormone treatments, fruits bagged for 25 days were unbagged and treated with water (control), 200 μM ABA, 200 μM GA, or 200 μM MeJA. Each treatment included three replicates, with three fruits per replicate. The fruits were rapidly frozen in liquid nitrogen and stored at −80 °C.

4.2. Genome-Wide Identification of SmHD-zip Genes in Eggplant

To identify HD-zip zip family genes genome-wide in eggplant, the amino acid sequences of 48 AtHD-zip proteins were collected from *Arabidopsis*, which were used as queries and searched against the eggplant genome database (http://www.eggplant-hq.cn/Eggplant/home/index, accessed on 25 February 2025) using the BLAST program (https://www.ncbi.nlm.nih.gov/, accessed on 25 February 2025). Then, the BLAST results were confirmed for the presence of the Hidden Markov Model (HMM) profiles of the HD and LZ domains using SMART. To investigate the characterization of HD-zip proteins in eggplant, we extracted and visualized their conserved domain sequences, and the Conserved Domain Database (CDD), a database of conserved domain alignments and models, was used to identify conserved domains within the candidate HD-zip protein sequences (https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml) (accessed on 10 December 2024). The information related to the basic physical and chemical properties of the SmHD-zip proteins was calculated using the ExPASy online tool (https://web.expasy.org/protparam/) (accessed on 2 December 2024).

4.3. Phylogenetic Tree Analysis and Gene Structure Analysis

To clarify the phylogenetic relationships of *HD-zip* genes in eggplant, we conducted a neighbor-joining (NJ) tree analysis using the full-length protein sequences of the identified 44 SmHD-zips, 48 AtHD-zips, and 58 SlHD-zips. The phylogenetic tree was constructed by MEGA11.0 [52]. Bootstrap analysis with 1000 replicates was performed, followed by phylogenetic tree visualization using iTOL v7 (https://itol.embl.de) (accessed on 5 January 2025). WebLogo (http://weblogo.berkeley.edu/logo.cgi) (accessed on 8 January 2025) was used to generate sequence logos of the conserved domains. Furthermore, the conserved motifs present in all *SmHD-zip* genes were identified using MEME (http://meme-suite.org/) (accessed on 2 January 2025). The UTR/CDS organization of the *SmHD-zip* genes was performed using the GSDS online tools (http://gsds.gao-lab.org/) (accessed on 2 January 2025).

4.4. Chromosome Duplication Events and Cis-Acting Elements Analysis

To explore the proposed regulatory functions of SmHD-zips, we retrieved the 2000 bp (upstream of the ATG) promoter regions of HD-zip genes to identify potential *cis*-acting elements. The *cis*-acting element was analyzed with PlantCARE following the method described previously [24]. To further analyze the evolutionary relationships among *SmHD-zip* genes in eggplant, we investigated the potential gene duplication events in the 44 *SmHD-zip* genes. For chromosome duplication events analysis, the 44 *SmHD-zip* genes were mapped by TBtools (https://github.com/CJ-Chen/TBtools/releases) (accessed on 12 January 2025) [53].

4.5. Quantitative Real-Time RT-PCR

Total RNA was extracted, and cDNA was synthesized, as described previously [24]. All primers of *SmHD-zip* genes for RT-qPCR in this study are listed in Supplementary Table S1. Quantitative real-time RT-PCR was performed on the ABI 7500 system, SYBR Taq, which was purchased from Yeasen Biotechnology (Shanghai, China) Co., Ltd. All RT-PCR experiments included three biological replicates. *SmActin* was used for delta CT analysis to normalize template levels. Relative gene expression levels were quantified using the $2^{-\triangle\triangle Ct}$ method [54].

5. Conclusions

In our study, the 44 *SmHD-zip* genes were identified at the whole-eggplant genome level, and their gene structures, conserved motifs, and expression profiles were comprehensively analyzed. The results revealed that *SmHD-zip* genes play significant roles in fruit development and ripening, mainly through their expression patterns under light exposure and in response to hormone level changes. Notably, several *SmHD-zip* genes were differentially expressed in response to hormone treatments, including ABA, GA, and MeJA, suggesting their roles in integrating light and hormone signaling pathways to regulate eggplant fruit ripening. In conclusion, this study provides a comprehensive foundation for future investigations into the biological functions of the *SmHD-zip* family in fruit development and ripening.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae11030261/s1, Figure S1: Chromosome distribution of eggplant HD-zip genes. The scale on the left is in megabases (Mb); Figure S2: Structures of eggplant HD-zip proteins; Figure S3: WebLogos showing the conserved domains in HD-zip proteins; Table S1: Primer sequences used for RT-qPCR.

Author Contributions: Y.W., C.J. and K.Z. designed the experiments. Y.W. and K.Z. wrote the manuscript. Y.M., H.Z. and K.C. contribute to basic bioinformatics analysis. Y.Z., Z.M., Y.J. and Y.L. performed the majority of the experiments. Y.W. and C.J. analyzed the data. All authors have read and agreed to the published version of the manuscript.

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