



## Article

# Genome-Wide Identification and Expression Analysis of Eggplant *DIR* Gene Family in Response to Biotic and Abiotic Stresses

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**Abstract:** Dirigent proteins (*DIR*) play important roles in the biosynthesis of lignins and lignans, defensive responses, secondary metabolism, and disease resistance in plants. The *DIR* gene family has been identified and studied in many plants. However, the identification of *DIR* gene family in eggplant has not been conducted yet. Therefore, in this study, based on the available genome information of eggplant, the *DIR* family genes in eggplant were identified with bioinformatics methods. The expression pattern analyses of eggplant *DIR* family genes in different organs and stresses were also conducted to understand their biological functions. The results showed that a total of 24 *DIR* genes were identified in the eggplant, which were divided into three subfamilies (*DIR-a*, *DIR-b/d*, and *DIR-e*). Synteny analysis of *DIR* genes among eggplant, *Arabidopsis*, and rice showed that 15 eggplant *DIR* genes were colinear with 18 *Arabidopsis* *DIR* genes, and 16 eggplant *DIR* genes were colinear with 15 rice *DIR* genes. Phylogenetic tree analysis showed that 19 pairs of orthologous genes were identified between eggplant and pepper. The *cis*-acting elements analysis implied that the eggplant *DIR* genes contained a lot of *cis*-elements associated with stress and hormone response. The organ-specific expression analysis of eggplant *DIR* family genes revealed that only the *SmDIR3* gene was highly expressed in all the 19 organs of eggplant. Some *SmDIR* genes, including *SmDIR7*, *SmDIR8*, *SmDIR11*, *SmDIR14*, *SmDIR18*, *SmDIR19*, *SmDIR20*, and *SmDIR23*, were not or were lowly expressed in the eggplant organs, while the other eggplant *DIR* family genes showed an organ-specific expression pattern. Furthermore, 19 of 24 *SmDIR* genes were differentially expressed in response to abiotic and biotic stresses. 5 *SmDIR* genes, including *SmDIR3*, *SmDIR5*, *SmDIR6*, *SmDIR12*, and *SmDIR22*, were differentially expressed under multiple types of abiotic and biotic stresses. Especially notable, the *SmDIR22* gene was differentially expressed under three types of abiotic stresses and two types of biotic stresses, which indicated that the *SmDIR22* gene plays an important role in the response to abiotic and biotic stresses. These results provide valuable evidence for a better understanding of the biological role of *DIR* genes in eggplant.

**Keywords:** eggplant; *DIR*; gene family; bioinformatics; expression analysis

## 1. Introduction

In nature, plants are easily influenced by pathogens, pests, severe temperatures, drought, salt, and heavy metals, which cause a dramatic reduction of crop yield and quality [1,2]. Plants usually respond to biotic and abiotic stresses by activating a variety of genes, including the dirigent protein (*DIR*) gene. The *DIR* gene is involved in the synthesis of lignins and lignans, which play a pivotal role against biotic and abiotic stresses in plants [3–5]. As an important compound, lignin confers stability and hydrophobicity to the plant vascular system and forms a barrier against microbial pathogens and pests [6,7]. Lignan has antifungal properties by inhibiting microbe-derived degradative enzymes such

as cellulases, laccases, polygalacturonases, and glucosidases [8–10]. Besides, lignans can also defend against insect attacks by disrupting the insect endocrine system [11]. *DIR* genes can regulate the biosynthesis of lignins and lignans, which indicates that *DIR* genes actively respond to biotic and abiotic stresses.

*DIR* proteins are widely distributed in almost all vascular plants, including ferns, gymnosperms, and angiosperms [12–14]. Previous studies reported that *DIR* proteins are divided into five subgroups, including *DIR-a*, *DIR-b*, *DIR-c*, *DIR-d*, and *DIR-e* [3]. With the increasing number of *DIR* proteins, another two new subgroups, *DIR-f* and *DIR-g*, were generated, and the *DIR-b* and *DIR-d* subfamilies are combined together as the *DIR-b/d* subgroup [12]. *DIR* proteins were first identified in *Forsythia suspense* [15]. Subsequently, the identification of *DIR* family genes were reported in a variety of monocotyledon and dicotyledon plants, such as *Arabidopsis thaliana* (L.) Heynh. [16], rice (*Oryza sativa* L.) [17,18], spruce (*Picea* spp.) [3,12], pear (*Pyrus bretschneideri*) [5], *Populus* [19], Chinese cabbage (*Brassica rapa* L.) [4], pepper (*Capsicum annuum* L.) [20], strawberry (*Fragaria vesca*) [21], Cucurbitaceae [22], and so on. Moreover, many studies reported that *DIR* family genes play important regulatory roles in the response to biotic and abiotic stresses, such as high temperature [13,18], low temperature [4,18], drought [4,13,18,23–25], salt [18,23,25,26], heavy metals [18], H<sub>2</sub>O<sub>2</sub> [13,23], and diseases [3,4,25,27–31]. In addition, *DIR* genes also actively respond to hormone stresses including ethylene, gibberellin (JA), salicylic acid (SA), abscisic acid (ABA), and so on [4,5,13,25].

Eggplant (*Solanum melongena* L.), one of the most important solanaceous crops, is widely cultivated across the world for its fruits, ranking third for total production and economic value in Solanaceae species, after potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum* L.) [32,33]. As early as 2014, the first genome sequencing project of eggplant was completed [34]. With the rapid development of genome sequencing technology, the improved eggplant genomes were constantly updated [35–38]. By using the high-quality genome information of eggplant, a great deal of research on gene family identification has been performed, such as the identification of R2R3-MYB [39], WRKY [40], NAC [41], ARF [42], and other gene families. However, the genome-wide identification of *DIR* gene family in eggplant was still not reported, which greatly limits the functional studies of *DIR* genes in eggplant.

Therefore, based on the high-quality genome information of eggplant, *DIR* family genes in eggplant (*SmDIR* genes) were identified for the first time in this study. The physicochemical characteristics, chromosome locations, gene structures, conserved motifs, phylogenetic tree, *cis*-acting elements, and synteny of *SmDIR* genes were analyzed via bioinformatic methods. Moreover, the analyses of organ-specific expression and stress-responsive gene expression patterns of *SmDIR* genes were conducted to preliminarily explore the biological functions of *DIR* family genes in eggplant. The results of the current study will provide future insight for further research on the biological functions of *SmDIR* genes, as well as a theoretical reference for the resistance breeding of eggplant.

## 2. Materials and Methods

### 2.1. Identification and Chromosomal Mapping of *DIR* Family Genes in Eggplant

The Hidden Markov Model (HMM) of dirigent domain (PF03018) was used to identify the putative *DIR* family genes in eggplant genome V4.1 [38] using HMMER 3.0 software [43] with E-value  $< 1 \times 10^{-5}$ . The protein sequences of candidate *SmDIR* genes were extracted with Fasta Extract program in TBtools software [44]. The Pfam (<http://pfam.xfam.org/>, accessed on 26 May 2022) [45] and SMART (<http://smart.embl.de/smart/batch.pl>, accessed on 26 May 2022) [46] websites were used to confirm the conserved dirigent domain. Genes containing the whole dirigent domain were chosen as the final confirmed members of *DIR* gene family in eggplant. The chromosomal locations of *SmDIR* genes were analyzed and visualized with TBtools software. The physicochemical characteristics such as the number of amino acids, molecular weight, theoretical isoelectric point, instability index, aliphatic index, and grand average of hydropathicity of each *SmDIR* protein were calculated with

the online tool ExPASy (<https://web.expasy.org/protparam/>, accessed on 27 May 2022). The subcellular localization of *SmDIR* genes were predicted with the online website CELLO (<http://cello.life.nctu.edu.tw/>, accessed on 27 May 2022) [47].

### 2.2. Gene Structure Analysis and Phylogenetic Tree Construction of DIR Family Genes in Eggplant

The exon/intron structures of *SmDIR* genes were drawn with GFF3 file (General Feature Format 3) of eggplant genome V4.1 using TBtools software. The conserved motifs of *SmDIR* proteins were analyzed using the MEME software (version = 5.4.1) [48] with the following parameters: maximum number of misfits, 10; and the optimum width of each motif, 6–100 amino acids. The 1.5 kb upstream sequences of the translation initiation codon of *SmDIR* genes were extracted from the eggplant genome V4.1. The sequences were then submitted to PlantCARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>, accessed on 27 May 2022) website [49] for *cis*-acting elements prediction. The phylogenetic tree of *DIR* family genes in eggplant, pepper [20] and *Arabidopsis* [16] was generated using MEGA 11 software [50] with the neighbor-joining method and 1000 bootstrap replications. The final phylogenetic tree was visualized using the online website Evolview (<https://www.evolgenius.info/evolview-v3/#login>, accessed on 27 May 2022) [51].

### 2.3. Gene Duplication and Synteny Analysis of DIR Family Genes

The tandem and segmental duplications of *SmDIR* genes were analyzed with MCScanX software [52]. The synteny analysis of *DIR* family genes among eggplant, *Arabidopsis* and rice was conducted with MCScanX software. The syntenic relationships of *DIR* family genes among eggplant, *Arabidopsis*, and rice were visualized with Circos software [53].

### 2.4. RNA-Seq Reanalysis of Eggplant Transcriptome Sequencing Data

The published eggplant transcriptome sequencing data were downloaded from the SRA database using the corresponding accession numbers (<https://www.ncbi.nlm.nih.gov/sra>, accessed on 4 June 2022). The downloaded SRA data were converted into Fastq data by using fasterq-dump.2.11.0 tool (<https://github.com/ncbi/sra-tools/wiki/HowTo:-fasterq-dump>, accessed on 10 June 2022). The read quality of Fastq data was then evaluated using the FastQC software (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>, accessed on 10 June 2022). The adapter sequences and low-quality bases were removed from the Fastq data with Trimmomatic software (version = 0.39) [54], finally obtaining the filtered clean data. The index of eggplant genome V4.1 was built using STAR software (version = 2.7.10a) [55]. The filtered clean data were mapped to eggplant genome V4.1, generating the SAM files. Next, the SAM files were directly converted into the sorted BAM files using the SAMtools software (version = 1.15) [56]. The expression levels of each gene were estimated with StringTie software (v2.2.1) [57]. Finally, the differential expression analysis was performed using the count matrix in DESeq2 software [58].

### 2.5. Organ-Specific Expression Analysis of DIR Family Genes in Eggplant

The transcriptome sequencing data of 19 kinds of eggplant organs, including cotyledon, radicle, root, stem, flower, pistil, leaf, bud, fruit peduncle, fruit skin, fruit flesh, fruit, and so on, were retrieved and downloaded from the SRA database (PRJNA328564) [35]. The organ-specific expression analysis of eggplant *DIR* family genes was conducted using the above methods. The heatmap was drawn using the Heatmap program in TBtools software.

### 2.6. The Expression Profiles Analysis of Eggplant DIR Family Genes under Stresses

The transcriptome sequencing data of eggplant under stress treatments including high temperature (PRJNA531285) [59], low temperature (PRJNA572318) [60], salt (PRJNA477924) [61], bacterial wilt (caused by *Ralstonia solanacearum*, PRJNA728497) [62], *Verticillium* wilt (PRJNA451240) [63], and *Tuta absoluta* (PRJNA695350) [64] were retrieved and downloaded from the SRA database. The expression profiles of eggplant *DIR* family

genes were analyzed using the above methods, and then drawn into heatmaps using TBtools software.

### 3. Results

#### 3.1. Genome-Wide Identification of DIR Family Genes in Eggplant

A total of 24 *DIR* family genes were identified in the eggplant genome (Table 1). These eggplant *DIR* family genes were renamed from *SmDIR1* to *SmDIR24* according to their chromosomal locations. The detailed information of eggplant *DIR* family genes was summarized in Table 1. The 24 *SmDIR* proteins ranged from 146 (*SmDIR11*) to 546 (*SmDIR17*) amino acid (aa) in length, and the corresponding molecular weights varied from 16.14 kDa (*SmDIR11*) to 59.69 kDa (*SmDIR17*). The isoelectric points (pI) of the 24 *SmDIR* proteins ranged from 4.34 (*SmDIR9*) to 9.85 (*SmDIR19*). Among the 24 eggplant *DIR* proteins, 14 *SmDIR* proteins were basic proteins (pI > 7), and 10 *SmDIR* proteins were acidic proteins (pI < 7). 20 *SmDIR* proteins were stable proteins (instability index less than 40), and the other 4 *SmDIR* proteins (*SmDIR1*, *SmDIR8*, *SmDIR9*, and *SmDIR20*) were unstable proteins (instability index greater than 40). The aliphatic indexes of 24 *SmDIR* proteins varied from 66.53 (*SmDIR16*) to 101.55 (*SmDIR22*). Most of *SmDIR* proteins (79%) were hydrophobic proteins (grand average of hydropathicity > 0), and only five *SmDIR* proteins (*SmDIR1*, *SmDIR3*, *SmDIR8*, *SmDIR16*, and *SmDIR20*) were hydrophilic proteins (grand average of hydropathicity < 0). Subcellular localization prediction showed that most of the *SmDIR* genes were located in the plasma membrane, and the other *DIR* family members were located in the extracellular space and mitochondrion. (Table 1).

Based on the GFF3 file of eggplant genome V4.1, the chromosomal distributions of 24 *SmDIR* genes were mapped across the eggplant chromosomes using TBtools software (Figure 1). In the eggplant genome, the 24 *SmDIR* family genes were unevenly distributed on 10 of 12 eggplant chromosomes. There were no *SmDIR* genes located on chromosomes 3 and 10. Chromosome 12 harbored the largest number of 4 *SmDIR* genes, whereas chromosomes 4, 7, and 9 contained only one *SmDIR* gene, respectively. The chromosomes 1, 2, 5, 6, and 11 had three *SmDIR* genes, respectively. Two *SmDIR* genes were positioned on chromosome 8. In our study, five pairs of tandem duplication genes on chromosome 1 (*SmDIR1/SmDIR2*), chromosome 5 (*SmDIR8/SmDIR9*), chromosome 8 (*SmDIR15/SmDIR16*), and chromosome 12 (*SmDIR21/SmDIR22*, *SmDIR22/SmDIR23*) were identified in eggplant *DIR* gene family.

#### 3.2. Phylogenetic Analysis of DIR Family Genes in Eggplant, Pepper and Arabidopsis

To investigate the evolutionary relationship of *DIR* family genes in eggplant, a phylogenetic tree was constructed with 24 *SmDIR* proteins, 24 pepper *DIR* proteins [20] and 25 *Arabidopsis* *DIR* proteins [16] by multiple sequence alignment (Figure 2). According to the clustering result of *Arabidopsis* *DIR* family genes, the phylogenetic tree was divided into three subgroups, including *DIR-a*, *DIR-b/d*, and *DIR-e*. Among them, the *DIR-b/d* subgroup harbored the largest number of *DIR* genes, containing a total of 40 *DIR* genes. The *DIR-e* subgroup had the second number of *DIR* genes, containing 19 *DIR* genes. The *DIR-a* subgroup had the least number of *DIR* genes, containing 14 *DIR* genes. In the phylogenetic tree, 19 pairs of orthologous genes were identified between eggplant and pepper, which were *SmDIR1/CaDIR17*, *SmDIR2/CaDIR16*, *SmDIR3/CaDIR5*, *SmDIR4/CaDIR8*, *SmDIR5/CaDIR13*, *SmDIR6/CaDIR6*, *SmDIR8/CaDIR10*, *SmDIR9/CaDIR19*, *SmDIR10/CaDIR23*, *SmDIR12/CaDIR22*, *SmDIR13/CaDIR12*, *SmDIR14/CaDIR14*, *SmDIR15/CaDIR2*, *SmDIR16/CaDIR1*, *SmDIR17/CaDIR7*, *SmDIR18/CaDIR4*, *SmDIR19/CaDIR9*, *SmDIR22/CaDIR24*, and *SmDIR24/CaDIR20*, respectively. The homologous genes clustered together in the evolutionary relationship possessed similar gene functions. Thus, the biological functions of *SmDIR* genes could be inferred from the molecular functions of pepper *DIR* family genes based on the clustering results of *DIR* family genes between eggplant and pepper.

**Table 1.** The physiochemical characteristics of *DIR* family genes in eggplant.

Gene Name	Gene ID	Number of Amino Acid (aa)	Molecular Weight (kDa)	Theoretical pI	Instability Index	Aliphatic Index	Grand Average of Hydropathicity	Subcellular Location
<i>SmDIR1</i>	SMEL4.1_01g003540.1	156	16.97	9.36	45.55	76.92	−0.108	Mitochondrion
<i>SmDIR2</i>	SMEL4.1_01g003550.1	343	36.23	6.13	11.54	95.54	0.171	Plasma Membrane
<i>SmDIR3</i>	SMEL4.1_01g031140.1	191	21.33	9.57	28.14	79.69	−0.017	Plasma Membrane
<i>SmDIR4</i>	SMEL4.1_02g003080.1	190	21.06	9.67	24.42	94.95	0.065	Mitochondrion
<i>SmDIR5</i>	SMEL4.1_02g009260.1	186	20.69	7.06	36.88	91.24	0.196	Plasma Membrane
<i>SmDIR6</i>	SMEL4.1_02g018810.1	194	21.19	9.08	28.43	93.87	0.111	Extracellular Space
<i>SmDIR7</i>	SMEL4.1_04g004510.1	190	21.37	9.42	29.75	91.79	0.022	Plasma Membrane
<i>SmDIR8</i>	SMEL4.1_05g003580.1	244	27.03	5.90	43.08	79.88	−0.167	Plasma Membrane
<i>SmDIR9</i>	SMEL4.1_05g003590.1	438	44.86	4.34	40.80	87.99	0.075	Extracellular Space
<i>SmDIR10</i>	SMEL4.1_05g019870.1	249	26.06	5.46	36.58	85.42	0.115	Plasma Membrane
<i>SmDIR11</i>	SMEL4.1_06g013950.1	146	16.14	8.82	27.09	91.51	0.135	Plasma Membrane
<i>SmDIR12</i>	SMEL4.1_06g020190.1	212	23.63	9.73	29.47	89.62	0.094	Plasma Membrane
<i>SmDIR13</i>	SMEL4.1_06g027190.1	372	38.41	4.75	26.34	96.02	0.230	Plasma Membrane
<i>SmDIR14</i>	SMEL4.1_07g016780.1	177	19.52	5.22	27.96	95.31	0.124	Plasma Membrane
<i>SmDIR15</i>	SMEL4.1_08g026590.1	190	21.28	7.79	13.38	88.84	0.088	Plasma Membrane
<i>SmDIR16</i>	SMEL4.1_08g026600.1	167	19.13	6.02	26.49	66.53	−0.298	Plasma Membrane
<i>SmDIR17</i>	SMEL4.1_09g022870.1	546	59.69	8.52	38.73	97.33	0.354	Plasma Membrane
<i>SmDIR18</i>	SMEL4.1_11g000960.1	168	18.76	6.90	15.68	99.76	0.123	Plasma Membrane
<i>SmDIR19</i>	SMEL4.1_11g019830.1	178	19.93	9.85	35.35	100.34	0.069	Plasma Membrane
<i>SmDIR20</i>	SMEL4.1_11g026460.1	369	38.79	4.63	42.99	84.80	−0.063	Extracellular Space
<i>SmDIR21</i>	SMEL4.1_12g003420.1	184	20.19	9.52	26.98	90.11	0.123	Mitochondrion
<i>SmDIR22</i>	SMEL4.1_12g003430.1	194	20.87	9.24	22.53	101.55	0.198	Extracellular Space
<i>SmDIR23</i>	SMEL4.1_12g003440.1	193	21.21	6.96	23.74	93.37	0.130	Extracellular Space
<i>SmDIR24</i>	SMEL4.1_12g003620.1	194	21.36	7.87	19.27	79.85	0.055	Extracellular Space

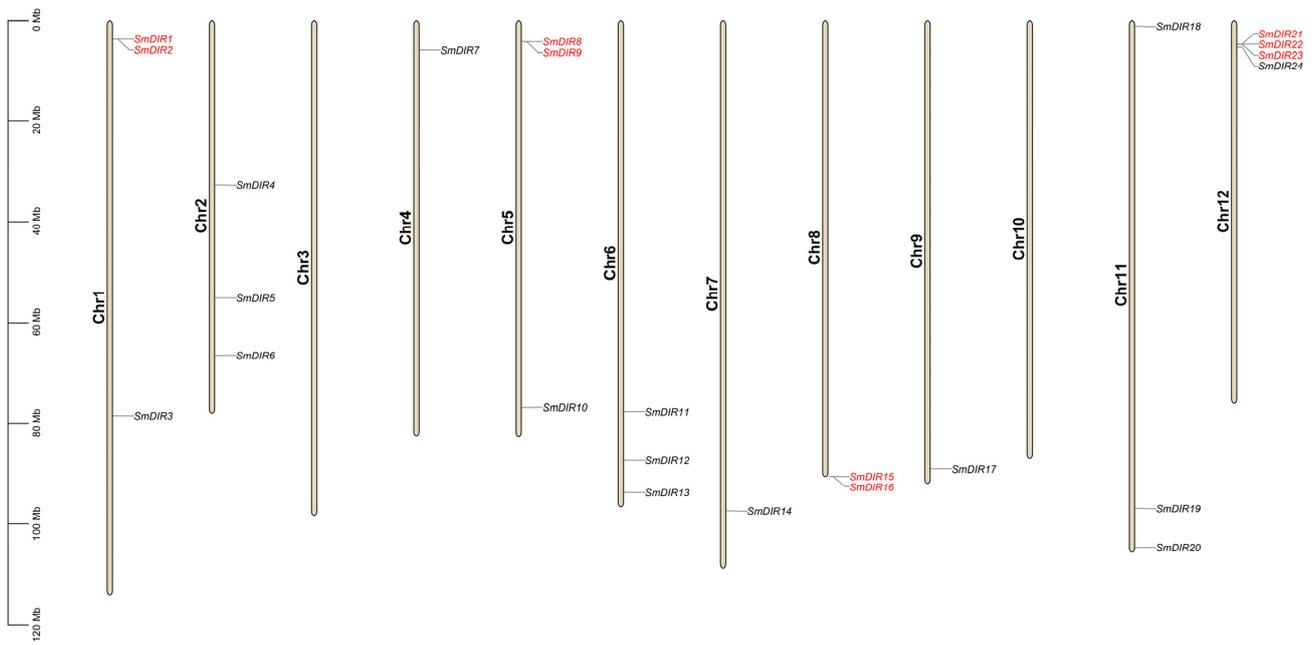


Figure 1. The chromosomal locations of *DIR* family genes in eggplant.

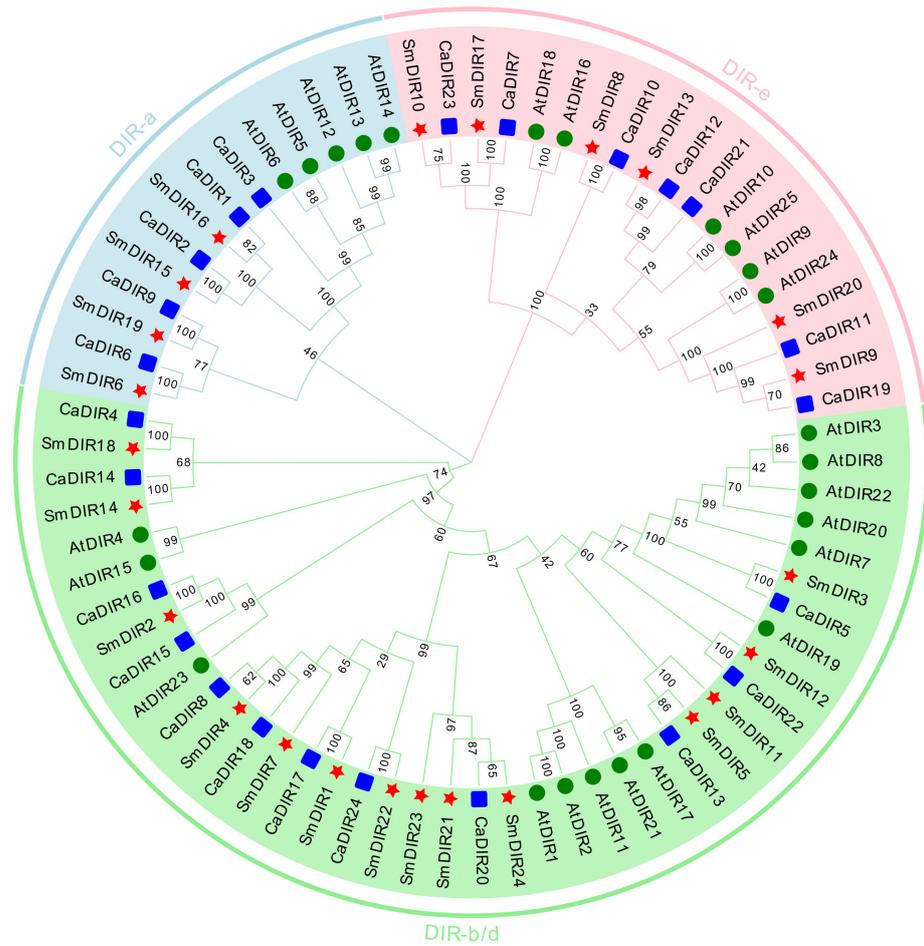
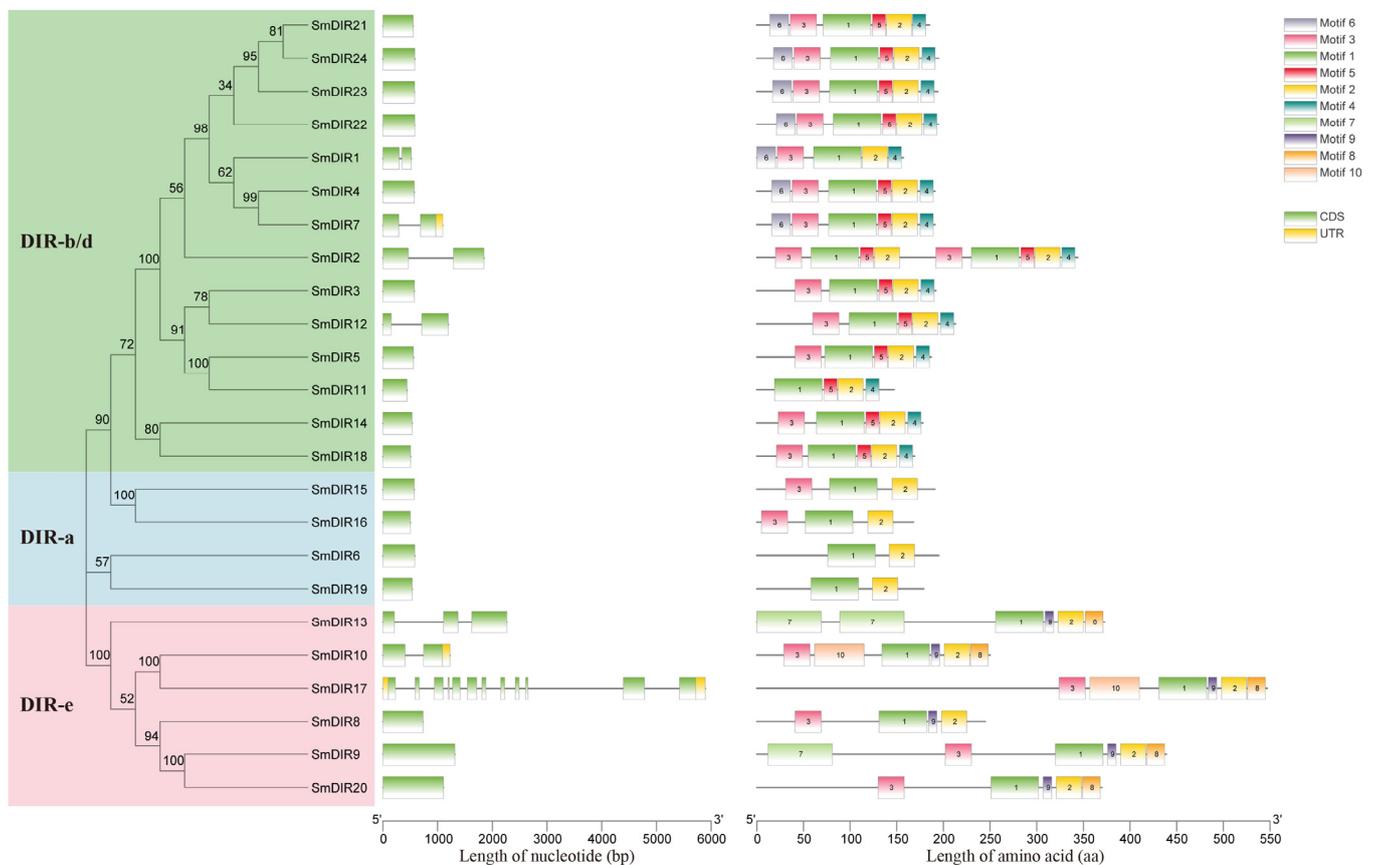


Figure 2. Phylogenetic analysis of *DIR* proteins from eggplant, pepper and *Arabidopsis*.

### 3.3. Gene Structure and Conserved Motifs Analyses of Eggplant DIR Genes

The diagrams of phylogenetic tree and gene structures of *SmDIR* genes were drawn with TBtools software. The results of phylogenetic tree analysis showed that 24 *SmDIR* genes were classified into three subgroups, namely *DIR-a*, *DIR-b/d* and *DIR-e* (Figure 3), which was consistent with the clustering result of eggplant, pepper and *Arabidopsis* DIR genes (Figure 2). Among them, there were fourteen *DIR* genes in the *DIR-b/d* subgroup, six *DIR* genes in the *DIR-e* subgroup, and four *DIR* genes in the *DIR-a* subgroup. The gene structure analysis showed that most of *DIR* genes in subgroup *DIR-b/d* contained one exon. All four *DIR* genes in the *DIR-a* subgroup contained only one exon. All *DIR* genes except *SmDIR10*, *SmDIR13*, and *SmDIR17* in subgroup *DIR-e* contained one exon (Figure 3). The results showed that most of the *SmDIR* genes (71%) contained only one exon and no introns, suggesting that the exon number of *SmDIR* genes was relatively conservative.



**Figure 3.** Exon-intron structures of *SmDIR* genes and conserved motifs of *SmDIR* proteins.

A schematic diagram of conserved motifs of *SmDIR* proteins was constructed with MEME analysis (Figure 3). A total of ten motifs were revealed in the eggplant *DIR* proteins (Table 2). The motif compositions of *SmDIR* proteins in different subgroups were different, while *SmDIR* proteins in the same subgroup shared the similar number, type, and order of motifs. For example, most of *SmDIR* proteins in the subgroup *DIR-b/d* contained motifs 3, 1, 5, 2, and 4, and exhibited the same order. In the subgroup *DIR-a*, motifs 1 and 2 were found in all *SmDIR* proteins and showed the same order. In the subgroup *DIR-e*, motifs 1, 9, 2, and 8 were found in all *SmDIR* proteins and showed the same order. These results indicated that the different distribution of conserved motifs in different subgroups might lead to the evolution of *SmDIR* gene functional diversity. The similar conserved motifs of *SmDIR* proteins in the same subgroup indicated that they have similar functions.

**Table 2.** The motifs information of *DIR* proteins in eggplant.

Motif	Sequence	Number of Amino Acid	Pfam Annotation
motif 1	FGTLTVIDDPLTIGPEPNSKJIGRAQGIYGSASQNGVSLLMALNFVFTGGKY	52	Dirigent
motif 2	YREMAIVGGTGKFRRLARGYATAKTYWFB	28	Dirigent
motif 3	HAKEKVTKLHFYFHDILSGKNPTAIQIAQ	29	-
motif 4	TGDAIVEYNVVVLHY	15	-
motif 5	GSTLSILGRNPVFHE	15	-
motif 6	MPTAQGIDLGPKAVEKWFKKL	21	-
motif 7	MAIIQNSNSFQLKVIISYLLILALTITFATAGRILDEEVATPTVPPNNDPPTDQPPVSGATAAGAAAGAT	70	-
motif 8	GQHTTDGNETILHITVYJTY	20	-
motif 9	DSLFFGVHR	10	-
motif 10	IYSGQIPFATPLGFQPPEDGVAIPNANGAMPTFNINGVPLGTGLAGTIFAGGNN	54	-

### 3.4. The Synteny Analysis of DIR Genes among Eggplant, Arabidopsis and Rice

To better understand the molecular functions of eggplant DIR family genes, the synteny analysis of DIR family genes among eggplant, *Arabidopsis* and rice was conducted. The results showed 29 syntenic relationships between 15 *SmDIR* genes (*SmDIR2*, *SmDIR3*, *SmDIR6*, *SmDIR9*, *SmDIR10*, *SmDIR11*, *SmDIR12*, *SmDIR13*, *SmDIR15*, *SmDIR17*, *SmDIR19*, *SmDIR20*, *SmDIR21*, *SmDIR23*, *SmDIR24*) genes and 18 *Arabidopsis* DIR genes (*AT4G13580*, *AT3G24020*, *AT1G58170*, *AT1G55210*, *AT2G39430*, *AT3G13650*, *AT3G55230*, *AT2G28670*, *AT5G49040*, *AT2G21100*, *AT1G65870*, *AT1G64160*, *AT3G58090*, *AT5G42500*, *AT4G23690*, *AT3G13662*, *AT5G42655*, *AT4G11430*). There were 27 syntenic relationships between 16 *SmDIR* genes (*SmDIR2*, *SmDIR3*, *SmDIR4*, *SmDIR5*, *SmDIR6*, *SmDIR8*, *SmDIR9*, *SmDIR10*, *SmDIR12*, *SmDIR13*, *SmDIR15*, *SmDIR16*, *SmDIR17*, *SmDIR21*, *SmDIR23*, *SmDIR24*) and 15 rice DIR genes (*Os04g42720*, *Os03g05030*, *Os03g17220*, *Os01g06250*, *Os01g65700*, *Os11g07830*, *Os12g07580*, *Os11g27620*, *Os11g07690*, *Os03g59440*, *Os07g44930*, *Os07g44250*, *Os07g44280*, *Os01g62030*, *Os07g44450*). It was found that five *SmDIR* genes (*SmDIR1*, *SmDIR7*, *SmDIR14*, *SmDIR18*, *SmDIR22*) were not colinear with either *A. thaliana* or rice, suggesting that these five *SmDIR* genes were conservative in eggplant. The synteny analysis of DIR family genes in eggplant showed that there were four pairs of *SmDIR* genes (*SmDIR1/SmDIR21*, *SmDIR1/SmDIR24*, *SmDIR8/SmDIR13*, *SmDIR10/SmDIR17*) with syntenic relationships, which were segmental duplication gene pairs (Figure 4).

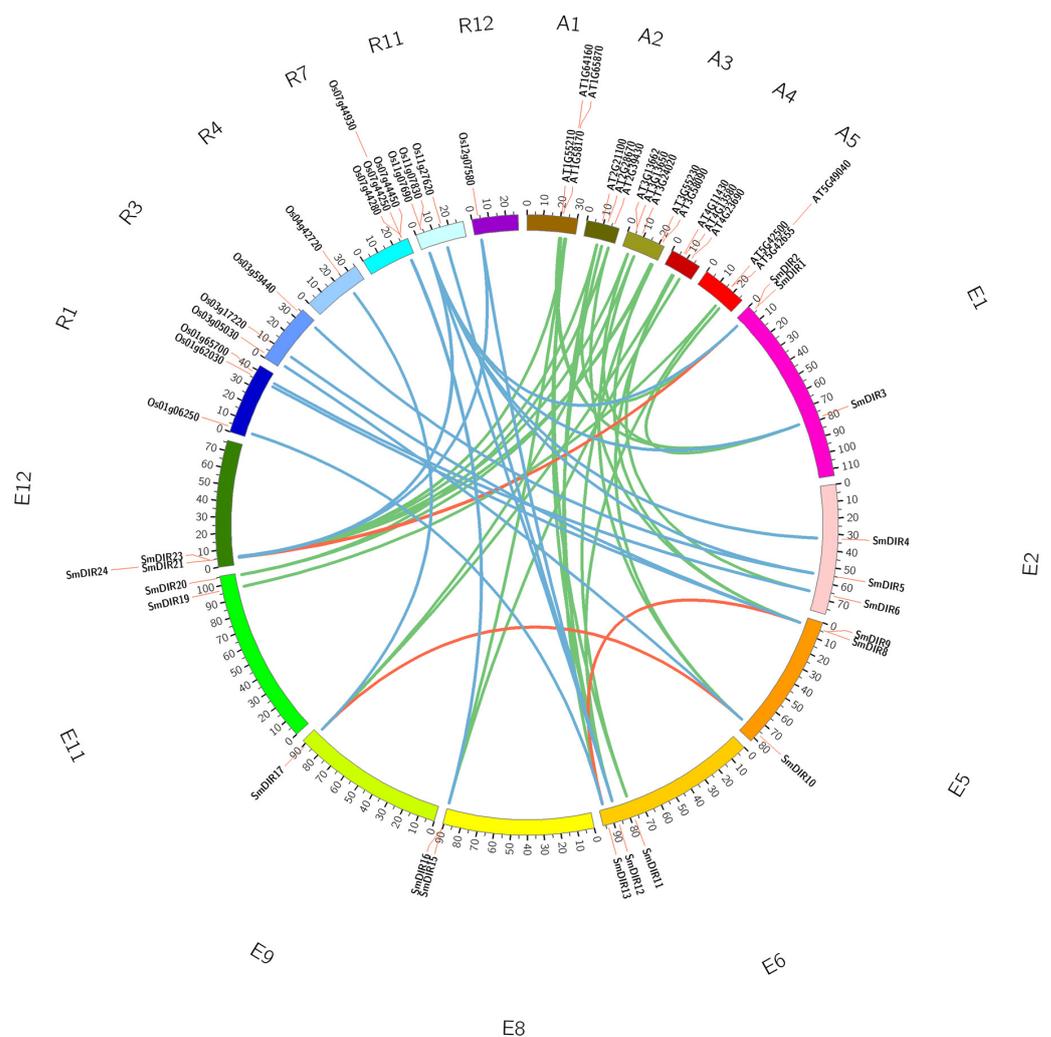
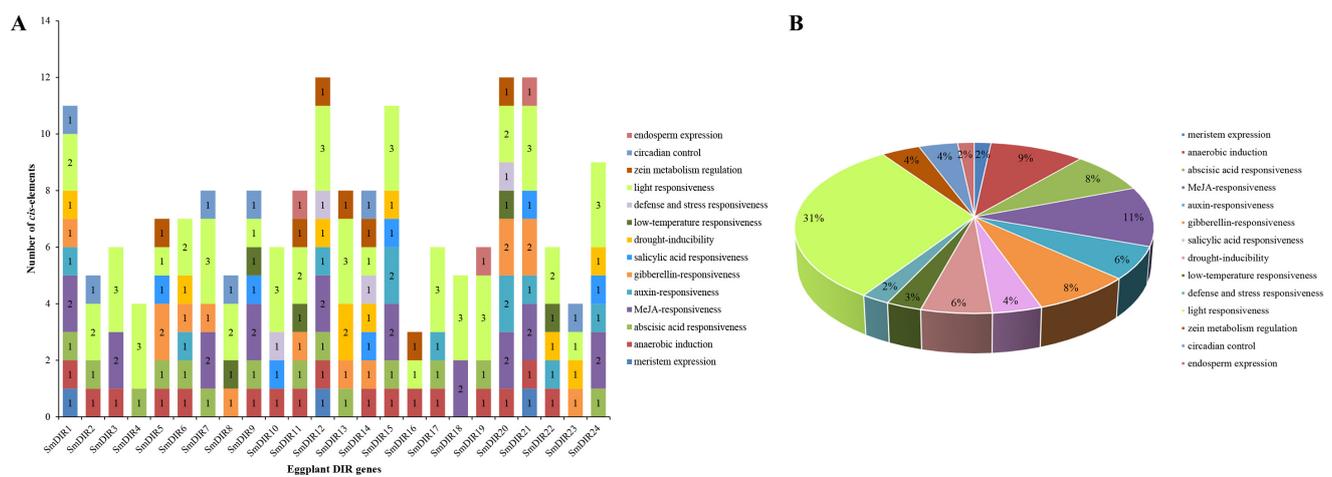


Figure 4. Syntenic relationships of DIR family genes among eggplant, *Arabidopsis* and rice.

### 3.5. The Cis-Acting Elements Analysis of Eggplant DIR Genes

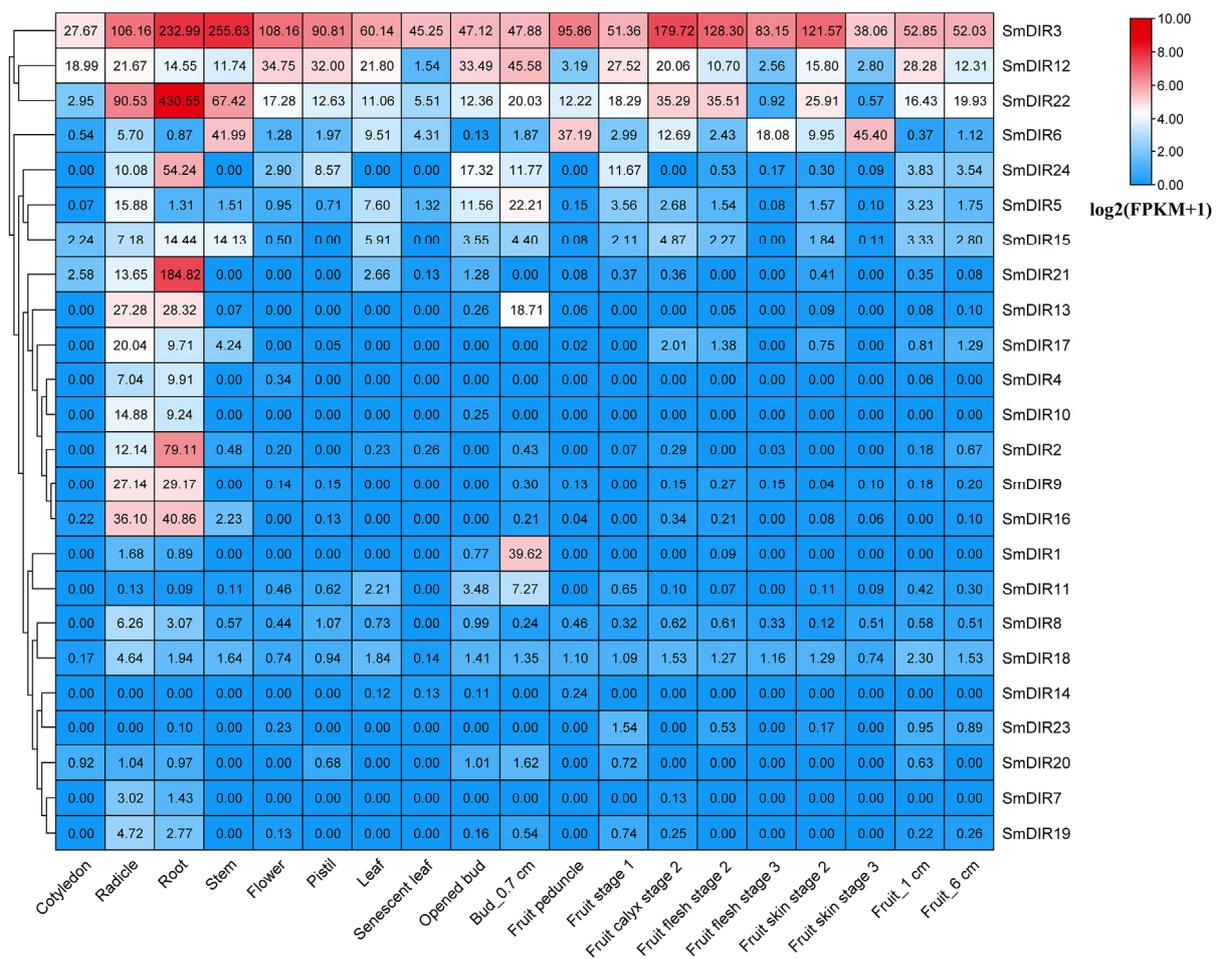
The 1.5-kb upstream sequences from the transcription start site of *SmDIR* genes were extracted to analyze their *cis*-acting elements in the promoters. The results showed that 14 kinds of *cis*-elements were identified (Figure 5). Among them, *cis*-elements related to light responsiveness were the major type, including ACE, AE-box, ATCT-motif, Box 4, Box II, G-box, GT1-motif, I-box, LAMP-element, and so on, accounting for 31% of total *cis*-elements. In addition, some other *cis*-elements were also identified, including *cis*-elements related to hormone response (abscisic acid, MeJA, auxin, gibberellin, salicylic acid), stress response (drought, low temperature, defense), circadian control, endosperm expression, and meristem expression. The results indicated that eggplant *DIR* genes play vital roles in plant growth and development.



**Figure 5.** Cis-elements analysis of the promoters of eggplant *DIR* family genes. (A) The types and numbers of various *cis*-elements in the promoters of each *SmDIR* gene. (B) The relative proportions of different kinds of *cis*-elements in the promoters of *SmDIR* genes are displayed by pie chart.

### 3.6. Organ-Specific Expression Analysis of Eggplant DIR Genes

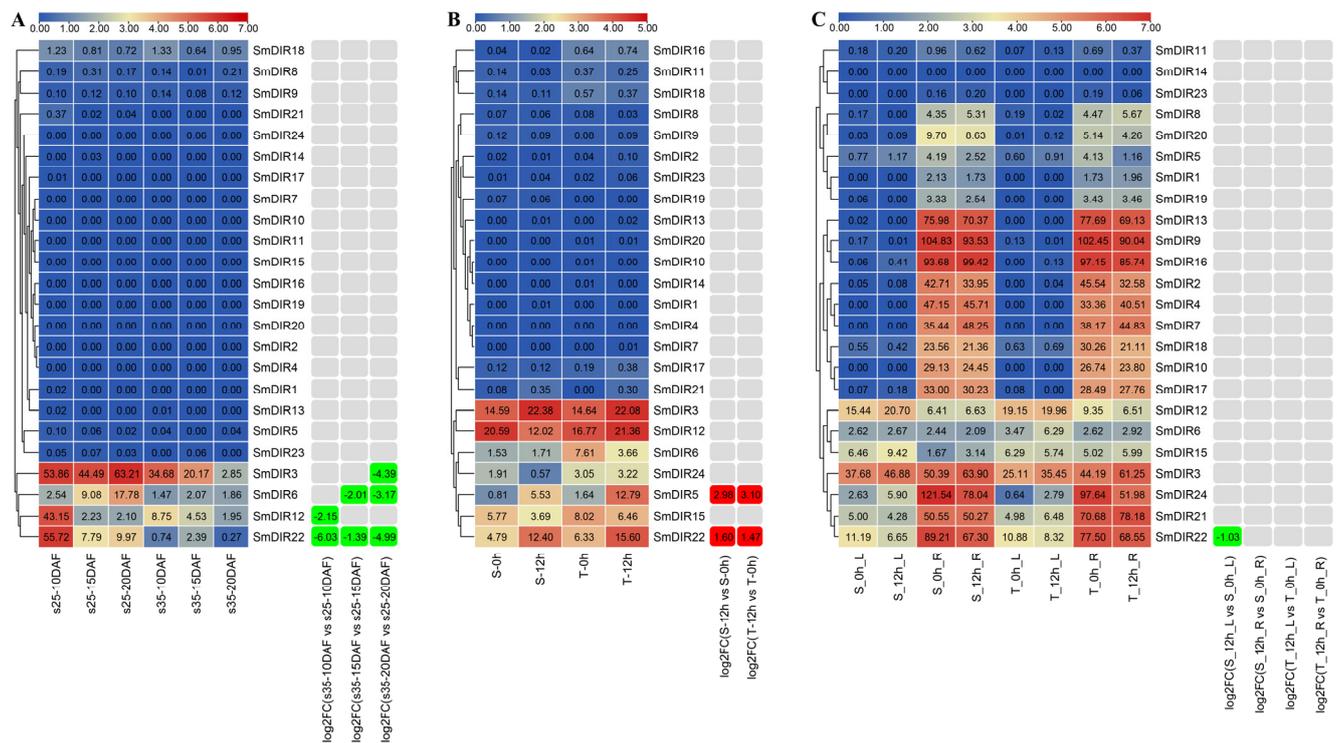
To analyze the organ-specific expression profiles of *SmDIR* genes in eggplant, the transcriptome sequencing data of 19 kinds of eggplant organs (accession number: PR-JNA328564) were reanalyzed with eggplant genome V4.1. The results indicated that the eggplant *DIR* gene *SmDIR3* was highly expressed in all the 19 organs of eggplant. Some *SmDIR* genes, including *SmDIR7*, *SmDIR8*, *SmDIR11*, *SmDIR14*, *SmDIR18*, *SmDIR19*, *SmDIR20*, and *SmDIR23*, were not or were lowly expressed in all the eggplant organs. *SmDIR12* gene was expressed in all eggplant organs, with the lower expression levels in senescent leaf, fruit peduncle, fruit flesh stage 3, and fruit skin stage 3. The *SmDIR22* gene was expressed in all eggplant organs, with the highest expression level in root and the lowest expression levels in fruit flesh and skin at stage 3. The *SmDIR6* gene was highly expressed in fruit skin stage 3, stem and fruit peduncle, but was lowly expressed in cotyledon and fruit. Some *SmDIR* gene was organ-specific expressed. For example, the *SmDIR1* gene was highly expressed in bud, but was lowly or not expressed in the other eggplant organs. *SmDIR13* gene was highly expressed in radicle, root and bud, but was lowly or not expressed in the other eggplant organs. The eggplant *DIR* genes *SmDIR2*, *SmDIR4*, *SmDIR9*, *SmDIR10*, *SmDIR17*, and *SmDIR21* were highly expressed in radicle and root, but were lowly expressed in the other organs (Figure 6). In summary, the expression patterns of *SmDIR* genes varied in different organs, suggesting a degree of organ specificity.



**Figure 6.** The expression heatmap of eggplant *DIR* family gene in different organs. The data in the boxes indicated the original FPKM values.

### 3.7. The Expression Profiles of Eggplant *DIR* Genes under Abiotic Stresses

To determine the expression profiles of eggplant *DIR* family genes under high temperature, low temperature, and salt stresses, the published transcriptome sequencing data (PRJNA531285, PRJNA572318, PRJNA477924) were re-analyzed with eggplant genome V4.1. Compared with the control, four eggplant *DIR* genes including *SmDIR3*, *SmDIR6*, *SmDIR12*, and *SmDIR22* genes were significantly down-regulated under high temperature stress. However, the expression patterns of these four *SmDIR* genes were different. Among them, the *SmDIR3* gene was significantly down-regulated only at 20DAF (days after flowering). *SmDIR12* gene was significantly down-regulated only at 10DAF. *SmDIR6* gene was significantly down-regulated at 15DAF and 20DAF. *SmDIR22* gene was significantly down-regulated at 10DAF, 15DAF, and 20DAF. The other 20 *SmDIR* genes were lowly or not expressed in the peel, and not affected by high temperature treatment (Figure 7A). Under low temperature stress, only two eggplant *DIR* genes *SmDIR5* and *SmDIR22* were significantly down-regulated in both sensitive and tolerant eggplant cultivars. Among the other twenty-two *SmDIR* genes, seventeen *SmDIR* genes were not or lowly expressed in the leaf, five *SmDIR* genes were not significantly regulated by low temperature treatment (Figure 7B). Under salt stress, only *SmDIR22* genes were significantly down-regulated in the sensitive eggplant leaf. The other 23 *SmDIR* genes were not significantly affected by salt stress (Figure 7C).

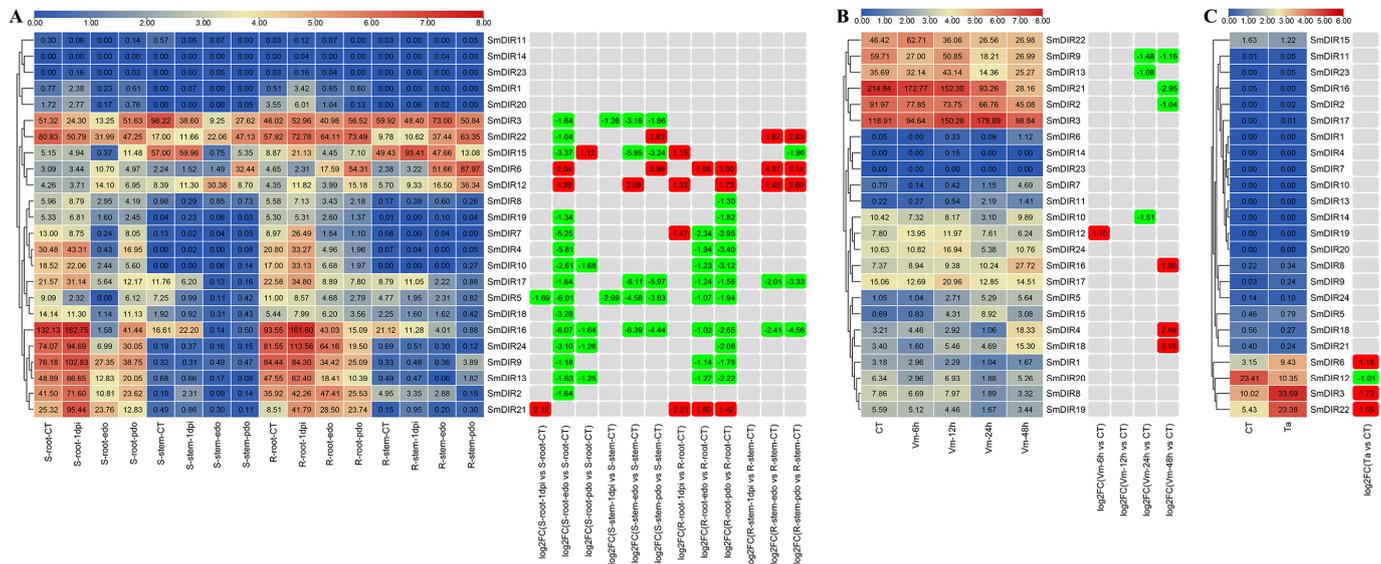


**Figure 7.** The expression heatmaps of eggplant *DIR* family genes under abiotic stress treatments. (A) The expression patterns of *SmDIR* genes under high temperature stress. s25: control treatment (25 °C); s35: high temperature treatment (35 °C); DAF: days after flowering. (B) The expression patterns of *SmDIR* genes under low temperature stress. S: low temperature-sensitive eggplant cultivar; T: low temperature-tolerant eggplant cultivar. 0 h: low temperature treatment for 0 h (control treatment); 6 h: low temperature treatment (4 °C) for 6 h. (C) The expression patterns of *SmDIR* genes under salt stress. S: salt-sensitive eggplant cultivar; T: salt-tolerant eggplant cultivar. 0 h: salt treatment for 0 h (control treatment); 12 h: salt treatment (200 mM NaCl) for 12 h; L: leaf; R: root. In each figure, the data in the boxes of left heatmap indicated the original FPKM values. Differentially expressed genes were highlighted by red (up-regulation) and green (down-regulation) color with  $\log_2$  (fold-change) values in the right table.

### 3.8. The Expression Profiles of Eggplant *DIR* Genes under Biotic Stresses

Based on the eggplant genome V4.1, the published transcriptome sequencing data under bacterial wilt (PRJNA728497), *Verticillium* wilt (PRJNA451240), and *Tuta absoluta* stresses (PRJNA695350) were re-analyzed to investigate the expression profiles of eggplant *DIR* genes under biotic stresses. Under bacterial wilt stress, only five *SmDIR* genes were not affected, which were also not or lowly expressed in the samples. The other 19 *SmDIR* genes exhibited significantly differential expression under bacterial wilt infection. Among them, three *SmDIR* genes, including *SmDIR6*, *SmDIR12*, and *SmDIR21*, were up-regulated in both resistant and susceptible eggplant cultivars. *SmDIR6* and *SmDIR12* genes were up-regulated in both root and stem, while *SmDIR21* was only up-regulated in the root. Thirteen *SmDIR* genes were down-regulated in response to bacterial wilt infection. Whereas the expression patterns of these 13 *SmDIR* genes were different. Only three of these 13 *SmDIR* genes, including *SmDIR2*, *SmDIR3*, and *SmDIR18*, were down-regulated in the susceptible eggplant cultivar. *SmDIR3* was down-regulated in the root and stem of the susceptible eggplant cultivar. *SmDIR2* and *SmDIR18* were only down-regulated in the root of susceptible eggplant cultivar. Only one *SmDIR* gene *SmDIR8* was down-regulated in the root of resistant eggplant cultivar. Two *SmDIR* genes including *SmDIR16* and *SmDIR17* were down-regulated in both root and stem in susceptible and resistant eggplant cultivars. Six *SmDIR* genes, including *SmDIR4*, *SmDIR9*, *SmDIR10*, *SmDIR13*, *SmDIR19*, and *SmDIR24*, were down-regulated in the root of both susceptible and resistant eggplant cultivars. The

*SmDIR5* gene was down-regulated in the root and stem of the susceptible eggplant line, but down-regulated only in the root of the resistant eggplant line. The expression patterns of three *SmDIR* genes (*SmDIR7*, *SmDIR15* and *SmDIR22*) were different in different samples, containing both up-regulation and down-regulation (Figure 8A).



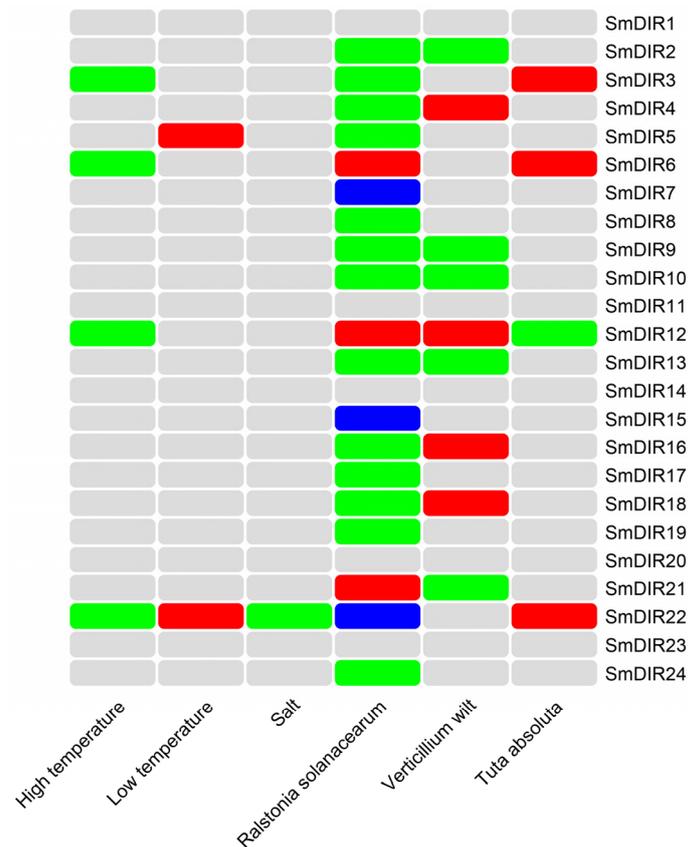
**Figure 8.** The expression heatmaps of eggplant *DIR* family genes under biotic stress treatments. (A) The expression patterns of *SmDIR* genes under bacterial wilt stress. S: susceptible eggplant cultivar; R: resistant eggplant cultivar; CT: control treatment; 1 dpi: 1 day post-inoculation; edo: early disease onset stage; pdo: peak disease onset stage. (B) The expression patterns of *SmDIR* genes under *Verticillium* wilt stress. CT: control treatment; Vm-6 h, Vm-12 h, Vm-24 h and Vm-48 h represented 6, 12, 24 and 48 h after *Verticillium* wilt infection. (C) The expression patterns of *SmDIR* genes under *Tuta absoluta* stress. CT: control treatment; Ta: *Tuta absoluta* infection. In each figure, the data in the boxes of the left heatmap indicated the original FPKM values. Differentially expressed genes were highlighted by red (up-regulation) and green (down-regulation) color with log<sub>2</sub> (fold-change) values in the right table.

Under *Verticillium* wilt stress, a total of nine *SmDIR* genes exhibited significantly differential expression compared to the control treatment. Among them, five *SmDIR* genes, including *SmDIR2*, *SmDIR9*, *SmDIR10*, *SmDIR13*, and *SmDIR21*, were down-regulated at 24 or 48 h after *Verticillium* wilt infection, while four *SmDIR* genes, including *SmDIR4*, *SmDIR12*, *SmDIR16*, and *SmDIR18*, exhibited significantly up-regulated expression after *Verticillium* wilt infection. *SmDIR4*, *SmDIR16*, and *SmDIR12* genes were significantly up-regulated at 48 h after *Verticillium* wilt infection. *SmDIR18* genes were significantly up-regulated at 6 h after *Verticillium* wilt infection (Figure 8B). Under *Tuta absoluta* stress, only four *SmDIR* genes were significantly differentially expressed compared to the control. Among them, three *SmDIR* genes, including *SmDIR3*, *SmDIR6*, and *SmDIR22*, were up-regulated expression, one *SmDIR* gene *SmDIR12* was down-regulated expression after *Tuta absoluta* infection (Figure 8C).

### 3.9. The Regulation Patterns Analysis of Eggplant *DIR* Family Genes under Stresses

To analyze the expression patterns of *SmDIR* genes under biotic and abiotic stresses, the differentially expressed genes were labeled and drawn into a heatmap (Figure 9). The results showed that 19 of 24 *SmDIR* genes were differentially expressed in response to stresses. Among them, five *SmDIR* genes, including *SmDIR3*, *SmDIR5*, *SmDIR6*, *SmDIR12*, and *SmDIR22*, were differentially expressed under multiple types of abiotic and biotic stresses, indicating that these five *SmDIR* genes actively participate in stress responses. Especially notable, the *SmDIR22* gene was differentially expressed under three types of

abiotic stresses and two types of biotic stresses, which could be considered as the favorable gene for the further studies. The other 14 of 24 *SmDIR* genes were only differentially expressed under biotic stresses. The regulation patterns analysis of eggplant *DIR* family genes could provide the theoretical references for further research on the biological function of eggplant *DIR* genes and the resistance breeding of eggplant.



**Figure 9.** The expression patterns heatmap of eggplant *DIR* family genes under abiotic and biotic stresses. Gray color represents that the *SmDIR* genes were not differentially expressed; red color represents up-regulated expression; green color represents down-regulated expression; blue color represents that the *SmDIR* genes exhibited both up-regulated and down-regulated expressions.

#### 4. Discussion

Since the *Arabidopsis thaliana* genome was published in 2000, more and more plant genomes have been sequenced and published, thanks to the rapid development of genome sequencing technology [65]. Based on the high-quality genome information available, the identification of many important gene families has gradually been carried out for different plants. *DIR* proteins have been found in almost all vascular plants and often appear as a gene family [3,12]. *DIR* proteins are a kind of gene family involved in the biosynthesis of cell wall lignins and lignans, which play important roles in plant growth and development, abiotic stress tolerance, biotic stress response, and secondary metabolism regulation [16,66]. The *DIR* gene family has been successively identified in model plants, including *Arabidopsis thaliana* [16] and rice [17,18], and subsequently reported in horticultural plants such as Chinese cabbage [4], pepper [20], strawberry [21], and Cucurbitaceae [22]. As the most important solanaceous crop, eggplant is one of important vegetable crops widely cultivated across the world. However, the identification of the *DIR* gene family in eggplant has not been performed. Therefore, in this study, the *DIR* gene families were identified in eggplant based on the high-quality eggplant genome V4.1 [38]. In addition, the expression profiles of eggplant *DIR* family genes in different organs and stress responses were conducted by reanalyzing the eggplant transcriptome sequencing data, which will provide theoretical

reference for further research on molecular functions of *DIR* genes and screen out the favorable *DIR* gene for eggplant resistance molecular breeding.

The number of *DIR* family genes varied greatly in different plants. In this study, a total of 24 members of the *DIR* gene family in eggplant were identified. Previous studies identified 25, 61, 35, 35, 40, 29, 24, 33, 22, 22, and 17 *DIR* family genes in *Arabidopsis* [16], rice [17,18], spruce [3,12], pear [5], *Populus* [19], Chinese cabbage [4], pepper [20], strawberry [21], watermelon, melon, and cucumber [22], respectively. The numbers of *DIR* genes in most plants that have finished the identification of their *DIR* gene family were more than the number of *DIR* genes in eggplant. The member of the *DIR* gene family in pepper was same with that of *DIR* genes in eggplant, which is probably because both pepper and eggplant belong to the crops of the Solanaceae family. The analysis of physicochemical characteristics showed that most of the *DIR* proteins in eggplant are stable proteins, which is similar to the physicochemical characteristics of *DIR* proteins in Chinese cabbage [4], pepper [20], strawberry [21], watermelon, melon, and cucumber [22]. Phylogenetic tree analysis of 24 eggplant *DIR* family genes were divided into three subgroups: *DIR-a*, *DIR-b/d*, and *DIR-e*, which was similar to the clustering results of phylogenetic analysis of *DIR* family genes in *Arabidopsis* [16], *Populus* [19], and pepper [20]. There were significant differences in gene structure among the *DIR* genes within three different subgroups, and the gene structures and conserved motifs of *DIR* genes in the same subgroup were similar. Most of eggplant *DIR* family genes (71%) only contained one exon and no introns, which were same with the exon/intron structures of *DIR* genes in other plants such as rice [17], pear [5], *Populus* [19], pepper [20], strawberry [21], watermelon, melon, and cucumber [22]. The analysis of gene duplication events of eggplant *DIR* family genes showed five pairs of tandemly duplicated genes and four pairs of segmentally duplicated genes, which revealed that the expansion of the *DIR* gene family in eggplant resulted from both tandem and segmental duplications. This phenomenon also existed in the *DIR* gene families of rice [17], pear [5], pepper [20] and strawberry [21], as well as some other gene families in other plants [67,68]. The synteny analysis of the *DIR* family genes in *Arabidopsis*, rice, and eggplant found that 15 eggplant *DIR* genes were collinear with *Arabidopsis* *DIR* genes, while 16 eggplant *DIR* genes were collinear with rice *DIR* genes, indicating that the eggplant *DIR* family genes have similar homology with *DIR* genes in both *Arabidopsis* and rice.

In recent years, many transcriptome sequencing projects in eggplant have been conducted with the rapid development of high-throughput sequencing technology, forming the eggplant transcriptome sequencing big data. Therefore, making good use of these transcriptome sequencing data could not only reduce the cost of research, but also enable in-depth mining of these data. Moreover, the biological functions of different gene families in eggplant could be studied using the eggplant transcriptome sequencing big data under different treatments. In this study, with the published eggplant transcriptome sequencing big data, the organ-specific expression patterns and the stress-responsive gene expression patterns of 24 eggplant *DIR* family genes were analyzed. The results showed that only *SmDIR3* was highly expressed in all 19 of the organs of eggplant. Some *SmDIR* genes, including *SmDIR7*, *SmDIR8*, *SmDIR11*, *SmDIR14*, *SmDIR18*, *SmDIR19*, *SmDIR20*, and *SmDIR23*, were not or were lowly expressed in the eggplant organs. While the other eggplant *DIR* family genes showed an organ-specific expression pattern. Previous studies have shown that *DIR* genes can contribute to the lignification of plant organs, suggesting that the *DIR* genes are essential for healthy plant growth. In our study, most of the *SmDIR* genes were specifically expressed in the radicle and root, indicating that these *SmDIR* genes play an important role in the development of radicle and root [69]. The organ-specific expression pattern of these *SmDIR* genes in different organs also cooperatively regulated the plant growth and development of eggplant.

In terms of the expression profiles of *SmDIR* genes in response to biotic and abiotic stress, 19 of 24 *SmDIR* genes were differentially expressed in response to stresses, which indicated that most of *SmDIR* genes were involved in response to stresses. Among them, five *SmDIR* genes, including *SmDIR3*, *SmDIR5*, *SmDIR6*, *SmDIR12*, and *SmDIR22*, were

differentially expressed under multiple types of abiotic and biotic stresses, indicating that these five *SmDIR* genes actively participate in stress responses. Especially notable, the *SmDIR22* gene was differentially expressed under three types of abiotic stresses and two types of biotic stresses. In addition, it was also found that the expression patterns of eggplant *DIR* family genes under biotic and abiotic stresses were different. The number of differentially expressed *DIR* family genes in response to abiotic stresses was less than the number of differentially expressed *DIR* family genes in response to biotic stresses. The number of up-regulated *DIR* genes in response to biotic stresses was more than that in response to abiotic stresses. These results support the hypothesis that *DIR* genes are associated with plant-pathogen/insect infection. After the infections by pathogen and insect, more *DIR* family genes would be significantly up-regulated to accumulate more lignin, thus preventing infections by pathogen and insect. Many studies have demonstrated the importance of *DIR* genes in plant-pathogen interactions [70,71]. In addition, some eggplant *DIR* genes also play an important role in abiotic stresses, especially under high temperature, low temperature, and drought, which is consistent with the types of *cis*-elements in the promoter of *DIR* genes. Whereas, most of the eggplant *DIR* genes were down-regulated in response to stresses, which is also worthy of further investigation.

## 5. Conclusions

In this study, a total of 24 *DIR* family genes were identified in eggplant, which were divided into three subgroups of *DIR-a*, *DIR-b/d*, and *DIR-e*. The gene structure and protein conserved motifs in the same subgroup were highly conservative, and the distributions of exon/intron and motifs differed among different subgroups. The organ-specific expression patterns and the stress-responsive gene expression patterns of eggplant *DIR* family genes were studied by re-analyzing the eggplant transcriptome sequencing data with eggplant genome V4.1. The results showed that the expression patterns of eggplant *DIR* family genes in different organs and stress responses were different, synergistically regulating the plant growth and development of eggplant. Among the eggplant *DIR* family genes, the *SmDIR22* gene was differentially expressed under three types of abiotic stresses and two types of biotic stresses, which indicated that the *SmDIR22* gene actively participated in stress responses. The results of the current study will provide a theoretical reference for further study of the biological functions of eggplant *DIR* family genes, and highlight *SmDIR22* as the favorable gene for molecular breeding of eggplant resistance.

**Author Contributions:** L.J. conceived the research and designed the experiments. K.Z. performed research, analyzed the data and wrote the manuscript. W.X., S.S. and D.Y. participated in downloading transcriptome sequencing data and helped with the bioinformatics analysis. F.Z., H.J. and C.Y. analyzed and interpreted the data. All authors have read and agreed to the published version of the manuscript.

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