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Identification and expression analysis of strigolactone biosynthetic and signaling genes in response to salt stress in soybean (*Glycine max*)

Yan-hua Qiao[1,a] , Wen-xiu Lu[2,a], Ru wang[3], Zaib_un Nisa[4], Yang Yu[5], Xiao-xia Jin[6], Li-jie Yu[7,*] and Chao Chen[8,*]

[1] School of Life Science and Technology, Harbin Normal University, Harbin 150025, P.R. China; QYH5505@163.com

[2] School of Life Science and Technology, Harbin Normal University, Harbin 150025, P.R. China; luwenxiu1996@sina.com

[3] School of Life Science and Technology, Harbin Normal University, Harbin 150025, P.R. China ; 1243109209@qq.com

[4] Plant stress physiology and molecular biology Lab, Department of Botany, GC Women, university Faisalabad, Pakistan; zaib.nisa@gcwuf.edu.pk

[5] Key Laboratory of Soybean Molecular Design Breeding, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin, China; yuyang_haqu@iga.ac.cn

[6] School of Life Science and Technology, Harbin Normal University, Harbin 150025, P.R. China; xiaoxia6195@126.com

[7] School of Life Science and Technology, Harbin Normal University, Harbin 150025, P.R. China; yulijie1961@126.com

[8] School of Life Science and Technology, Harbin Normal University, Harbin 150025, P.R. China; chchao@hrbnu.edu.cn

* Correspondence: chchao@hrbnu.edu.cn; yulijie1961@126.com

a: These authors contributed equally to this work.

Abstract: Strigolactones (SLs) are a novel emerging plant hormones, which play important roles in regulating plant organ development and environmental stress tolerance. Even though the SL related genes have been identified and well characterized in some plants. The information of SL related genes in soybean is not fully established yet, especially in response to salt stress. In this study, we identified nine SL biosynthesis genes: two D27, two CCD7, two CCD8, and three MAX1, and seven SL signaling genes: two D14, two MAX2 and three D53 in soybean genome. We found that SL biosynthesis and signaling genes are conserved during evolution in different species. Syntenic analysis of these genes revealed their location on nine chromosomes as well as existence of ten pairs of duplication genes. Moreover, plant hormone and stress-responsive elements were identified in the promoter regions of SL biosynthesis and signaling genes. By using quantitative real-time PCR (qRT-PCR), we confirmed that SL genes have different tissue expression in roots, stems and leaves. Further, we also explored the expression profiles of SL biosynthesis and signaling genes under salt stress. These results suggested that SL signaling genes may play important regulatory roles in response to salt stress. In conclusion, we identified and provided valuable information on the soybean SL biosynthesis and signaling genes, and established a foundation for further functional analysis of soybean SL related genes in response to salt stress.

Keywords: Soybean; Strigolactones; Biosynthesis and signaling genes; Expression patterns; Salt stress

1. Introduction

Strigolactones, as novel emerging hormones, are a class of terpeniod lactone derived from carotenoid [1]. SLs were first identified as a root secretory component for germination of parasite

weed Striga lutea and isolated from root exudates in cotton [2, 3]. Studies have been demonstrated that SLs play important roles in regulating plant growth and development, including shoot branching, soybean nodulation, early seedling development and root growth [4-6].

SL transduction pathways include biosynthesis and signaling. The four mainly major enzymes in SL biosynthetic pathway have been identified: a *cis*/*trans*-carotene isomerase DWARF27 (D27), two carotenoid cleavage dioxygenase (CDD7 and CDD8), and a cytochrome P450 monooxygenase (MAX1) [7, 8]. Recently, researchers also found that oxidoreductase-like enzyme (LBO) could inhibit shoot branching in *Arabidopsis*, which may play important roles in the later steps of strigolactone biosynthesis [9]. In *Arabidopsis*, *Fragaria vesca* and rice, the three mainly SL signaling pathway enzymes have been investigated: a SL receptor α/β hydrolyzyme (D14), a ubiquitin-related protein F-box leucine-rich repeat (LRR) protein (D3/MAX2), and a transcriptional repressor Clp ATPase family protein (D53/ MXL6/7/8) [10, 11].

Out of which, biosynthesis of SL begins with the trans-β-carotene, which is catalyzed by D27 to 9-cis-β-carotene. Next, the CCD7 and CCD8 convert 9-cis-β-carotene into carlactone, the biosynthetic precursor for a class of SLs [12]. Further, in the presence of SLs, D14 can hydrolyze SL molecules and trigger a spatial structure change, which form a complex with D53 and D3 [13, 14]. Studies also discovered that transcriptional co-repressor transcription factor Ideal Plant Architecture 1 (IPA1) and transcriptional co-repressor TOPLESS (TPL)-related protein (TPR2) are major components in SL signaling [15, 16]. For example, in the absence of SLs, D53 can interact with TPR2 and IPA1 to repress the expression of downstream IPA1-regulated genes with no SL response [13].

Previous studies on SLs have focused on their roles in response to various environmental stresses, such as drought, salt and osmotic stresses [17-19]. Furthermore, some SL biosynthetic and signaling genes have been identified to play significant roles in environmental stresses. For example, SL-deficient and SL-signaling max mutants *max2*, *max3* (*CDD7*) and *max4* (*CDD8*) exhibited hypersensitivity towards drought and salt stress in *Arabidopsis* [20]. Overexpression of *Sapium sebiferum MAX2* in *Arabidopsis* enhanced plant tolerance to osmotic, drought and salt stresses [11]. *OaMAX2* could restore the drought-tolerant phenotype of *atmax2* mutant [21]. Soybean is considered as crop with vegetable oil and abundant protein. As a moderately salt-tolerant crop, salt stress exerts serious effects to soybean yield in the world. Although prior work have identified *MAX1*/2/3/4 genes in soybean, and clarified the function of *GmMAX3* in soybean nodulation [22]. However, the whole soybean SL biosynthesis and signaling genes have not been identified so far. Moreover, little is known about the soybean SL biosynthesis and signaling genes in response to environment stresses, especially salt stress.

Hence, in this study, we obtained sixteen SL biosynthesis and signaling genes, and identified their chromosomal locations and gene-duplication, promoter cis-elements and expression patterns in different tissues under salt stress. These results suggested that SL biosynthesis and signaling genes may play important roles in soybean development and salt stress responses. Our results will also establish a foundation for further research concerning the potential roles of the soybean SL biosynthesis and signaling genes in salt stress response.

2. Results

2.1 Identification of SL biosynthetic genes CCD7, CCD8, MAX1 and D27 in soybean

SLs play crucial roles in plant response to salt stress [16]. Hence, in this study, we intended to identify the soybean SL biosynthetic and signaling genes as well as to investigate their expression profiles. To get all SL biosynthetic members in soybean, we used CCD7, CCD8, MAX1 and D27 amino acid of rice and *Arabidopsis* as query sequences to establish a Hidden Markov model, respectively. Furthermore, we searched the soybean protein database in NCBI by using HMM profile. As a consequence, we obtained two D27 (*GmD27a* and *GmD27b*), two *CCD7* (*GmCCD7a* and

1 **Table 1.** Protein information of SL biosynthesis and signaling genes in soybean

Gene ID	Gene Name	Amino acid residues	MW (kDa)	pI	Description	Similarity with *Arabidopsis*	
Glyma.02G143300	*GmD27a*	249	27881.44	8.46	SL biosynthetic gene	*DWARF27/D27*	*AT1G03055*
Glyma.10G031100	*GmD27b*	256	28730.57	9.29	SL biosynthetic gene	*DWARF27/D27*	*AT1G03055*
Glyma.U016700	*GmCCD7a*	618	69.37356	9.00	SL biosynthetic gene	*CCD7/MAX3*	*AT2G44990*
Glyma.01G073200	*GmCCD7b*	614	69.31318	7.99	SL biosynthetic gene	*CCD7/MAX3*	*AT2G44990*
Glyma.04G084100	*GmCCD8a*	566	63.35102	6.35	SL biosynthetic gene	*CCD8/MAX4*	*AT4G32810*
Glyma.06G085800	*GmCCD8b*	563	62.97967	6.22	SL biosynthetic gene	*CCD8/MAX4*	*AT4G32810*
Glyma.04G052100	*GmMAX1a*	548	61773.46	8.96	SL biosynthetic gene	*CYP711A1/MAX1*	*AT2G26170*
Glyma.06G052700	*GmMAX1b*	548	61846.45	8.92	SL biosynthetic gene	*CYP711A1/MAX1*	*AT2G26170*
Glyma.17G227500	*GmMAX1c*	532	60178.94	8.8	SL biosynthetic gene	*CYP711A1/MAX1*	*AT2G26170*
Glyma.14G089000	*GmD14a*	266	29591.82	5.72	SL signaling gene	*D14*	*AT3G03990*
Glyma.17G235300	*GmD14b*	269	29938.20	5.78	SL signaling gene	*D14*	*AT3G03990*
Glyma.06G277000	*GmMAX2a*	718	80333.59	5.66	SL signaling gene	*MAX2/ORE9/PPS*	*AT2G42620*
Glyma.12G128600	*GmMAX2b*	711	79146.65	5.79	SL signaling gene	*MAX2/ORE9/PPS*	*AT2G42620*
Glyma.02G226900	*GmD53a*	1061	117572.88	6.80	repressor of SL signalling	*D53*	*AT1G07200*
Glyma.14G193900	*GmD53b*	1094	120699.50	6.16	repressor of SL signalling	*D53*	*AT1G07200*
Glyma.18G062300	*GmD53c*	1089	120473.93	7.52	repressor of SL signalling	*D53*	*AT1G07200*

GmCCD7b), two CCD8 (*GmCCD8a* and *GmCCD8b*), and three *MAX1* (*GmMAX1a*, *GmMAX1b* and *GmMAX1c*) genes by removing incomplete domains and overlapping genes. The protein information of SL biosynthetic genes, including the ID, amino acid residues, molecular weight (MW) and isoelectric point (pI) are presented in Table 1.

Previous studies have identified SL biosynthetic and signaling genes in some species, such as *Arabidopsis*, rice, *Medicago truncatula* and *Fragaria vesca* [10, 11]. To further study the conserved domains of soybean SL biosynthetic genes, we performed a MEME online analysis of these proteins with *Arabidopsis*, rice, *Medicago truncatula* and *Fragaria vesca* biosynthetic proteins, respectively. The results showed that all CDD8 (GmCDD8a/b, MtCDD8a/b, AtCDD8, FvCDD8 and OsCDD8) and MAX1 (GmMAX1a/b, MtMAX1, AtMAX1, FvMAX1 and OsMAX1a/b/c) proteins contain same motifs, respectively (Figure S1-2). GmD27a/b, AtD27, FvD27 and OsD27 almost contain same motifs, except that OsD27 did not include motif 4 at C-terminal ((Figure S3). Consistently, GmCDD7a/b, MtCDD7, AtCDD7 and FvCDD7 all contain motifs 1-8 except OsCDD7 (Figure S4). Collectively, these studies indicating that the SL biosynthetic genes are conserved during evolution among different species.

2.2 Identification of SL signaling genes D14, MAX2 and D53 in soybean

Similarly, to identify SL signaling members in soybean, we used D14, MAX2 and D53 amino acid of rice and *Arabidopsis* as query sequences to establish a Hidden Markov model, respectively. As a consequence, we obtained two *D14* (*GmD14a* and *GmD14b*), two *MAX2* (*GmMAX2a* and *GmMAX2b*) and three D53 (*GmD53a*, *GmD53b* and *GmD53c*) genes by removing incomplete domains and overlapping genes. The protein information of SL signaling genes are presented in Table 1. Further, the MEME online analysis showed that MAX2 (GmMAX2a/b, MtMAX2, OsMAX2 and AtMAX2) and D53 (GmD53a/b/c, AtD53, FvD53a/b and OsD53a/b) proteins also have identical motifs composition composed, respectively (Figure S5-6).

D14 is the SL receptor protein in the SL signal pathway [14, 23]. Remarkably, KAI2/HTL/D14-Like (D14L), as a close homolog of D14, is the receptor protein in the KAR signal pathway [24]. In the present study, we identified seven complete domain proteins in soybean protein database by using D14 amino acid in rice and *Arabidopsis* as query sequences. To further identify D14 proteins in soybean, we constructed the phylogenetic tree using these identified proteins, D14 and D14L proteins in other species. The phylogenetic tree was classified into two clades: clade A and clade B (Figure 1A). The KAR receptors *OsD14L*, *AtD14L*, *PpKAI2* and five soybean genes were clustered in clade A according to their bootstrap values. However, the SL receptors *AtD14*, *MtD14*, *FveD14* and other two soybean genes were clustered in clade B. Therefore, we named clade B the D14 clades, and *GmD14a* and *GmD14b* belong to *D14* genes in soybean. To further identify the difference of D14 and D14L, we studied the conserved domains by using MEME online analysis (Figure 1B). The results revealed that all D14 and D14L proteins have identical motif compositions (motifs 1-7). However, only D14L proteins contain motif 8, suggesting the functional differences of D14L and D14 proteins.

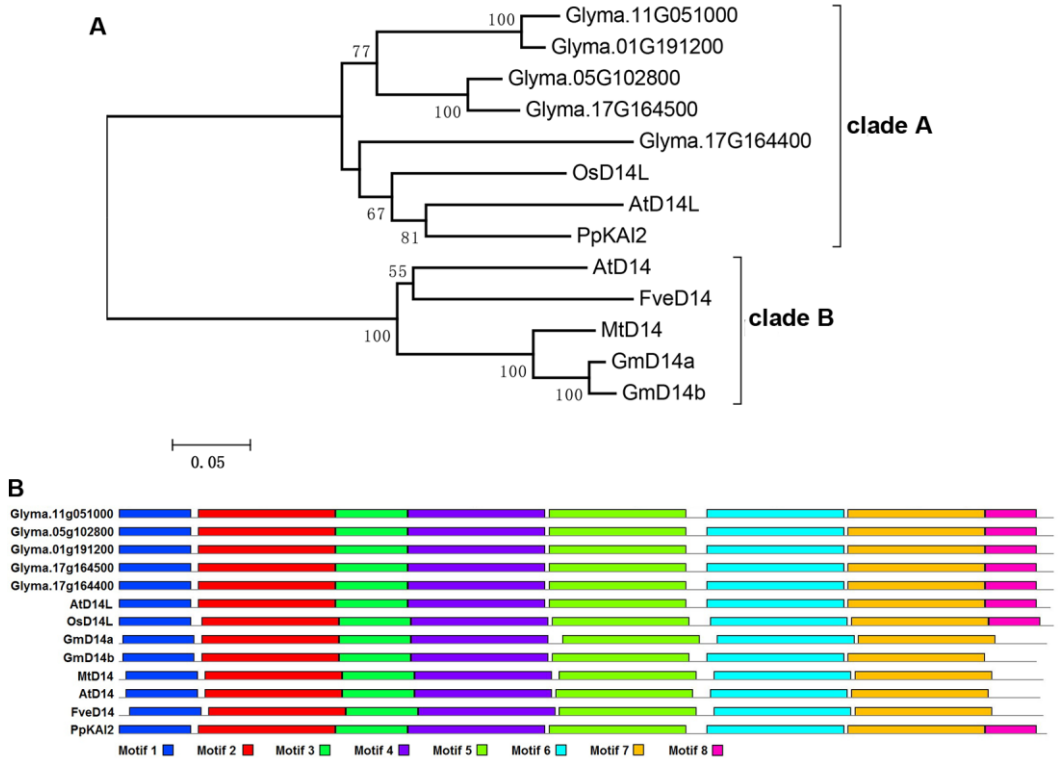

Figure 1. The phylogenetic and conserved domain analysis of D14 and homologous proteins. **A** The phylogenetic relationships of D14 and D14L was identified from soybean, *Arabidopsis*, rice, *Medicago sativa*, woodland strawberry and *Physcomitrella patens*. **B** The motif composition of D14 and D14L proteins were identified using MEME 5.0 software, and the motifs were displayed by boxes of different colors.

2.3 Chromosomal locations and syntenic analysis of SL biosynthetic and signaling genes in soybean

In order to identify the genome distribution of SL biosynthetic and signaling genes, their physical locations and potential genome duplication events were determined using the syntenic analysis. The results showed that the genes were randomly mapped to ten chromosomes (Figure 2). Moreover, we found that 12 genes were located near the edges of the chromosomes except for *GmMAX2b*, *GmD27a*, *GmCCD7a* and *GmCCD7b*.

Gene duplication events have contributed to the plant functional diversity and evolution of novel functions [25, 26]. This study identified that each close homologue of biosynthetic and signaling genes shared the high amino acid similarity. For example, the sequences of GmD27a and GmD27b proteins share the similarity of 96.8 % and GmD14a and GmCCD7b share the similarity of 97.8 %. The results of this analysis also showed that eleven pair of duplication genes were identified, and the duplication genes were linked to each other (Figure 2).

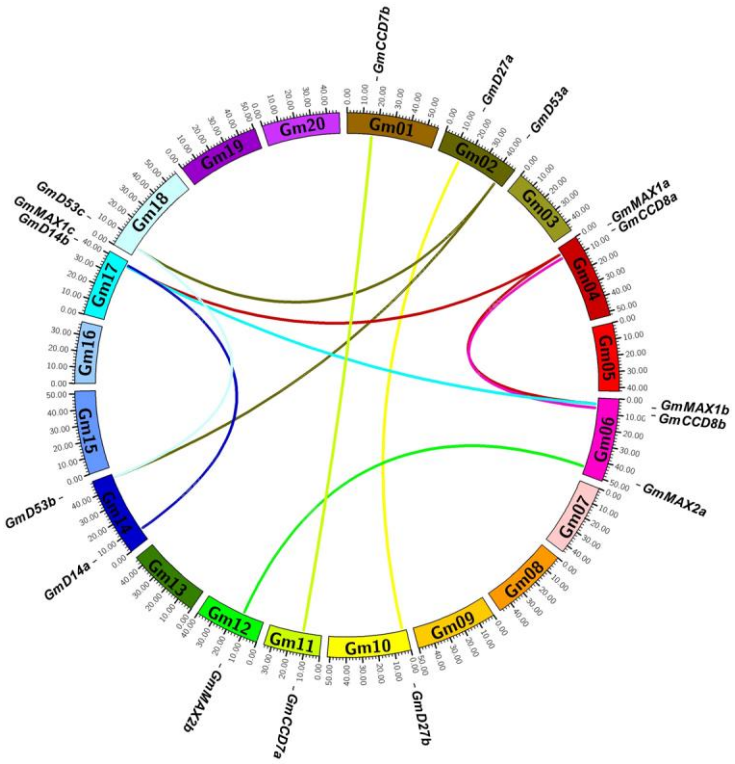

Figure 2. Chromosomal locations and syntenic analysis of SL biosynthesis and signaling genes in soybean. The chromosomes are indicated with different color as a circle. The pair of duplications pairs connected by different color lines.

2.4 Analysis of cis-acting elements of SL biosynthetic and signaling genes promoters in soybean

Cis-acting elements are important molecular switches to regulate various biological processes, such as hormone responses and environmental stress responses [27]. To explore the potential roles of SL biosynthetic and signaling genes in plant response to development stages or environment stresses, we analyzed the promoters of these genes using PLANTCARE online database. Our analyses demonstrated that there are various putative hormone and abiotic stress-related *cis*-acting elements in the promoter region of these genes, including ABRE (ABA), MeJA (methyl jasmonate), SA (salicylie acid), GA (gibberellin), Defense and Stress, and Anaerobic-responsive elements (Table 2). For example, most of genes contain ABRE responsive elements except for *GmCCD7a/b*, *GmD14a/b* and *GmD53b/c*. *GmMAX1a/b* contain large numbers of ABRE, MeJA and Anaerobic responsive elements than other genes. Moreover, we also found that the responsive elements in a pair of duplication genes have large differences. This indicated that the duplication genes may play different roles in plant response to stresses or development stages.

Table 2. Distribution of cis-acting elements on the promoters of SL biosynthesis and signaling genes in soybean

Name	ABRE	MeJA	SA	GA	Auxin	Defense and Stress	Anaerobic induction
GmD27a	4	1	3	0	0	1	2
GmD27b	3	12	1	1	1	2	0
GmCCD7a	0	4	2	0	0	1	3
GmCCD7b	0	1	0	0	0	1	1
GmCCD8a	1	0	1	2	1	0	3
GmCCD8b	3	0	0	1	0	1	2
GmMAX1a	8	6	2	1	1	0	10
GmMAX1b	5	8	0	0	1	0	7
GmMAX1c	3	6	1	0	2	0	1
GmD14a	0	2	0	3	0	1	0

GmD14b	0	0	1	2	0	0	2
GmMAX2a	2	0	1	0	0	0	2
GmMAX2b	4	0	0	0	0	0	4
GmD53a	3	0	2	2	0	1	0
GmD53b	0	4	0	1	1	1	5
GmD53c	0	0	2	3	1	0	1

2.5 Tissue-specific expression analysis of SL biosynthetic and signaling genes

The tissue-specific expression patterns are useful to explore the potential roles of a gene under certain environmental conditions or developmental stages. To explore the expression patterns of SL biosynthetic and signaling genes, we analyzed their expression in roots, stems and leaves by qRT-PCR assays. As shown in Figure 3, the expression of SL biosynthetic genes showed significant variations in three tissues. For example, *GmCCD7a* and *GmCCD7b* displayed a high expression in leaves (Figure 3C-D). *GmMAX1a*, *GmMAX1b* and *GmMAX1c* showed very high expression levels in roots (Figure 3G-I). However, we found that almost all SL signaling genes expressed relatively higher levels in leaves in comparison with roots (Figure 4). Moreover, some duplication genes showed a relatively similar expression patterns, such as *GmCCD7a* and *GmCCD7b*, *GmD14a* and *GmD14b* (Figure 3C-D, Figure 4A-B). Notably, *GmD27a* and *GmD27b*, *GmCCD8a* and *GmCCD8b* showed a relatively different expression patterns (Figure 3A-B, E-F). In conclusion, these results suggested that SL biosynthetic and signaling genes displayed tissue-specific expression patterns, which indicating their potential roles in plant.

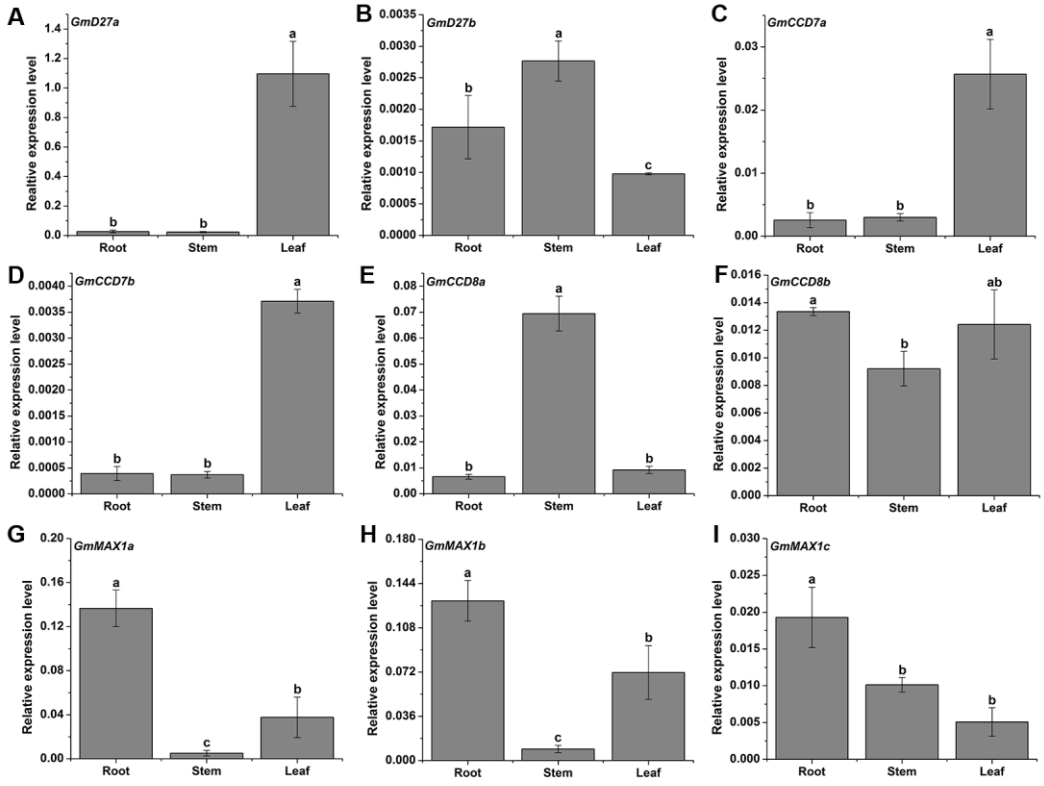

Figure 3. Tissue-specific expression of SL biosynthesis genes in soybean. **A-I** The expression levels of SL biosynthetic genes (*GmD27a*, *GmD27b*, *GmCCD7a*, *GmCCD7b*, *GmCCD8a*, *GmCCD8b*, *GmMAX1a*, *GmMAX1b* and *GmMAX1c*). The relative expression levels were measured using qRT-PCR, and *GAPDH* was used as an internal control. Statistical analyses were performed using the $2^{-\Delta CT}$ method and one-way ANOVA followed by Duncan's test.

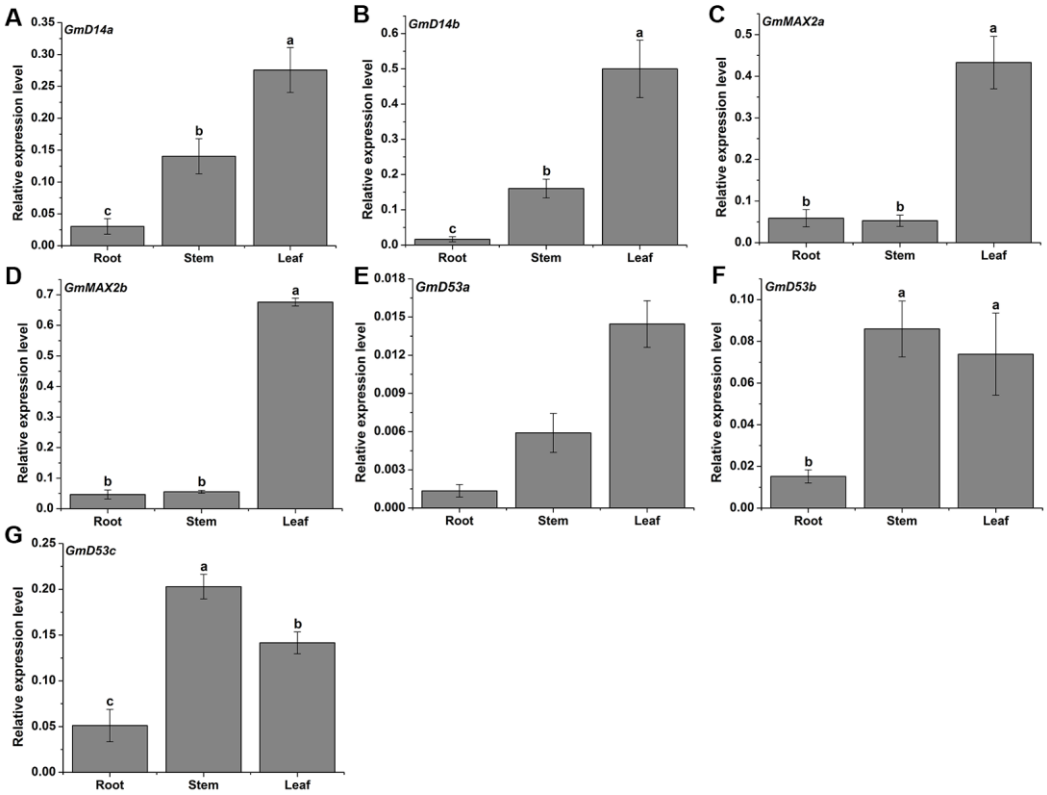

Figure 4. Tissue-specific expression of SL signaling genes in soybean. **A-G** The expression levels of SL signaling genes (*GmD14a*, *GmD14b*, *GmMAX2a*, *GmMAX2b*, *GmD53a*, *GmD53b* and *GmD53c*). The relative expression levels were measured using qRT-PCR, and *GAPDH* was used as an internal control. Statistical analyses were performed using the $2^{-\Delta CT}$ method and one-way ANOVA followed by Duncan's test.

2.6 Expression analysis of SL biosynthetic and signaling genes in response to salt stress

Previous studies showed that some *Arabidopsis* SL genes played positive regulatory roles in response to salt stress [11, 20]. To assess the potential roles of SL biosynthetic and signaling genes in salt stress response in soybean, we further analyzed their expression patterns under salt stress by using qRT-PCR analyses. The results showed that the expression of two up-regulated (*GmCCD7b* and *GmMAX1c*) and six down-regulated (*GmCCD8a*, *GmCCD8b*, *GmD27a*, *GmD27b*, *GmMAX1a* and *GmMAX1b*) SL biosynthetic genes were identified under salt stress (Figure 5). Specifically, we found *GmMAX1c* showed a significantly high expression level, which had a contrary expression with *GmMAX1a and GmMAX1b* (Figure 5G-I). Also, we identified six up-regulated (*GmD14a*, *GmD14b*, *GmMAX2a*, *GmMAX2b*, *GmD53a* and *GmD53b*) SL signaling genes, and only *GmD53c* showed slight decrease in expression under salt stress (Figure 6). The above results indicated that SL genes may participate in plant responses to salt stress in soybean.

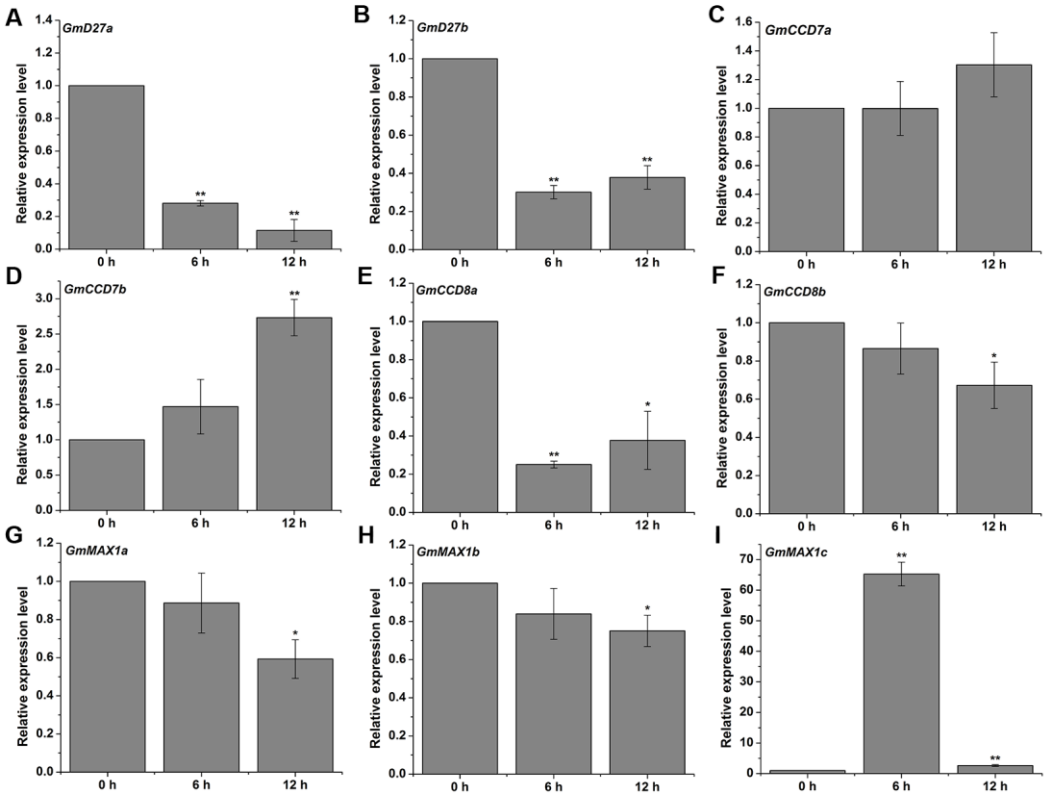

Figure 5. Expression analysis of soybean SL biosynthesis genes in response to salt stress. **A-I** The expression levels of SL biosynthetic genes (*GmD27a*, *GmD27b*, *GmCCD7a*, *GmCCD7b*, *GmCCD8a*, *GmCCD8b*, *GmMAX1a*, *GmMAX1b* and *GmMAX1c*). The seedlings were treated with 200 mM NaCl for 0, 6 and 12 h. The transcript data results were analyzed using the $2^{-\Delta\Delta CT}$ method and Student's t-test (*P < 0.05 and **P < 0.01).

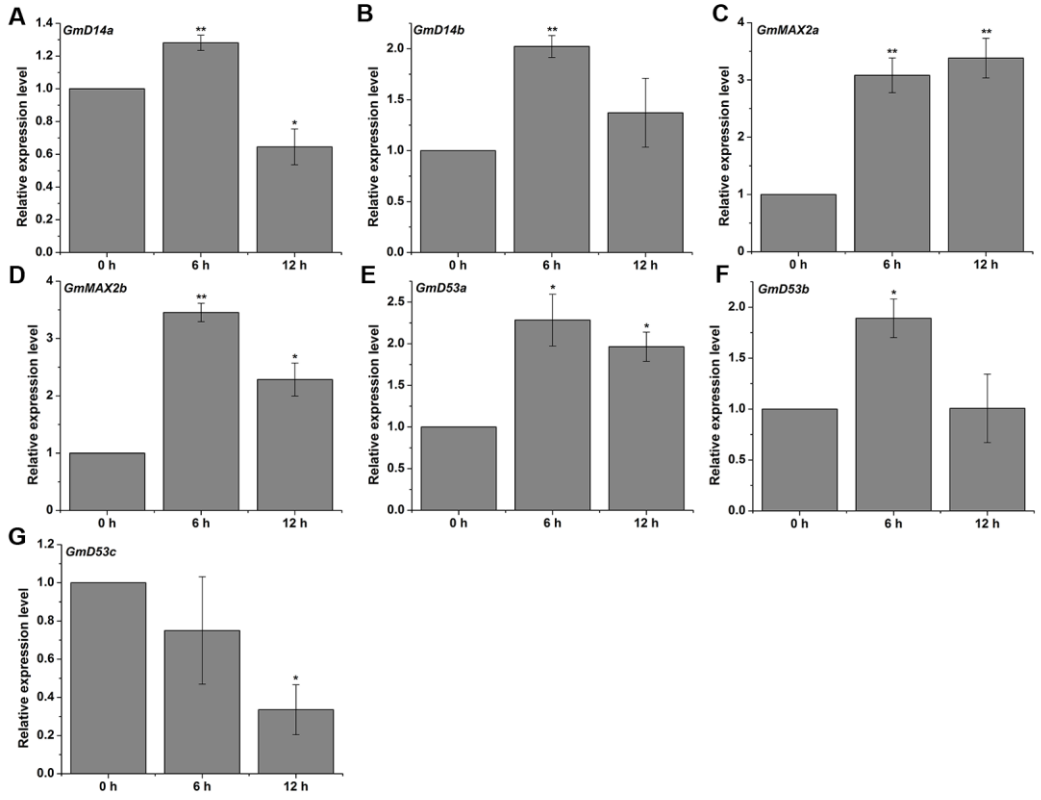

Figure 6. Expression analysis of soybean SL signaling genes in response to salt stress. **A-G** The expression levels of SL signaling genes (*GmD14a*, *GmD14b*, *GmMAX2a*, *GmMAX2b*, *GmD53a*, *GmD53b* and *GmD53c*). The

seedlings were treated with 200 mM NaCl for 0, 6 and 12 h. The transcript data results were analyzed using the $2^{-\Delta\Delta CT}$ method and Student's t-test (*P < 0.05 and **P < 0.01).

3. Discussion

Previously, studies have showed that SL hormones play crucial roles in regulating plant development and stress tolerance [28, 29]. The SL biosynthetic and signaling genes also have been identified in some species, such as *Arabidopsis*, rice and petunia [12]. However, the soybean SL related genes have not been identified, and the roles of these genes in regulating plant salt stress tolerance are rarely studied in soybean. Hence, we identified and investigated the SL biosynthesis and signaling genes, and identify their potential roles in response to salt stress.

In this study, we totally identified 16 SL biosynthetic and signaling genes, according to the rice and *Arabidopsis* SL-related genes (Table 1). *D27*, *CDD7*, *CDD8* and *MAX1* are essential for SL biosynthesis, and *D14*, *MAX2* and *D53* for SL signal transduction [10]. In soybean, the SL biosynthesis and signaling genes almost shared the identical motifs composition with these genes, respectively (Figure 1, Figure S1–6). This indicated SL biosynthetic and signaling genes are conserved during evolution in different species. Previous studies showed that *Arabidopsis* have one each of SL-related genes [30]. However, we found that all soybean SL biosynthesis and signaling genes existed in pair of duplication genes, such as *GmD27a* and *GmD27b*, *GmMAX1a*, *GmMAX1b* and *GmMAX1c*(Figure 2). In plants, the gene duplication will exist novel gene and gene rearrangement, which is an important evolutionary mechanism for plants to adapt to diverse environments [31]. Thus, our results indicated that the SL biosynthetic and signaling genes may existed large gene duplications in soybean genome. Consistent with others plants, petunia has two *MAX2* genes (*PbMAX2A* and *PbMAX2B*) [32], and woodland strawberry contains two *MAX1* genes (*FveMAX1a* and *FveMAX1b*) [11].

SL and KAR signal pathway are two different signal transduction pathways with distinct roles in plant development [12, 33]. D14L is the receptor protein in the KAR signal pathway, which has a close homolog of D14 [24]. In the present study, we also identified five soybean D14L proteins by using rice and *Arabidopsis* D14 amino acid as query sequences. However, the phylogenetic analysis showed that D14L and D14 displayed distinct evolutionary relationship (Figure 1A). Even though D14 and D14L proteins have highly identical motif compositions (Figure 1B), the difference of D14 and D14L motif may suggest the potential distinct ligand specificities of D14L and D14 proteins [24].

Although natural SLs were mainly isolated from the roots in some plants, SLs are synthesized in shoots and roots [34, 35]. However, SL biosynthetic genes have no tissue specific expressions. For example, the expression of *AtCCD7* and *AtD27* were low in root, whereas *AtCCD8* had a high expression in shoot [24]. In woodland strawberry, *FevCCD7*, *FevCCD8* and *FevMAX1* showed high expression levels in stems and low expression in leaves, which showed a contrary expression of *FevD27* [11]. In the present study, we also found the diverse expressions of SL biosynthetic genes in three tissues (Figure 3). In addition, the duplication genes showed a relatively different expression patterns. Taken together, the expression patterns of these genes provide no sufficient guidance on where the SL are mainly synthesized, and the further work is needed in the future.

By contrast, the expression of SL signaling genes *GmD14a/b*, *GmMAX2a/b* and *GmD53a* was relatively higher in leaves, compared with stems and roots (Figure 4). In line with these studies, the high expression of SL signaling genes in leaves was also detected in other species [11, 15, 36, 37]. Studies have shown that SLs play important roles during leaf senescence [38]. SLs could significantly improve leaf chlorophyll contents, increase the activities of peroxidase and superoxide dismutase under salt stress [16]. In addition, overexpression of the *Sapium sebiferum MAX2* gene in *Arabidopsis* could improve drought and salt resistance by regulating redox homeostasis in leaf [11]. These findings indicated the potential roles of SL signaling genes in development and stress responses in leaf.

SLs elicit their control on plant development and environmental response via a direct or indirect influence of other hormones, such as ABA, MeJA, SA, GA and auxin [39, 40]. For example, the crosstalk of SLs and auxin could regulate the root elongation in *Festuca arundinacea Schreb* under heat

stress [29]. In rice, SL-deficient and SL-insensitive mutant with high ABA content had a greater tolerance under salt stress [41]. In the present study, we investigated that the promoter of SL biosynthetic and signaling genes contain various putative hormones-related *cis*-acting elements (Table 2), indicating the potential roles of these genes in relation with plant hormones. Interestingly, we also found that 14 genes contain anaerobic induction-related *cis*-acting elements. However, little information is given for the roles of SLs in response to anaerobic stress. This finding implies that SLs might participate in anaerobic stress responses in soybean.

Studies portray SLs as positive regulators in response to abiotic stresses. Exogenous application of SLs can act as positive regulators of osmotic, drought and salinity stresses [16, 29, 42]. In *Arabidopsis*, *AtMAX2*, *AtMAX3* and *AtMAX4* have been identified to play positive roles in response to salt stress [20]. Consistently, our results also uncovered the differential expression responses of SL-related genes to salt stress. Among SL biosynthetic genes, only *GmCCD7b* and *GmMAX1c* showed greatly increased expression (Figure 5D, I), while the *GmMAX1c* duplication genes *GmMAX1a* and *GmMAX1b* displayed decreased expression (Figure 5G-H). In line with this, the SL signaling genes *GmD53a/b* and *GmD53c* also showed a contrary expression (Figure 6E-G). One possible reason may be presence of different *cis*-acting elements (Table 2), indicating these genes might take part in different pathways. Among SL signaling genes, *GmD14a/b*, *GmMAX2a/b* and *GmD53a/b* exhibited significantly increased expression under salt stress (Figure 6A-F). In addition, the previous studies have focused on the roles of *MAX2* genes under salt stress [11, 21]. This suggested that SL signaling genes may play important regulatory roles in response to salt stress.

4 Materials and methods

4.1 Identification of SL biosynthesis and signaling genes in soybean genome

To get all SL related genes in soybean genome, the amino acid sequences of *Arabidopsis* and rice SL biosynthesis and signaling genes were used as queries to establish a Hidden Markov model, respectively[12]. Then, the HMM profiles were used to blast the proteins by using the soybean genome database. The Pfam (http://pfam.xfam.org) and SMART (http://smart.embl-heidelberg.de) online databases were used to remove incomplete conserved domain and overlapping proteins. Finally, the sequence of sixteen SL biosynthesis and signaling proteins were obtained. The molecular weight (MW) and isoelectric point (pI) values of these proteins were predicted using ExPASy online software (http://au.expasy.org/tools/pi_tool.html).

4.2 Phylogenetic, conserved motif, chromosomal locations and syntenic analyses

The conserved motifs of soybean SL biosynthesis and signaling proteins were identified with other plants, including *Arabidopsis*, rice, *Medicago sativa* and woodland strawberry, by submitting the protein sequences to MEME on line website (http://meme-suite.org), respectively. The neighbor-joining phylogenetic tree was constructed using D14 and D14L proteins in soybean, *Arabidopsis*, rice, *Medicago sativa*, woodland strawberry and *Physcomitrella patens*.

4.3 Analyses of cis-acting elements of soybean SL biosynthesis and signaling gene promoters

The promoter regions of soybean SL biosynthesis and signaling genes were obtained from the PHYTOZOME online database (http://www.phytozome.org). The *cis*-acting elements of promoters were detected using the PLANTCARE online database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html).

4.4 Plant material, growth condition and salt stress treatment

The soybean (DN50) seeds were sterilized in 75% alcohol for 1-2 min followed by several times washing with sterile water. After germination of two days in the dark, the aseptic seedlings were transferred and grown in Hoagland's nutrient solution at 22-28℃ room temperature and 16-h light/8-h dark environmental conditions. Following growth for up to twelve days, the entire root tissue, stem and all leaves except cotyledons were collected. For salt stress treatment, twelve days after sowing, the seedlings were transferred and grown in Hoagland's nutrient solution with 200

mM NaCl for 0, 6 and 12 h. The seedlings were harvested as three biological replicates, and stored at -80 °C.

4.5 Tissue-specific expression and transcript data analysis of SL biosynthesis and signaling genes under salt stress

The samples stored at -80 °C were used for total RNA extraction using the OminiPlant RNA isolation kit (Kangwei). The cDNA was synthesized by First Stand cDNA Synthesis kit (Toyobo) according to the protocol. Quantitative real-time PCR was performed using UtraSYBR Mixture (Baioleibo), with *GmGADPH* as internal control. The *GmGADPH* and SL biosynthesis and signaling gene primers are listed in supplemental Table S1. The tissue-specific expression results were analyzed using the $2^{-\Delta CT}$ method and one-way ANOVA followed by Duncan's test. The RNA transcript data results under salt stress were analyzed using the $2^{-\Delta\Delta CT}$ method and Student's t-test. Three biological and technical replicates were obtained and analyzed.

5. Conclusions

In conclusion, in this study, we identified nine SL biosynthesis genes: two *D27*, two *CCD7*, two *CCD8*, and three *MAX1*, and seven SL signaling genes: two *D14*, two *MAX2* and three *D53* in soybean genome. We also identified their conserved domain, chromosomal locations and gene-duplication as well as promoter *cis*-elements. The results suggested that SL biosynthesis and signaling genes are conserved during the course of evolution among different species, exist with large gene duplications, plant hormone and stress-related *cis*-elements. In addition, we confirmed that SL biosynthesis and signaling genes may play important regulatory roles in response to plant development and salt treatment. Our results will also provide valuable information on the soybean SL biosynthesis and signaling genes, established a foundation for further research concerning the potential roles of these genes in salt stress response.

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Abbreviations

SLs	Strigolactones
qRT-PCR	quantitative real-time PCR
D27	DWARF27
MW	molecular weight
pI	isoelectric point
D14L	D14-Like
ABA	ABRE
MeJA	methyl jasmonate
SA	salicylie acid
GA	gibberellin

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