Genome-Wide Analysis of Fatty Acid Desaturases in Soybean (*Glycine max*)

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Abstract Fatty acid desaturases can introduce double bonds into the hydrocarbon chains of fatty acids to produce unsaturated fatty acids. In the present study, 29 full-length desaturase genes were identified from soybean genome by a thorough annotation exercise. A comprehensive analysis was performed to characterize phylogeny, chromosomal locations, structures, conserved motifs, and expression patterns of those genes. The soybean genes were phylogenetically clustered into nine subfamilies with the *Arabidopsis* counterparts, *FAB2*, *FAD2*, *FAD3*, *FAD5*, *FAD6*, *FAD7*, *FAD8*, *SLD1*, and *DES1*. Twenty-nine desaturase genes were found to be distributed on at least 15 of the 20 soybean chromosomes. The gene structures and motif compositions were considerably conserved among the subfamilies. The majority of desaturase genes showed specific temporal and spatial expression patterns across

different tissues and developmental stages based on microarray data analyses. The study may provide new insights into the origin and evolution of fatty acid biosynthesis pathways in higher plants. Additionally, the characterization of desaturases from soybean will lead to the identification of additional genes for genetic modification of plants to produce nutritionally important fatty acids.

Keywords *Glycine max* · Genome · Fatty acid desaturase · Phylogenetic analysis

Abbreviations

FAB2	Stearoyl-ACP desaturase
FAD2	Microsomal $\Delta 12$ desaturase
FAD3	Microsomal $\omega 3$ desaturase
FAD5	$\Delta 7$ Desaturase
FAD6	Plastidial $\Delta 12$ desaturase
FAD7	Plastidial $\omega 3$ desaturase
FAD8	Plastidial $\omega 3$ desaturase
ADS	$\Delta 9$ Desaturase
SLD1	Sphingolipid $\Delta 8$ desaturase
DES1	Sphingolipid $\Delta 4$ desaturase
D6D	$\Delta 6$ Desaturase
D5D	$\Delta 5$ Desaturase
DesC	Cyanobacterial $\Delta 9$ desaturase
DesA	Cyanobacterial $\Delta 12$ desaturase
DesB	Cyanobacterial $\omega 3$ desaturase

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Introduction

Fatty acids, a major source of energy in plant, are the main compositions of plant membrane lipids and seed storage

lipids (Topfer et al. 1995; Ohlrogge and Browse 1995). The oxidative stability and nutritional value of seed oils, which are already being considerable of commercial importance, are also affected by the levels of fatty acids (Pattee et al. 2002).

The biosynthesis of polyunsaturated fatty acids (PUFAs) in different organisms involves a variety of pathways, which are catalyzed by a complex series of desaturation and elongation steps (Li et al. 2010). Fatty acid desaturases are enzymes that introduce double bonds into the hydrocarbon chains of fatty acids (Singh et al. 2002). They contain three conserved histidine regions with the exception of stearyl-acyl carrier protein (ACP) gene, which contains just two histidine regions (Tao et al. 2006; Shilman et al. 2010). Histidine-rich boxes are thought to form a part of the diiron center where oxygen activation and substrate oxidation occur (Chi et al. 2008a, b; Lu et al. 2009). The desaturation processes take place in both the plastidial membrane and the endoplasmic reticulum (ER) membrane through two different pathways (Ohlrogge and Browse 1995). The genes for ER- and plastid-derived desaturases have been characterized from many plant species to date, capable of introducing double bonds at various locations on the fatty acyl chain (Chi et al. 2008a, b; Lu et al. 2010; Chen et al. 2010).

Soybean (*Glycine max*) cultivated for both high seed protein (42%) and oil contents (23%) since the beginning of humanity has long been one of the most important economical crops all over the world (Dornbos and Mullen 1992; Saski et al. 2005; Zhang et al. 2008a; Vijay et al. 2009). The functional and nutritional value of soybean oil is determined to a large extent by the degree of saturation of triacylglycerol fatty acids. With better understanding of fatty acid metabolic pathways, many desaturase genes with different regioselectivities have been cloned from soybean. A plastidial ω 6 desaturase, designated as *GmFAD6* (Glyma02g36460), has been cloned from soybean (William et al. 1994). Three different soybean omega-6 desaturase genes, *GmFAD2-1*, *GmFAD2-2*, and *GmFAD2-3*, have been reported (Heppard et al. 1996; Tang et al. 2005; Li et al. 2007). *GmFAD2-1A* (Glyma10g42470) and *GmFAD2-1B* (Glyma20g24530) genes, specifically expressed in developing seeds, played a predominant role in determining the PUFA content of seed storage oil. In contrast, *GmFAD2-2* and *GmFAD2-3* were constitutively expressed in both vegetative tissue and developing seeds. Recent soybean genomic analyses have shown that *GmFAD2-2* gene exists as three alleles, *GmFAD2-2A* (Glyma19g32930), *GmFAD2-2B* (Glyma19g32940), and *GmFAD2-2C* (Glyma03g30070; Schlueter et al. 2007). Transcript abundance of *GmFAD2-2C* was observed to increase approximately eightfold in pods grown in cool conditions relative to those in warmer

conditions. The third gene, *GmFAD2-3*, was also constitutively expressed in both vegetative and developing seed tissues but showed no significant changes in transcript levels in cold-stressed leaves (Li et al. 2007). Bilyeu et al. (2003) identified three independent microsomal omega-3 fatty acid desaturases in soybean, *GmFAD3A* (Glyma14g37350), *GmFAD3B* (Glyma02g39230), and *GmFAD3C* (Glyma18g06950). *GmFAD3A* was significantly upregulated in developing seeds whereas *GmFAD3B* and *GmFAD3C* levels remained relatively low in that tissue. A significant downregulation of α -linolenic acid was achieved by siRNA-mediated silencing of three active members of the *FAD3* family in soybean (Flores et al. 2008). Furthermore, the silencing was stably inherited in engineered soybean lines. Three alleles of *GmFAB2* have been identified and characterized from soybean. Transcripts of the *GmFAB2A* (Glyma07g32850) and *GmFAB2B* (Glyma02g15600) were detected most abundantly in developing seeds; however, differences in transcript abundance between A and B were not dramatic (Byfield et al. 2006). High levels of *GmFAB2C* (Glyma14g27990) transcripts were expressed only in developing seeds, and mutations in this gene from two soybean germplasm sources, mutants A6 (30% stearate) and FAM94-41 (9% stearate), resulted in elevated seed stearic acid (Zhang et al. 2008b). Two FAD7 paralogous genes have been identified in soybean, *GmFAD7-1* (Glyma18g43210) and *GmFAD7-2* (Glyma07g18350; Andreu et al. 2010). Both *GmFAD7-1* and *GmFAD7-2* genes were expressed in all soybean tissues examined, displaying their highest mRNA accumulation in leaves. This expression profile contrasted with *GmFAD3A* and *GmFAD3B* mRNA accumulation, which was very low in this tissue.

Currently, the soybean genome has been sequenced, approximately 975 Mb captured in 20 chromosomes, and its gene prediction and annotation are publicly available. Soybean is a well-documented paleopolyploid for which there is molecular evidence of genome duplications that occurred at approximately 59 and 13 Ma ago (Schmutz et al. 2010). The availability of the genome and annotated genes facilitates comparative genomic studies which will help to address major plant biology questions in silico and provide an effective means of gene discovery in focused metabolic situations (Bowman et al. 2007). In the present study, orthologous genes encoding enzymes of fatty acid desaturation from soybean were identified. The identification of novel desaturases will be helpful for the reconstruction of the pathways involved in unsaturated fatty acid biosynthesis and metabolic engineering of fatty acid synthesis in soybean. Moreover, the characterization of desaturases from soybean will provide ample candidate genes for the production of nutritionally important fatty acids in transgenic plants.

Materials and Methods

Gene Retrieval and Annotation

The genome of *G. max* was downloaded from the Phytozome database (<http://www.phytozome.net/soybean>), while the *Arabidopsis* data were available from The Arabidopsis Information Resource (TAIR version 9.0). An initial set of query proteins, including well-characterized and putative fatty acid desaturases identified from *Arabidopsis*, soybean, and other plants, were obtained from GenBank database (National Center for Biotechnology Information). Each protein in this query dataset was used to search the potential novel sequences in soybean and *Arabidopsis* for which whole genome sequences were available by BLASTP and TBLASTN programs, with an *E*-value $<10^{-10}$. The searches were iterated until convergence. As for the incorrectly predicted genes, manual reannotation was performed using online web server FGENESH (<http://linux1.softberry.com/berry.phtml>; Salamov and Solovyev 2000). The putative desaturase genes across soybean genome were listed in Table 1. The other amino acid sequences beyond soybean were retrieved from GenBank and summarized in Table 2. The *Arabidopsis* and soybean proteome data included alternatively splicing variants. We counted these splicing variants of the two organisms as one gene in our statistics in Tables 1 and 2. For instance, Glyma20g24530 had five known splicing variants (Glyma20g24530.1, Glyma20g24530.2, Glyma20g24530.3, Glyma20g24530.4, and Glyma20g24530.5); only one protein was included in our phylogenetic analyses; we counted them as one single gene in Table 1.

Chromosomes Mapping and Gene Structure Prediction

Fatty acid desaturase genes were mapped on chromosomes by determining their chromosomal positions given in the Phytozome soybean database. Gene structure display server program (Guo et al. 2007) was used to illustrate exon/intron organization for individual desaturase gene by comparison of the cDNAs with their corresponding genomic DNA sequences from Phytozome (<http://www.phytozome.net/soybean>).

Conserved Motif Scanning

To identify the conserved motifs, Multiple Expectation Maximization for Motif Elicitation (MEME) version 2.2 (Bailey and Elkan 1995) was employed with a set of parameters as follows: number of repetitions—any, maximum number of motifs—20, and optimum motif width set to ≥ 6 and ≤ 200 . The motifs obtained were annotated using SMART (<http://smart.embl-heidelberg.de/>) and National Center for Biotechnology Information Conserved Domain Database search program.

SSR Loci Screening

The SSR loci were screened in the 41 fatty acid desaturase genes by MISA software (MicroSATellite, <http://pgrc.ipk-gatersleben.de/misa/>). The di-, tri-, tetra-, and penta-nucleotide SSR motifs were used in the sequence analysis. SSR motifs, which repeated more than seven times for di-nucleotide, five for tri-nucleotide, four for tetra-nucleotide, and penta-nucleotide, were identified (Thiel et al. 2003).

Phylogenetic Analysis

Sequence alignments were generated using the ClustalW program (Thompson et al. 1994). The produced files with “*.aln” extension were opened by BioEdit at the option of “Graphic View.” Conserved histidine-rich boxes were identified manually from the BioEdit sequence editor. Phylogenetic analyses were conducted using MEGA version 4 (Tamura et al. 2007). The neighbor-joining phylogenetic tree was constructed with default parameters using complete protein sequences of desaturase genes. Bootstrap with 1,000 replicates was used to establish the confidence limit of the tree branches. Three different protein targeting prediction programs were used to estimate the putative subcellular locations of the candidate proteins: IPSORT (<http://hc.ims.u-tokyo.ac.jp/iPSORT/>), TARGETP (<http://www.cbs.dtu.dk/services/TargetP/>), and PREDOTAR (<http://urgi.versailles.inra.fr/predotar/predotar.html>).

Coexpression Analysis

The gene coexpression patterns were examined using tools at the Genevestigator web site (Zimmermann et al. 2005; <https://www.genevestigator.ethz.ch/>). For genes with more than one probe set, the highest of expression values was considered. When several genes had the same probe set, then they were considered to have same level of transcript abundance. The expression data were gene-wise normalized and hierarchical clustered based on Pearson coefficients with average linkage in the Genesis (version 1.75) program (Sturn et al. 2002).

Results

Identification and Organization of Fatty Acid Desaturase Genes

Forty-one desaturase genes were predicted and annotated from the soybean genome using BLASTP and TBLASTN programs with the query sequence. The candidate genes identified in this study were listed in Table 1. Twelve partial

Table 1 Candidate genes for fatty acid desaturase enzymes in soybean

No.	Gene symbol	Locus tag	Genome location	Gene length (bp)	Protein length (aa)	Extron number	Characterized gene symbol	<i>Arabidopsis</i> ortholog locus	<i>Arabidopsis</i> locus description	Identity (%)	E-value
1	SLD1.1	Glyma01g05470.1	5305194–5307172	1,979	450	1		AT2G46210.1	SLD1.1	77	0
2	FAD8.1	Glyma01g29630.1	39945843–39949387	3,545	452	8		AT5G05580.1	FAD8	70	0
		Glyma01g29630.2	39945843–39949387	3,545	453	8		AT5G05580.1	FAD8	70	0
3	SLD1.2	Glyma02g11820.1	10061635–10063322	1,688	451	1		AT2G46210.1	SLD1.1	76	0
4	FAB2.1	Glyma02g15600.1	14104551–14108401	3,851	391	3	FAB2B	AT2G43710.1	FAB2.2	81	0
5	FAD6.1	Glyma02g36460.1	41942650–41948200	5,551	441	10	FAD6	AT4G30950.1	FAD6	74	0
6	FAD3.1	Glyma02g39230.1	44497345–44501526	4,182	380	8	FAD3B	AT5G05580.1	FAD3	73	e-165
7	FAD2.1	Glyma03g30070.1	38041194–38047360	6,167	383	2	FAD2-2C	AT3G12120.1	FAD2	77	0
		Glyma03g30070.2	38041208–38047360	6,153	384	1		AT3G12120.1	FAD2	77	0
8	FAD8.2	Glyma03g07570.1	8155170–8158680	3,511	453	8		AT5G05580.1	FAD8	70	0
9	FAB2.2	Glyma07g32850.1	37734526–37738422	3,897	402	3	FAB2A	AT2G43710.1	FAB2.2	81	0
10	FAD5.1	Glyma07g03370.1	2368570–2370178	1,609	337	6		AT3G15850.1	FAD5	75	e-145
11	FAD7.1	Glyma07g18350.1	18262673–18265740	3,068	453	8	FAD7-1	AT3G11170.1	FAD7	67	0
12	SLD1.3	Glyma08g41120.1	41164980–41167316	2,337	448	1		AT2G46210.1	SLD1.1	73	0
13	FAD5.2	Glyma08g22730.1	17244861–17246951	2,091	390	5		AT3G15850.1	FAD5	63	e-149
14	FAD2.2	Glyma09g17170.1	20913906–20915066	1,161	386	1		AT3G12120.1	FAD2	72	e-172
15	FAD2.3	Glyma10g42470.1	49416645–49418380	1,736	387	2	FAD2-1A	AT3G12120.1	FAD2	69	e-165
16	FAD3.2	Glyma11g27190.1	26805260–26808430	3,171	381	8		AT5G05580.1	FAD3	69	e-164
17	DES1.1	Glyma13g31440.1	33848800–33850851	2,052	324	2		AT4G04930.1	DES1	79	e-155
18	SLD1.4	Glyma14g10890.1	9085877–9087926	2,050	448	1		AT2G46210.1	SLD1.1	62	e-172
19	FAB2.3	Glyma14g27990.1	34322879–34325575	2,697	337	2	FAB2C	AT1G43800.1	FAB2.1	79	e-160
20	FAD3.3	Glyma14g37350.1	46647783–46651952	4,170	376	8	FAD3A	AT5G05580.1	FAD3	71	e-165
21	DES1.2	Glyma15g07900.1	5561199–5563177	1,979	324	2		AT4G04930.1	DES1	82	e-160
22	SLD1.5	Glyma17g34640.1	38625376–38627403	2,028	448	1		AT2G46210.1	SLD1.1	62	e-175
23	FAD6.2	Glyma17g08220.1	6090607–6091631	1,025	211	3		AT4G30950.1	FAD6	68	6e-74
24	SLD1.6	Glyma18g15260.1	15343498–15345414	1,917	326	1		AT2G46210.1	SLD1.1	74	e-155
25	FAD7.2	Glyma18g43210.1	52649022–52651597	2,576	453	8	FAD7-2	AT3G11170.1	FAD7	67	0
26	FAD3.4	Glyma18g06950.1	5619528–5622303	2,776	380	8	FAD3C	AT5G05580.1	FAD3	72	e-167
27	FAD2.4	Glyma19g32940.1	40627566–40632255	4,690	383	2	FAD2-2B	AT3G12120.1	FAD2	77	0
28	FAD2.5	Glyma19g32930.1	40622192–40623183	992	320	2	FAD2-2A	AT3G12120.1	FAD2	63	e-133
29	FAD2.6	Glyma20g24530.1	34177085–34179657	2,573	387	2	FAD2-1B	AT3G12120.1	FAD2	68	e-163
		Glyma20g24530.2	34176265–34179657	3,393	388	2		AT3G12120.1	FAD2	68	e-163
		Glyma20g24530.3	34177927–34179657	1,731	388	2		AT3G12120.1	FAD2	68	e-163
		Glyma20g24530.4	34176265–34179657	3,393	388	2		AT3G12120.1	FAD2	68	e-163
		Glyma20g24530.5	34176265–34179657	3,393	380	1		AT3G12120.1	FAD2	68	e-163

30	/	Glyma01g10120.1	13009086–13010193	1,108	171	5	AT5G05580.1	FAD3	69	5e-061
31	/	Glyma03g30050.1	38033607–38034511	905	272	3	AT3G12120.1	FAD2	43	3e-059
32	/	Glyma03g30060.1	38037143–38037511	369	110	2	AT3G12120.1	FAD2	68	5e-031
33	/	Glyma06g38430.1	41343359–41343739	381	127	1	AT3G12120.1	FAD2	77	9e-061
34	/	Glyma09g14360.1	16319097–16319246	240	50	1	AT3G12120.1	FAD2	80	9e-022
35	/	Glyma10g20450.1	25937324–25937704	381	116	2	AT3G12120.1	FAD2	69	2e-051
36	/	Glyma12g13970.1	12623850–12624319	470	87	2	AT4G30950.1	FAD6	77	2e-014
37	/	Glyma13g08970.1	9872718–9877985	5,268	387	6	AT1G43800.1	FAB2.1	52	e-105
38	/	Glyma13g08990.1	9921786–9922355	570	190	1	AT1G43800.1	FAB2.1	63	3e-064
39	/	Glyma13g27910.1	31024611–31025503	893	125	4	AT4G30950.1	FAD6	60	7e-015
40	/	Glyma15g20600.1	18498232–18499998	1,767	176	4	AT4G30950.1	FAD6	64	3e-070
41	/	Glyma15g23200.1	22244290–22245053	764	254	1	AT3G12120.1	FAD2	79	e-145

sequences existed in the obtained soybean putative desaturase genes, including six *FAD2*, three *FAD6*, two *FAB2*, and one *FAD3* gene. To facilitate the classification and phylogenetic analyses of different types of desaturases, these partial sequences were excluded in the following study. The remaining 29 full-length genes contained typical histidine-rich boxes (Table 3), which accorded with the standard of different types of desaturase genes. For example, all the genes from *FAD6* subfamily matched the standard for plastidial $\Delta 12$ desaturase, i.e., GHDCXH, HX₂HH, and HXPHH; two histidine-boxes of *FAB2* genes were consistent with these of plastidial stearyl-ACP desaturases that were represented as EENRHG, DEKRHE. Compared to *Arabidopsis*, soybean appeared to possess multiple copies of the gene due to the consequence of repeated rounds of genome duplication followed by limited sequence loss (Schmutz et al. 2010).

Figure 1 showed the distribution of the fatty acid desaturase genes on different soybean chromosomes. As for *Arabidopsis thaliana*, 18 fatty acid desaturase genes were found to distribute on five chromosomes. Seven genes were located on chromosome 3, four on chromosome 2, three on chromosome 5, and two on chromosomes 1 and 4. As shown in Fig. 1, 29 soybean desaturase genes were found to be distributed on at least 15 of the 20 chromosomes. Substantial clustering of these genes was evident on several chromosomes. The maximum number of genes was located on chromosome 2 as four. Three genes were located on chromosomes 7, 14, and 18; two on chromosomes 1, 3, 8, 17, and 19; and one on chromosomes 9, 10, 11, 13, 15, and 20.

Different members of many fatty acid gene subfamilies were located in different soybean chromosomes. This was observed for the *FAB2*, *FAD2*, *FAD3*, *FAD7*, *FAD8*, *DES1*, and *SLD1* gene subfamilies. For instance, different gene members of *FAB2* subfamily were located on chromosomes 2, 7, and 14, respectively. For the *SLD1* subfamily, different members were located on chromosomes 1, 2, 8, 14, 17, and 18, respectively. Almost all the members of each subfamily were distributed in different chromosomal regions except that *GmFAD2.4* and *GmFAD2.5* were tandemly duplicated within the same small chromosomal region. This indicated that fatty acid desaturase genes were distributed widely in the soybean genome and they might originate from different ancestors.

Gene Structures and Distribution of Conserved Motifs

The MEME motif search tool was employed to identify the conserved motifs presenting in soybean and *Arabidopsis* fatty acid desaturases (Fig. 2), and 20 distinct motifs were identified. Most of the motifs belonged to the coiled coil region (transmembrane) and a region of unknown com-

Table 2 List of organisms and desaturase protein sequences analyzed in this study (except for the above sequences from soybean genome)

Organisms	Accession	Gene symbol
<i>Arabidopsis thaliana</i>	AT1G43800.1	FAB2.1
	AT2G43710.1	FAB2.2
	AT2G43710.2	FAB2.2
	AT3G02610.1	FAB2.3
	AT3G02620.1	FAB2.4
	AT3G02630.1	FAB2.5
	AT5G16230.1	FAB2.6
	AT5G16240.1	FAB2.7
	AT1G06080.1	ADS.1
	AT2G31360.1	ADS.2
	AT3G12120.1	FAD2
	AT3G12120.2	FAD2
	AT4G30950.1	FAD6
	AT3G11170.1	FAD7
	AT5G05580.1	FAD8
	AT2G29980.1	FAD3
	AT2G29980.2	FAD3
	AT3G15850.1	FAD5
	AT2G46210.1	SLD1.1
	AT3G61580.1	SLD1.2
<i>Chlamydomonas reinhardtii</i>	AT4G04930.1	DES1
	EDP04705	FAB2
	EDP04777	FAD2
	EDP03637	FAD6
	EDP09401	FAD7
<i>Homo sapiens</i>	EDP02613	FAD5
	BAG35237	ADS
	AAD20018	D6D
<i>Rattus norvegicus</i>	AAF29378	D5D
	EDL94279	ADS
	BAA75496	D6D
<i>Synechocystis</i> sp. PCC 6803	AAG35068	D5D
	BAA03982	DesC
	BAA18169	DesA
<i>Brassica napus</i>	BAA02924	DesB
	AAF78778	FAD2
	AAA50157	FAD6
<i>Borago officinalis</i>	CAA11857	SLD1
	AAG43277	SLD1
	AAD01410	D6D
<i>Synechococcus</i> sp. PCC 7002	AAF21445	DesA
	AAB61352	DesB
<i>Chlorella vulgaris</i>	BAB78716	FAD2
	BAB78717	FAD3
<i>Ostreococcus lucimarinus</i>	ABO95591	FAB2
	ABP00213	FAD5
<i>Mortierella alpina</i>	CAB38177	ADS

Table 2 (continued)

Organisms	Accession	Gene symbol
<i>Acheta domesticus</i>	AAK25797	ADS
<i>Synechococcus elongatus</i> PCC 7942	ABB58591	DesC
<i>Haematococcus pluvialis</i>	ABP57425	FAB2
<i>Arachis hypogaea</i>	ACI45450	FAB2
<i>Lotus corniculatus</i>	AAY78547	FAB2
<i>Linum usitatissimum</i>	CAA07349	FAB2
<i>Bassia scoparia</i>	AAL26877	FAB2
<i>Cyprinus carpio</i>	CAB57858	ADS
<i>Caenorhabditis elegans</i>	AAF97550	ADS
<i>Drosophila simulans</i>	CAB52475	ADS
<i>Gallus gallus</i>	CAA42997	ADS
<i>Saccharomyces cerevisiae</i>	AAA34826	ADS
<i>Cyanidioschyzon merolae</i>	BAA28834	ADS
<i>Phaeodactylum tricornutum</i>	AAW70158	ADS
<i>Oryza sativa</i>	EEE57453	DES1
<i>Limnanthes douglasii</i>	AAG28599	D5D
<i>Danio rario</i>	Q9DEX7	D5D/D6D
<i>Echium pitardii</i>	AAL23581	D6D
<i>Thalassiosira pseudonana</i>	AY817154	SLD1
<i>Helianthus annuus</i>	CAA60621	SLD1

plexity which represented the typical domains of desaturase as indicated by SMART. No common motifs were found in all the members of the fatty acid desaturase family. All proteins belonging to *FAD2* subfamily had the motifs 2, 5, 6, 15, 16, and 17, except for *GmFAD2.5*, which did not have motif 17. All *FAD3/FAD7/FAD8* subfamily had the motifs 2, 3, 5, and 14, except for *GmFAD7.2*, which had another motif 13. All *FAB2* subfamily members possessed the motifs 1, 8, 9, 13, and 20, whereas the motif 9 was absent in *GmFAB2.3*. All *SLD1* proteins had the motifs 4, 6, 10, 12, 14, and 18, except for *GmSLD1.6*, which was lack of motifs 10 and 18. The conserved motifs 7 and 11 were presented only in the *FAD5/ADS* and *DES1* subfamily members, respectively.

Genes in the same subfamily had more similar exon/intron structures than those genes in the other subfamilies (Fig. 2). The *FAD3/FAD7/FAD8* subfamily members mostly had up to eight exons whereas the *FAB2* subfamily members possessed two to four exons. Members of *DES1* subfamily had two exons, compared to the *FAD5/ADS* subfamilies, which possessed five to six exons. All of *FAD2* and *SLD1* subfamily members had one exon, except for *GmFAD2.5*, which possessed two exons. The *FAD6* subfamily members usually possessed ten exons, whereas three exons were present in *GmFAD6.2*.

Table 3 Conserved histidine-rich boxes of fatty acid desaturases in soybean

Name	H-box1	H-box2	H-box3
FAB2	WTRAWTAENRHGDL	AA(S)DEKRHE	/
FAD2	GVVWIAHECGHHAFS	VPYFSWKYSHRRHHSNTGSL	ITD(G)THVA(V)HHLFSTMPH
FAD6	FVIGHDCAHRSFS	EPWRFKHDRHHAKTN	HDINVHIPHHISP
FAD3/FAD7/FAD8	TMFWALFVLGHDCGHGSFS	YHGWRISHRTHHQN(H)HGH	HHDIGTHVIHHLFPQIPHYHL
FAD5	GITLSFHRNLSHRSFK	QGNPIDWVSTHRYHHQFCD	GEGWHNNHHAFEYSAR
SLD1	GHDSGHY	WWKWITHNAHHIA(S)CNSLD	GGLQFQLEHHLPRLPR
DES1	NLFLAIHELSHNLAF	TFQKYHLEHHRFQGVGDID	VGYHNEHHDFFRIPG

SSR Markers Development and Marker-Assisted Breeding

During the last decade, microsatellites or SSRs have been proven to be useful markers in plant genetic research and have been used for marker-assisted breeding purposes (Varshney et al. 2005). SSR markers have some advantages, such as (1) highly informative, (2) locus-specific and frequently show co-dominant inheritance, (3) adaptable to high-throughput genotyping, and (4) simple to maintain and distribute (Yu et al. 2004). Unfortunately, the development of SSR markers is expensive, labor-intensive, and time-consuming if SSRs are developed from genomic libraries (Senthilvel et al. 2008). With the completion of soybean genomic sequences, the development of SSR markers will be facilitated in a large-scale level.

By MISA analysis, there were eight SSR loci derived from seven different genes out of 41 candidate genes involved in fatty acid biosynthesis (Table 4). All these SSRs could be divided into four kinds of motifs, such as di-

nucleotide, tri-nucleotide, tetra-nucleotide, and penta-nucleotide. The di- and tri-nucleotide motifs were the most frequent motifs in eight SSR loci. These SSR loci were distributed in the 5' UTR, intron, and exon. Five SSR loci were located in the 5' UTR, two in the intron, and one in the exon. These SSR markers could help geneticists and breeders identify fatty acid biosynthesis genes in different accessions and cultivars in order to screen the available soybean crops which hold nutritional value of seed oils.

Phylogenetic Analysis of Fatty Acid Desaturase Genes from Soybean Genome

The polyunsaturated fatty acids are synthesized by two distinct pathways in plants, known as the prokaryotic and eukaryotic pathways, which are located within the plastid and the endoplasmic reticulum, respectively (Sato and Moriyama 2007). Therefore, two major classes of desaturases exist: soluble and membrane-bound desaturases.

Fig. 1 Chromosomal locations of soybean fatty acid desaturase genes. Only those chromosomes bearing fatty acid desaturase genes (15) were represented. Centromeric region was drawn in black. Genes were plotted according to their sequence positions along the chromosomes.

Chromosome numbers corresponded to soybean linkage groups as follows: 1 D1a, 2 D1b, 3 N, 7 M, 8 A2, 9 K, 10 O, 11 B1, 13 F, 14 B2, 15 E, 17 D2, 18 G, 19 L, 20 I

Fig. 2 The conserved motifs and exon/intron structures of the soybean and *Arabidopsis* fatty acid desaturase genes. Schematic representation of motifs identified in soybean fatty acid desaturase proteins using MEME motif search tool. Each motif was represented by a number in colored box. Length of box did not correspond to

length of motif. Boxes represented the exons and lines represented introns. The sizes of exons and introns could be estimated using the scale at bottom. The numbers above the boxes and lines indicated the splicing phases of the fatty acid desaturase sequences, 0 referred to phase 0, 1 to phase 1, and 2 to phase 2

The soluble desaturases are analyzed separately from membrane-bound desaturases because soluble desaturases are restricted to higher plants and show no evolutionary relationship with the more widely distributed membrane desaturases (Shanklin and Cahoon 1998). The desaturase

genes detected in soybean belonged to four subfamilies so far identified in other angiosperms: stearyl-ACP desaturase subfamilies, $\Delta 7/\Delta 9$ desaturase subfamilies, $\Delta 12/\omega 3$ desaturase subfamilies, and “front-end” desaturase subfamilies ($\Delta 4$, $\Delta 6$, and $\Delta 8$ desaturases).

The plant stearyl-ACP desaturase is the only soluble desaturase identified to date. All other desaturases identified in plants, algae, animals, and fungi are integral membrane proteins (Singh et al. 2002; Luo et al. 2009). There were seven prospective stearyl-ACP desaturases in *Arabidopsis* while soybean had five homologous genes that consisted of three full-length and two partial ones. The phylogenetic tree (Fig. 3) showed that the prospective stearyl-ACP desaturases from soybean and *Arabidopsis* were grouped with those from higher plants and distinct from those of green algae. It may suggest that stearyl-ACP desaturases in green algae and higher plants arose by independent gene duplication events. *GmFAB2.2*

Table 4 SSR loci in fatty acid desaturase genes

Locus tag	SSR type	SSR length	SSR location
Glyma01g05470.1	(AG)13	26	5' UTR
Glyma19g32940.1	(TC)9	18	Intron
Glyma02g15600.1	(TAT)7	21	Intron
Glyma03g30050.1	(AT)13	26	Extron
Glyma07g32850.1	(TTA)7	21	5' UTR
Glyma07g32850.1	(CT)17	34	5' UTR
Glyma08g22730.1	(AACAC)5	25	5' UTR
Glyma02g39230.1	(AGC)5	15	5' UTR

Fig. 3 Neighbor-joining tree based on the deduced amino acid sequences of stearoyl-acyl carrier protein desaturase homologs. Sequences from soybean and *Arabidopsis* genomes were shown by their locus tags and gene symbols. Other sequences were shown by their accession numbers, strain names, and gene symbols. Bootstrap values from neighbor-joining analyses were listed to the left of each node, with values more than 50 are shown

(*GmFAB2A*, Glyma07g32850) and *GmFAB2.1* (*GmFAB2B*, Glyma02g15600) fell into a group and were constitutively expressed in both vegetative tissues and throughout seed development, whereas *GmFAB2.3* (*GmFAB2C*, Glyma14g27990), clustered with *FAB2.1* from *Arabidopsis*, were expressed only in developing seeds.

As shown in the phylogenetic tree, all of the membrane-bound desaturases fell into three distinct subfamilies: the $\Delta 7/\Delta 9$ desaturase subfamily, $\Delta 12/\omega 3$ desaturase subfamily, and “front-end” desaturase subfamily (Fig. 4). Based on functional criteria and the position of the clade integrated by $\Delta 9$ desaturases, $\Delta 9$ desaturase was assumed to be the ancestor of the remaining desaturases (Alonso et al. 2003). The $\Delta 7/\Delta 9$ desaturase subfamily was clustered into two clades. In clade I, two $\Delta 7$ -homologous genes from soybean (*GmFAD5.1* and *GmFAD5.2*) as well as three genes (one $\Delta 7$ and two $\Delta 9$) from *Arabidopsis* grouped with $\Delta 7$ homologs from green algae, while the genes of cyanobacteria were placed in a basal position. Therefore, the $\Delta 9$ desaturase might arise by independent gene duplication events in plant and green algae branches, and the cyanobacterial $\Delta 9$ desaturase was identified as the origin of plant/green algae $\Delta 7/\Delta 9$ desaturase. In clade II, one $\Delta 9$ -homologous gene from *Cyanidioschyzon merolae* was grouped with those from fungi, apart from the subgroup comprised of genes from diatoms and animals.

In cluster I of the $\Delta 12/\omega 3$ desaturase subfamily, two putative chloroplastic $\Delta 12$ desaturases from soybean (*GmFAD6.1* and *GmFAD6.2*) and one from *Arabidopsis* formed a group with the desaturase genes from higher plants and green algae. Moreover, the $\Delta 12$ desaturases from cyanobacteria were situated at the basal position. In cluster II, six microsomal $\Delta 12$ desaturase genes from soybean as well as one gene from *Arabidopsis* clustered

Fig. 4 Neighbor-joining tree of membrane desaturases. Sequences from soybean and *Arabidopsis* genomes were shown by their locus tags and gene symbols. Other sequences were shown by their accession numbers, strain names, and gene symbols. Bootstrap values from neighbor-joining analyses were listed to the left of each node, with values more than 50 are shown

with genes from higher plants and green algae. Among FAD2-like genes that have been reported in soybean, two seed-specific genes *GmFAD2.3* (*GmFAD2-1A*, Glyma10g42470) and *GmFAD2.6* (*GmFAD2-1B*, Glyma20g24530) clustered together; two housekeeping genes *GmFAD2.1* (*GmFAD2-2C*, Glyma03g30070) and *GmFAD2.4* (*GmFAD2-2B*, Glyma19g32940) were grouped with *FAD2* gene from *Arabidopsis* and set apart from *GmFAD2.5* gene (*GmFAD2-2A*, Glyma19g32930). The *GmFAD2.5* gene was not amplified from soybean by Schlueter et al. (2007), suggesting that *GmFAD2.5* was either not expressed in tissue surveyed in this experiment or that this gene copy was no longer functional.

In cluster III, the $\omega 3$ desaturases from cyanobacteria were placed in a basal position. Eight putative $\omega 3$ desaturases from soybean (four *FAD3*, two *FAD8*, and two *FAD7*) and three from *Arabidopsis* were grouped with their respective microsomal or chloroplastic $\omega 3$ desaturases from higher plants and set apart from enzymes from green algae. It was speculated that $\omega 3$ desaturases might arise by independent gene duplication events from a $\Delta 12$ desaturase ancestor, and the cyanobacterial $\Delta 12$ desaturase was identified as the origin of the plant $\omega 3$ desaturases, including both chloroplast and ER isozymes. Four alleles of *GmFAD3* were separated into two groups: *GmFAD3.1* (*GmFAD3B*, Glyma02g39230) and *GmFAD3.3* (*GmFAD3A*, Glyma14g37350), *GmFAD3.2* and *GmFAD3.4* (*GmFAD3C*, Glyma18g06950). Although *GmFAD3.1* and *GmFAD3.3* showed high sequence similarity, *GmFAD3.3* was the only gene that predominantly expressed in developing seeds (Bilyeu et al. 2003).

The “front-end” desaturases ($\Delta 4$, $\Delta 6$, and $\Delta 8$ desaturases) formed a separate clade (Fig. 4), and their phylogeny was complicated. The sphingolipid $\Delta 4$ desaturases from higher plants integrated into one group. In the other group, one subgroup was constituted by $\Delta 6$ desaturases from higher plants and $\Delta 8$ -sphingolipid desaturases from higher plants and algae. Four putative $\Delta 8$ -sphingolipid desaturases from soybean (*GmSLD1.1*, *GmSLD1.2*, *GmSLD1.3* and *GmSLD1.6*), and two homologous genes from *Arabidopsis* integrated with those of higher plants, while two other soybean $\Delta 8$ -sphingolipid desaturases (*GmSLD1.4* and *GmSLD1.5*) clustered with $\Delta 6$ desaturases of higher plants. The other subgroup was integrated by $\Delta 5$ and $\Delta 6$ desaturases from animals.

Coexpression Pattern Analysis

Currently, there are 2,886 soybean and 4,013 *Arabidopsis* microarray chips available for query on the Genevestigator web site. The online analysis tools allow a large range of questions to be asked about gene expression during developmental stages, stress conditions, or by tissue/organ specificity (Grennan 2006). In this study, gene coexpression pattern

analysis with 20 soybean and 16 *Arabidopsis* fatty acid desaturase genes was performed by the GENEVESTIGATOR program. Nine soybean desaturase genes and two *Arabidopsis* genes did not have their corresponding probe sets in the dataset (Table 5). The relatively low coverage of soybean desaturases in the datasets analyzed maybe partially due to the fact that these arrays were mainly derived from ESTs rather than offered whole genome coverage. From microarray based expression analysis, it was evident that 20 soybean and 16 *Arabidopsis* desaturase genes showed specific temporal and spatial expression patterns across different tissues and developmental stages (red and blue boxes, Fig. 5).

Figure 5a clustered organ-localized expression of the desaturase genes of *Arabidopsis*. The expression levels of *AtSLD1.1*, *AtSLD1.2*, and *AtDES1* were very high in pollen and stamens relative to the expression of these genes in other organs suggesting a potentially specialized function in pollen development and/or function. *AtFAD2*, *AtFAD3*, and *AtFAB2.5* were extensively expressed among organs tested. *AtFAD3* was expressed dominantly in callus, imbedded seed and endodermis cortex of root; *AtFAD2* had relatively high expressions in pollen and stem, whereas *AtFAB2.5* expressed strongly in stem and seed suspensor. *AtFAD5*, *AtFAD6*, *AtFAD7*, and *AtFAB2.2* had similar expression patterns, showing higher transcript abundance in seedling cotyledons, stem, adult leaf, and leaf primordia. *AtFAB2.1* preferentially expressed in callus, endodermis cortex, and lateral root cap of roots, whereas *AtADS.2* exhibited specifically high transcript accumulation in chalazal endosperm and testa of seed. Figure 5c reported expression of the *Arabidopsis* desaturase genes over nine stages of plant development. *AtFAB2.3*, *AtFAB2.6*, *AtSLD1.1*, and *AtDES1* RNAs were found in low abundance over all stages, whereas *AtFAD5* had relatively high expression. *AtADS.1* and *AtFAB2.7* transcripts were relatively rare; *AtADS.1* expression was limited to primarily germinated seeds (Fig. 5c “stage 1”), whereas *AtFAB2.7* was largely restricted to mature siliques (Fig. 5c “stage 9”). *AtFAD6* and *AtFAD7* shared the characteristic of having maximal expression very early and expressing at their lowest levels at the latest developmental stage. *AtADS.2*, *AtFAB2.2*, *AtFAB2.5*, *AtFAD2*, *AtFAD3*, and *AtSLD1.2* had similar expression behavior over the developmental stages with higher expression in developed flower (Fig. 5c “stage 7”); *AtFAB2.2*, *AtFAD2*, and *AtFAD3* were expressed at their highest levels at the earliest developmental stage; in addition, *AtADS.2*, *AtFAB2.2*, *AtFAD2.5*, and *AtFAB3* had relatively high expression at the latest developmental stage.

Figure 5b showed the organ-specific coexpression between different desaturase genes of soybean. Six gene pairs (*GmSLD1.1/GmSLD1.2*, *GmFAD8.1/GmFAD8.2*, *GmFAD6.1/GmFAD6.2*, *GmSLD1.3/GmSLD1.6*, *GmDES1.1/GmDES1.2*, *GmSLD1.4/GmSLD1.5*) had the

Table 5 Selected fatty acid desaturase genes used for coexpression analysis in GENEVESTIGATOR

Organism	Gene symbol	Locus tag	UniGene number	Microarray probe
<i>Glycine max</i>	SLD1.1	Glyma01g05470.1	Gma.20380	GmaAffx.93332.1.S1_at
	FAD8.1	Glyma01g29630.1	Gma.11043	Gma.11043.1.S1_at
	SLD1.2	Glyma02g11820.1	Gma.20380	GmaAffx.93332.1.S1_at
	FAB2.1	Glyma02g15600.1	Gma.32339	Gma.6256.1.S1_S_at
	FAD6.1	Glyma02g36460.1	Gma.49	Gma.49.1.S1_at
	FAD3.1	Glyma02g39230.1	Gma.50724	/
	FAD2.1	Glyma03g30070.1	Gma.25376	GmaAffx.93278.1.S1_S_at
	FAD8.2	Glyma03g07570.1	Gma.11043	Gma.11043.1.S1_at
	FAB2.2	Glyma07g32850.1	Gma.6256	GmaAffx.43163.1.S1_at
	FAD5.1	Glyma07g03370.1	Gma.22389	/
	FAD7.1	Glyma07g18350.1	Gma.9403	/
	SLD1.3	Glyma08g41120.1	Gma.7596	Gma.7596.1.S1_at
	FAD5.2	Glyma08g22730.1	Gma.22389	/
	FAD2.2	Glyma09g17170.1	/	/
	FAD2.3	Glyma10g42470.1	Gma.18367	Gma.1839.1.S1_at
	FAD3.2	Glyma11g27190.1	Gma.8463	/
	DES1.1	Glyma13g31440.1	Gma.5077	Gma.5077.1.A1_at
	SLD1.4	Glyma14g10890.1	Gma.19021	GmaAffx.87656.1.S1_at
	FAB2.3	Glyma14g27990.1	Gma.36064	/
	FAD3.3	Glyma14g37350.1	Gma.30186	Gma.10564.1.1.S1_at
	DES1.2	Glyma15g07900.1	Gma.5077	Gma.5077.1.A1_at
	SLD1.5	Glyma17g34640.1	Gma.19021	GmaAffx.87656.1.S1_at
	FAD6.2	Glyma17g08220.1	Gma.49	Gma.49.1.S1_at
	SLD1.6	Glyma18g15260.1	Gma.7596	Gma.7596.1.S1_at
	FAD7.2	Glyma18g43210.1	Gma.9403	/
	FAD3.4	Glyma18g06950.1	Gma.26862	Gma.8463.1.S1_at
	FAD2.4	Glyma19g32940.1	Gma.5046	GmaAffx.90006.1.A1_at
	FAD2.5	Glyma19g32930.1	/	/
	FAD2.6	Glyma20g24530.1	Gma.1839	Gma.1839.2.S1_a_at
<i>Arabidopsis thaliana</i>	FAB2.1	AT1G43800.1	At.43113	260869_at
	FAB2.2	AT2G43710.1	At.22018	260570_at
	FAB2.3	AT3G02610.1	At.41109	258473_s_at
	FAB2.4	AT3G02620.1	At.41106	/
	FAB2.5	AT3G02630.1	At.18818	258485_at
	FAB2.6	AT5G16230.1	At.31698	246498_at
	FAB2.7	AT5G16240.1	At.26326	246502_at
	ADS.1	AT1G06080.1	At.342	260957_at
	ADS.2	AT2G31360.1	At.14455	263249_at
	FAD2	AT3G12120.1	At.23898	256277_at
	FAD6	AT4G30950.1	At.1931	253547_at
	FAD7	AT3G11170.1	At.272	256417_s_at
	FAD8	AT5G05580.1	At.273	/
	FAD3	AT2G29980.1	At.271	266865_at
	FAD5	AT3G15850.1	At.5631	258250_at
	SLD1.1	AT2G46210.1	At.36539	266592_at
	SLD1.2	AT3G61580.1	At.120	251323_at
	DES1	AT4G04930.1	At.4051	255276_at

◀ **Fig. 5** xThe coexpression analysis of selected fatty acid desaturase genes. The *Arabidopsis* and soybean desaturase genes were analyzed for organ-specific and developmental coexpression patterns by GENEVESTIGATOR program. The hierarchical clustering was calculated by Pearson correlation. **a, c** Hierarchical clustering of organ-specific and developmental expression profiles of 16 desaturase genes of *Arabidopsis*. **b, d** Hierarchical clustering of organ-specific and developmental expression profiles of 20 desaturase genes of soybean. In soybean six gene pairs (*SLD1.1/SLD1.2*, *FAD8.1/FAD8.2*, *FAD6.1/FAD6.2*, *SLD1.3/SLD1.6*, *DES1.1/DES1.2*, *SLD1.4/SLD1.5*) shared the same expression patterns with respect to the organs and developmental stages examined, respectively

same probe sets and shared the same expression patterns, respectively. *GmFAD2.3*, *GmFAD2.6*, and *GmFAD3.4* had dedicated expression in seeds; the expression patterns of *GmFAD2.3* and *GmFAD2.6* were similar, expressing very strongly in cotyledon parenchyma, whereas *GmFAD3.4* showed the highest transcript abundance in inner integument of testa among tissues tested. *GmFAD2.1*, *GmFAD8.1/GmFAD8.2*, and *GmFAD6.1/GmFAD6.2* had different tissue-specific expression patterns, mainly expressed in trifoliolate leaf. The remaining eight desaturase genes were largely restricted in their expression to seeds, mainly expressed higher in embryo. Figure 5d summarized developmental expression profiles of desaturase genes of soybean over three stages. *GmFAD3.3*, *GmFAB2.1*, and *GmSLD1.3/GmSLD1.6* mRNAs were found in high abundance over all stages. *GmFAD2.3* and *GmFAD2.6* expressions were limited to bean development stage (Fig. 5d “stage 3”); in contrast, *GmFAD2.1* and *GmFAD6.1/GmFAD6.2* were largely restricted to main shoot growth stage (Fig. 5d “stage 1”). The expression levels of *GmFAD3.4*, *GmFAB2.2*, *GmSLD1.4/GmSLD1.5*, and *GmDES1.1/GmDES1.2* remained relatively low at the initial stage but showed dramatic increases in abundance during later stages, whereas *GmFAD8.1/GmFAD8.2* and *GmSLD1.1/GmSLD1.2* showed the highest transcript abundance in the main shoot growth stage and decreased thereafter.

It was noteworthy that several desaturase orthologs between *Arabidopsis* and soybean showed strikingly consistent expression patterns, which lent further supporting to the existence of functional conservation between these two species. For example, the expression profiles of homologous pair *GmFAD6.1* and *GmFAD6.2* highly resembled the expression pattern of their closest ortholog *AtFAD6* in *Arabidopsis*, with preferential expressions in photosynthetic tissues. However, there still existed some differences. Both *GmFAD7* paralogous genes (*GmFAD7-1* and *GmFAD7-2*) have been functionally characterized and expressed in all soybean tissues analyzed (Andreu et al. 2010), whereas in *Arabidopsis* no *AtFAD7* mRNA was detected in roots (Yadav et al. 1993; Nishiuchi et al. 1997).

Many genes encoding both microsomal and plastidial fatty acid desaturases have been isolated and studied from soybean. These genes showed very distinct tissue-specific expression

patterns, which were in good agreement with the above microarray profiles. For example, in accordance with previous findings, *GmFAD2.3* (*GmFAD2-1A*, *Glyma10g42470*) and *GmFAD2.6* (*GmFAD2-1B*, *Glyma20g24530*) were specifically expressed in developing seeds (Heppard et al. 1996). Consistent with the microarray profiles, *GmFAD2.4* (*GmFAD2-2B*, *Glyma19g32940*) was expressed in all tissues surveyed; *GmFAD2.1* (*GmFAD2-2C*, *Glyma03g30070*) had constitutively weak expression levels in seed grown in warm conditions, and it showed upward of an eightfold increase in expression in developing seeds grown in cooler conditions (Schlueter et al. 2007).

Conclusion

Contribution of fatty acid desaturase family for the functional properties of both animal fats and vegetable oils is well documented (Kinney et al. 2002). However, only a limited number of fatty acid desaturase genes have been functionally validated in soybean by now. In the present study, a comprehensive analysis including phylogeny, chromosomal locations, gene structures, conserved motifs, and expression profiling of desaturase family in soybean was performed. We identified a sum of 29 full-length desaturase genes in soybean genome. Twelve additional partial desaturase sequences could represent additional genes. The desaturase genes in *Arabidopsis* were phylogenetically clustered into ten distinct subfamilies. Representatives of all except *ADS* subfamily were found in soybean. The exon/intron structures and motif compositions of desaturases were highly conserved in each subfamily, indicative of their functional conservation. The comprehensive annotation undertaken in this investigation improved our understanding of the involvement of these genes in fatty acid synthesis in soybean and provided the source for selection of candidate genes for functional validation studies.

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