Identification and expression analysis of the *Glycine max* CYP707A gene family in response to drought and salt stresses

Yan Zheng, Yingyi Huang, Weihao Xian, Jinxiang Wang* and Hong Liao

State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Root Biology Center, South China Agricultural University, Guangzhou 510642, China

* For correspondence. E-mail jinxwang@scau.edu.cn

Received: 30 November 2011 Returned for revision: 6 March 2012 Accepted: 16 April 2012 Published electronically: 1 July 2012

**Background and Aims** Abscisic acid (ABA) plays crucial roles in plants’ responses to abiotic stresses. ABA 8′-hydroxylation controlled by CYP707A genes has been well studied in Arabidopsis and rice, but not in legumes. The aims of the present study were to identify and functionally analyse the soybean CYP707A gene family, and to explore their expression patterns under dehydration and salt stresses.

**Methods** A complementation experiment was employed to verify the function of soybean CYP707A1a in ABA catabolism. Genomic and cDNA sequences of other soybean CYP707A genes were isolated from the Phytozome database based on soybean CYP707A1a. The structure and phylogenetic relationship of this gene family was further analysed. The expression patterns of soybean CYP707A genes under dehydration and salt stress were analysed via quantitative real-time PCR.

**Key Results** Over-expression of GmCYP707A1a in the actv707a2 T-DNA insertion mutant decreased its sensitivity to ABA, indicating that GmCYP707A1a indeed functions as an ABA 8′-hydroxylase in higher plants. The soybean genome contains ten CYP707A genes. Gene structure and phylogenetic analysis showed high conservation of ten GmCYP707A genes to the other CYP707A genes from monocots and dicots. Seed imbibition induced expression of A1a, A1b, A2a, A2b, A2c, A3a and A5 in embryo, and expression of A1a, A1b, A2a and A2b in cotyledon. Dehydration induced expression of A1a, A1b, A2b, A2c, A3a, A3b, A4a, A4b and A5 both in roots and in leaves, whereas rehydration stimulated transcription of A2a, A2b, A3b, A4a and A5 in roots, and only A3b and A5 in leaves. Expression of all soybean CYP707A genes was induced either by short- or by long-term salt stress.

**Conclusions** The first biological evidence is provided that GmCYP7071a encodes an ABA 8′-hydroxylase through transgenic studies. Ten soybean GmCYP707A genes were identified, most of them expressed in multiple soybean tissues, and were induced by imbibition, dehydration and salinity.

**Key words:** Abscisic acid, ABA catabolism, *Glycine max*, CYP707A gene family, drought, salt stress.

**INTRODUCTION**

Abscisic acid (ABA) is an important phytohormone in plants, which regulates root and seed development, seed germination, and biotic and abiotic stress responses (Cutler et al., 2010). ABA interacts with other phytohormones to mediate plant performance under biotic and abiotic stress conditions (Cutler et al., 2010). ABA modulates expression of many genes at transcription and post-transcription levels (Finkelstein et al., 2002). After several decades of investigation, three groups identified PYR1/PYL1/RCAR as ABA receptors (Park et al., 2009). Abscisic acid (ABA) is an important phytohormone in plants, which regulates root and seed development, seed germination, and biotic and abiotic stress responses (Cutler et al., 2010). ABA interacts with other phytohormones to mediate plant performance under biotic and abiotic stress conditions (Cutler et al., 2010). ABA modulates expression of many genes at transcription and post-transcription levels (Finkelstein et al., 2002). After several decades of investigation, three groups identified PYR1/PYL1/RCAR as ABA receptors (Park et al., 2009). Abscisic acid, ABA catabolism, plant hormone, ABA 8′-hydroxylase.

Generally, the level of ABA is elevated under multiple stresses in plants, such as drought and salt (Jia et al., 2002; Seiler et al., 2011). Recent studies suggest that ABA plays an important role under drought stress, and it is found to be significantly enhanced during seed-set and grain-filling events (Govind et al., 2011). In maize, ABA accumulates more in roots than in leaves under salt treatment (Jia et al., 2002). The level of ABA is tightly controlled by biosynthesis and catabolism. The ABA biosynthesis pathways have been widely identified in a number of plants. The precursor of ABA is carotetiol (C40), followed by a two-step conversion of the intermediate xanthoxin to ABA via ABA aldehydehydroxylase (Zeevaart et al., 1999; Zhou et al., 2004). ABA is mainly oxidized to 8′-hydroxy-ABA (8′-OH-ABA) catalysed by ABA 8′-hydroxylase, which is unstable and can be re-arranged to phaseic acid (PA), and finally reduced to dihydrophaseic acid (DPA) (Nambara and Marion-Poll, 2005).
ABA is reported to promote water uptake and flow in *Planta*, possibly through its effects on aquaporin (Hose et al., 2000). ABA contents in xylem sap increased dramatically, acting as a signal molecule to arrive in leaf epidermis guard cells through long-distance transport under drought stress (Davies and Zhang, 1991). Stomatal aperture is regulated by ABA, which both promotes closure of stomata and inhibits opening of stomata (Schroeder et al., 2001). Consistent with this, over-expression of *AtNCED3*, a key enzyme for ABA biosynthesis, increases Arabidopsis tolerance to drought resulting from reduced transpiration rate in leaves (Iuchi et al., 2001), but over-expression of ABA 8'-hydroxylase decreases ABA levels and produces ABA-deficient phenotypes (Nitsch et al., 2009), indicating ABA homeostasis is crucial for plants to cope with water stress. On the other hand, ABA stimulates accumulation and transport of proline, betaine, Na⁺, K⁺, Ca²⁺, and Cl⁻ (Chen and Murata, 2002; Younis et al., 1994), which can strengthen tolerance of plants to drought or osmotic stress.

In developing seeds, a higher ABA content promotes biosynthesis of storage proteins (Xiong and Zhu, 2003). During germination, ABA plays an antagonistic role with gibberellin (Lee et al., 2002). Salt stress results in hyperosmolarity, which in turn induces ABA biosynthesis (Xiong and Zhu, 2003). Other molecular studies indicate that biosynthesis of ABA is dependent on the regulation of ABA biosynthesis genes such as ZEP, NCED, AAO3, MCSU and SDR1 (Xiong and Zhu, 2003; Tan et al., 1997; Taylor et al., 2000; Qin and Zeevaart, 2002; Gonzalez-Guzman et al., 2002). However, previous studies have focused on the behaviour of ABA synthesis-related genes at transcription and post-transcript level in plant tissues at developmental stages and under stressful conditions, whereas ABA turnover is another important facet of ABA homeostasis in plant tissues. More extensive studies are therefore required regarding the ABA hydroxylation catalysed by ABA hydroxylase.

In recent years, the key enzymes of the ABA 8'-hydroxylation reaction have been intensively studied in plants. All are members of the cytochrome P450 (CYP) superfamily, named CYP707A. Four Arabidopsis CYP707A genes, encoding ABA 8'-hydroxylase, were isolated (Kushiro et al., 2004); later two from barley (Millar et al., 2006), three from rice (Saika et al., 2007), and three and five from bean and maize, respectively (Yang and Zeevaart, 2006; Vallabhaneni and Wurtzel, 2010), were determined. These studies established that the CYP707A gene family was mainly regulated at the transcriptional level and controlled ABA level under various stresses.

It has been shown that levels of ABA change dramatically in response to dehydration and subsequent rehydration (Umezawa et al., 2006). In *Phaseolus vulgaris*, CYP707A3 transcripts significantly increased during dehydration, no changes of mRNA levels of CYP707A1 and CYP707A2 were found, while mRNA levels of CYP707A1 and CYP707A2 were rapidly increased by rehydration of water-stressed leaves (Yang and Zeevaart, 2006). Furthermore, high salinity induces ABA accumulation in various plants. Strong induction of CYP707A1 and CYP707A4 transcripts and moderate increases of CYP707A2 and CYP707A3 mRNA levels were observed in Arabidopsis subjected to 250 mM NaCl stress (Saito et al., 2004).

Although the mechanism of ABA 8'-hydroxylation has been well studied in model plant species, such as Arabidopsis and rice, the roles of ABA catabolism in oil crops remain unclear. Legume crops such as soybean are the main sources of edible oils. Increasing legume yield under drought, salinity and other stressful conditions is of importance, and this can be achieved by fine-tuning ABA levels using biotechnology approaches. Hence we need to understand the details of ABA catabolism directly regulated by ABA 8'-hydroxylase. *GmCYP707A1a* might function as an ABA 8'-hydroxylase in soybean, as determined by gene expression analysis (Wang et al., 2010), but direct biological evidence for this is still lacking. Therefore, in this study, we first verified the function of *GmCYP707A1a* as an ABA 8'-hydroxylase through a complementation experiment using an Arabidopsis atcyp707a2 T-DNA insertion mutant; we then identified the soybean CYP707A gene family, and explored the expression patterns of this under dehydration and salt stresses.

**MATERIALS AND METHODS**

Database search and bioinformatics analysis of CYP707A genes in soybean genome

A tBlast search was performed with the known amino acid sequence of one soybean (*Glycine max*) protein CYP707A1a (Glyma09g35250), which was identified in our previous study and used as seed sequence (Wang et al., 2010), at the Phytozone database (http://www.phytozone.org). Nine candidates showed high levels of identity and similarity to *GmCYP707A1a*. The predicted amino acid sequence of these ten CYP707As was each used as a query sequence for a single round of Blastp searches at the Phytozone website. No further predicted CYP707As were found, suggesting that the total number of predicted CYP707As in the soybean genome was likely to be ten. The ten soybean CYP707As (*GmCYP707As*) were named according to their identity and similarity to *GmCYP707A1a*. In addition, we also used blast searches in TIGR database (http://planutta.jcvi.org/index.php) to extract expressed sequence tag (EST) sequences of this family.

The genomic and cDNA sequences of *GmCYP707A* members were retrieved from the Phytozone website. The structure of *CYP707A* genes from Arabidopsis, soybean and *Medicago* was constructed at the Gene Structure Display Server (GSDS) website (http://gsds.cbi.pku.edu.cn/index.php) (Guo et al., 2007). In addition, a phylogenetic tree of CYP707As from Arabidopsis, soybean, maize, *Medicago*, sorghum, common bean, black cottonwood, castor bean, *Physcomitrella patens* and rice based on entire amino acid sequence alignments using ClustalX (Kohl and Bachhawat, 2003; Thompson et al., 2002) was constructed via the neighbour-joining method with 1000 bootstrap replicates with the MEGA 5.0 program (Tamura et al., 2011) and identity and similarity analysis results were displayed via GeneDoc 2.6 (Karl et al., 1997). Complete deletion was used to deal with gaps or missing data in the sequences. The distance between sequences was estimated after Poisson correction. The numbers of exons and introns and total exon and intron length were extracted from the Phytozone website. The first exon length was extracted from the GSDS website.
Plant materials and treatments

The soybean genotype used in this study was BX10. For the seed germination experiment, soybean seeds were immersed in double distilled water in a sterile Petri dish, and embryos and cotyledons were harvested after 0, 1, 6 and 24 h. All the soybean plants were sand-cultured in a greenhouse. For the gene expression experiments, soybean seeds were germinated in a paper pouch for 1 week, and then transplanted into sand irrigated with full-strength nutrient solution [250 μM KH₂PO₄, 3000 μM KNO₃, 2000 μM Ca(NO₃)₂, 250 μM MgSO₄, 25 μM MgCl₂, 12.5 μM H₂BO₃, 1 μM MnSO₄, 1 μM ZnSO₄, 0.25 μM CuSO₄, 0.25 μM (NH₄)₆Mo₇O₂₄ and 25 μM Fe- Na-EDTA, pH 5.8] every 2 d. Uniform seedlings with fully developed first trifoliate leaves were treated in sand as indicated below. For analysis of tissue specificity of gene expression, leaves and roots of BX10 were separately harvested after the second trifoliate leaves had fully developed; for the drought treatment, soybean with the first trifoliate leaves expanded was not irrigated with nutrient solution for 3 d, and the leaves and roots were then separately harvested at 0, 2, 4 and 8 d. For the rehydration treatment, the soybean plants were first treated in drought conditions for 11 d, and then rehydrated with nutrient solution at the indicated times. The leaves and roots were separately harvested after 1, 6 and 24 h of rehydration. For salt treatment, soybean seedlings were cultured in nutrient solution containing 300 mM NaCl, and then rehydrated with nutrient solution at the indicated times.

Vector construction and transformation of atcyp707a2 mutant

The Arabidopsis thaliana ecotype Columbia-0 (Col-0) was used as wild-type in this study. The mutant allele of atcyp707a2 was obtained from the SALK stock of Arabidopsis T-DNA insertion lines (Salk_083966). The full-length soybean CYP707A1a open reading frame (ORF) was obtained by PCR amplification. The forward primer was: 5′-AAAAAGTACCATCGGAACTACA GCACCATGTTTTCC-3′, with KpnI site, and the reverse primer was: 5′-AAAACATAATTCTAATTTGGTGG GAAATAATGTG-3′ with SpeI digest site. After digestion, the GmCYP707A1a ORF was inserted into vector pOx, which contains the maize ubiquitin promoter (Peng et al., 1999), and named pOx-GmCYP707A1a. The atcyp707a2 mutant was transformed through floral dipping with Agrobacterium GV3101 harboring pOx-GmCYP707A1a (Clough and Bent, 1998). Transformed plants were selected in hygromycin-containing media. Single-copy homozygous transformed plants were used for further study. Control and transformed Arabidopsis seeds were surface sterilized with 70% ethanol for 10 min and then with 10% NaClO for 5 min before being washed five times with sterilized water. The plants were grown in growth chambers with a 16/8-h photoperiod at 22/18 °C, 75% relative humidity and 100 μm s⁻¹. Seed germination rate was determined under the microscope. Primary root length was measured with ImageJ 1.32 (Abramoff et al., 2004) and the number of lateral root was counted under the microscope.

RNA extraction and quantitative real-time PCR

Total RNA was separately extracted from embryo, cotyledon, roots and leaves of soybean plants by using RNAiso™ Plus reagent (TaKaRa, http://www.takara-bio.com) according to the manufacturer’s instructions. RNA samples were treated with RNaFree DNase I (Invitrogen, http://www.invitrogen.com) to avoid amplification from genomic DNA. The first cDNA strand was synthesized from total RNA using MMLV-reverse transcriptase (Promega, USA, http://www.promega.com) according to the protocol from the supplier. Quantitative real-time PCR (qRT-PCR) was performed using SYBR® Premix EX Taq™ (TaKaRa). All the reactions were done in a Rotor-Gene 3000 (Cobert Research, Australia, http://www.corbettlifescience.com/). The soybean housekeeping gene, Glyma10g38460, a calcium/calmodulin-dependent kinase-related protein, was used as an endogenous control to normalize the samples (Libault et al., 2008). Based on the genomic and cDNA sequence of each CYP707A from Arabidopsis and soybean, qRT-PCR primer pairs (listed in Supplementary Data Table S1) were designed with Primer-BLAST via the NCBI website to specifically amplify the fragments of CYP707A genes (http://www.ncbi.nlm.nih.gov/tools/primer-blast). Reaction conditions for thermal cycling were: 95 °C for 1 min, 40 cycles of 95 °C for 15 s, 56–60 °C for 15 s (based on the primer’s melting temperature), and 72 °C for 30 s. The annealing temperature (56–60 °C) was adjusted to amplify the individual GmCYP707A. Fluorescence data were collected at the end of the cycle at 72 °C.

Statistical analyses

All results are from three biologically independent experiments with similar results. All data were analysed using Origin 7.5 (OriginLab Corporation, USA, http://www.originlab.com/) for calculating means and standard errors, and SAS 6.2 (SAS Institute, USA, http://www.sas.com/) for ANOVA analyses.

RESULTS

Functional analysis of GmCYP707A1a

The results in Fig. 1A and B showed T-DNA insertion only decreased the transcription level of AtCYP707A2a, indicating that atcyp707a2 was a knock-down mutant but not a knock-out mutant; and two independent lines of ectopic over-expression of GmCYP707A1a driven by the maize ubiquitin promoter (Peng et al., 1999) in atcyp707a2 were successful. Expression of the remaining AtCYP707A members in 14-d-old Arabidopsis seedlings (Fig. 1A) and germinating seeds after 4 d of imbibition (Fig. 1B) was detectable. These results indicated there was no significant difference between wild-type and GmCYP707A1a over-expression lines in atcyp707a2 background regarding the expression of endogenous AtCYP707A1, A3 and A4.

It is well documented that ABA inhibits primary root growth and lateral root formation (De Smet et al., 2006). Hence we analysed primary root length and lateral root number to quantify the ABA sensitivity of different lines. Compared with Col-0, the T-DNA knock-down mutant of atcyp707a2 was more sensitive to ABA: primary root length decreased by 27.2 %, and lateral root number decreased by 55.3 %. In contrast to the mutant, two GmCYP707A1a over-expression lines rescued the sensitivity of atcyp707a2 to ABA, as indicated by a 53.3 and 81.9 % increase of primary root length, and a 100 and 130.8 % increase of lateral root number, respectively (Fig. 1C, D), almost recovering to wild-type level.
Moreover, under normal conditions (no ABA treatment) germination rates were not significantly different (Fig. 1E), but after 6 d of 0.5 μM ABA application, 61.1% of atcyO707a2 seeds failed to germinate, whereas germination rates of the wild-type and AtCYP707A2 overexpression line were as high as 72.2 and 84.4%, respectively. Compared with atcyO707a2, two GmCYP707A1a overexpression lines showed 82.7 and 72.8% germination, values similar to those of Col-0 (Fig. 1F). Compared with Col-0 and atcyO707a2, the transcripts of AtCYP707A1, A3 and A4 were not altered in OE-A1a-1 and OE-A1a-2 lines (Fig. 1B), indicating that GmCYP707A1a plays a similar and direct role to AtCYP707A2 in seed germination.

**Identification and characterization of CYP707A genes in soybean**

The soybean genome sequence was released in 2010 through de novo sequencing (Schmutz et al., 2010). Based on the amino
acid sequence of GmCYP707A1a, which was reported to interact with GmWNK1 (Wang et al., 2010), we used the soybean genome in BLASTP searches of the Phytozome database. We identified a further nine GmCYP707A member candidates (Table 1). The genome locus, full-length cDNAs and amino acid sequences were downloaded from the Phytozome website. We used BLAST searches for ESTs on the TIGR website to verify GmCYP707A members and found that some ESTs or tentative contigs matched GmCYP707A1a, GmCYP707A1b, GmCYP707A2a, GmCYP707A2b, GmCYP707A3a, GmCYP707A3b, GmCYP707A4a and GmCYP707A4b at nearly 100% (Table 1), but we did not find ESTs that closely matched GmCYP707A5a, GmCYP707A5b or GmCYP707A5c (Table 1).

To better understand the evolutionary origin of these genes, phylogenetic analysis was carried out using the putative amino acid sequences of plant CYP707A family members annotated in the Phytozome website from Arabidopsis, soybean, maize, Medicago, sorghum, common bean, black cottonwood, castor bean, rice and Hyscomitrella patens. Based on the phylogenetic tree, CYP707As in Planta could be grouped into two subgroups (Fig. 2A). Each group contained CYP707As from monocot and dicot species, indicating that the common ancestor of this gene family appeared before the monocots and dicots evolved. But in the secondary subgroup, all monocotyledonous CYP707As formed a single clade, and all dicotyledonous CYP707As belonged to another clade, implying the existence of a father gene from some CYP707A genes from monocots and dicots. Physcomitrella patens represents a classic older multicellular plant. The existence of CYP707A (ABA 8'-hydroxylase) in Physcomitrella patens (Fig. 2A) indicates that ABA catabolism through hydroxylase is conserved in non-flowering plants and terrestrial flowering higher plants. Consistent with the identity and similarity analysis, GmCYP707A1s and GmCYP707A2s are grouped into subgroup 1a with short evolutionary distance, indicating they might come from a common ancestor in dicots. GmCYP707A3s and GmCYP707A4s are located in subgroup Ib with short evolutionary distance. And GmCYP707A5 belongs to another clade with a long distance to other soybean family members (Fig. 2A).

Alignment of CYP707As from Arabidopsis, Medicago and soybean indicated high conservation of CYP707As across species (Fig. 2B). The conserved motif of CYP707As was PFG[NSD][TVIA][HR][SAM]CPG, the highly conserved cysteine residue of which was the heme iron ligand, which had been proved to be essential for CYP catalysis (Kushiro et al., 2004).

Levels of sequence similarity and identity between the GmCYP707A proteins are presented in Table 2. The GmCYP707A proteins were grouped into five subgroups, A1–A5, based on identity and similarity with GmCYP707A1a (Table 2). A1 had two members with 97% identity. A2 had three members showing approx. 75% identity to A1a; members of both A3 and A4 had nearly 50% identity to A1a, and the A5 subgroup contained only one member, which showed 53% identity to A1a (Table 2).

We also examined the exon–intron boundaries of CYP707A gene families among soybean, Arabidopsis and Medicago through the GSDS website (http://gds.cbi.pku.edu.cn/index.php) (Guo et al., 2007). As shown in Fig. 3 and Table 3, all members of the CYP707A gene family from the three dicots contained multiple introns with a similar and conserved structure. Based on the gene structures, soybean CYP707A gene family members were grouped into three subgroups. Members of subgroup 1 included A1a, A1b, A2a, A2b and A2c with seven exons and six introns. Subgroup 2 consisted of A4b and A5 with eight exons and seven introns, and subgroup 3 contained A3a, A3b and A4a with nine exons and eight introns (Fig. 3 and Table 3).

Expression pattern analysis of GmCYP707As in multiple tissues

We hypothesized that ABA catabolism in tissues was directly regulated by GmCYP707As. To investigate whether the expression patterns of CYP707As were tissue-specific, we detected the mRNA level of these ten family members in embryos, cotyledons, roots and leaves via qRT-PCR. The results showed that under normal condition, expression levels of A1a and A1b were high in roots but almost absent in leaves, consistent with the previous report that GmCYP707A1a is a root-specific gene (Wang et al., 2010). A2a, A2b and A2c transcripts could be detected both in roots and in leaves, but expression levels were not very high. On the other hand, the expression levels of A3b, A4a and A4b were higher in leaves than in roots, even being undetectable for A5 in roots, suggesting that A5 is a leaf-specific gene. The A3a gene was highly expressed in both roots and leaves (Fig. 4A, B).

Table 1. General information relating to the ten GmCYP707A genes and their deduced proteins

<table>
<thead>
<tr>
<th>Proposed name</th>
<th>Locus name</th>
<th>TIGR ESTs</th>
<th>Gene location</th>
<th>ORF (bp)</th>
<th>Number of amino acids</th>
<th>Predicted size (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GmCYP707A1a</td>
<td>Glyma0g35250</td>
<td>TA48921_3847</td>
<td>Gm09: 41452092–41454893 (--)</td>
<td>1407</td>
<td>468</td>
<td>53.7</td>
</tr>
<tr>
<td>GmCYP707A1b</td>
<td>Glyma0g35660</td>
<td>TA48920_3847</td>
<td>Gm01: 48174716–48177618 (--)</td>
<td>1404</td>
<td>467</td>
<td>53.3</td>
</tr>
<tr>
<td>GmCYP707A2a</td>
<td>Glyma16g08340</td>
<td>TA11162_3847</td>
<td>Gm16: 7617252–7620328 (--)</td>
<td>1407</td>
<td>468</td>
<td>53.1</td>
</tr>
<tr>
<td>GmCYP707A2b</td>
<td>Glyma16g20490</td>
<td>CX709855</td>
<td>Gm16: 23098403–23101117 (--)</td>
<td>1278</td>
<td>428</td>
<td>49.1</td>
</tr>
<tr>
<td>GmCYP707A3a</td>
<td>Glyma14g14310</td>
<td>not found</td>
<td>Gm17: 11061147–11065184 (--)</td>
<td>1287</td>
<td>482</td>
<td>55.2</td>
</tr>
<tr>
<td>GmCYP707A3b</td>
<td>Glyma17g36070</td>
<td>TA59987_3847</td>
<td>Gm17: 40077601–40081864 (+)</td>
<td>1539</td>
<td>512</td>
<td>58.7</td>
</tr>
<tr>
<td>GmCYP707A4a</td>
<td>Glyma0g14290</td>
<td>TA71162_3847</td>
<td>Gm16: 23098403–23101117 (--)</td>
<td>1491</td>
<td>496</td>
<td>57.1</td>
</tr>
<tr>
<td>GmCYP707A4b</td>
<td>Glyma0g33560</td>
<td>TA48921_3847</td>
<td>Gm09: 41452092–41454893 (--)</td>
<td>1400</td>
<td>479</td>
<td>54.9</td>
</tr>
</tbody>
</table>

The locus name, gene location, ORF length and protein size were extracted from the Phytozome website (http://www.phytozome.org/index.php); the + and – in parentheses indicate the sense and antisense strand of DNA; information relating to the TIGR tentative contigs or expressed sequence tags (ESTs) were extracted from the TIGR website (http://plantta.jcvi.org/index.php).
FIG. 2. (A) Phylogenetic analysis of CYP707A proteins from Arabidopsis, soybean, maize, Medicago, sorghum, common bean, black cottonwood, castor bean, Physcomitrella patens and rice. This was constructed by the neighbour-joining method with 1000 bootstrap replicates in the MEGA 5.0 software. The scale bar represents 0.1 substitutions per site (B) Alignment of CYP707A from Arabidopsis, Medicago and soybean. The thick black line at the bottom of the figure shows the highly conserved motif of PFG[NSD][TVIA][HR][SAM]CPG. NCBI accession numbers are as follows: GmCYP707A1a (ABQ65856), GmCYP707A1b (ABC68415), GmCYP707A2a, GmCYP707A2b, GmCYP707A3a, GmCYP707A3b, GmCYP707A4a, GmCYP707A4b, GmCYP707A5, AtCYP707A1 (AEE84162), AtCYP707A2 (AEC08210), AtCYP707A3 (AED95234), AtCYP707A4 (AEE76215), GRMZM2G002142 (ACN34951), GRMZM2G065928 (ACF78881), GRMZM2G126505 (ACN28537), GRMZM2G179147 (ACN25205), Os02g47440 (BAF34848), Os08g36860 (BAF23935), Os09g28390 (BAF25280), Sb02g026600 (EER99010), Sb04g030660 (EES05661), Sb07g022990 (EES15128), Medtr4g129760, Medtr4g129800, Medtr5g025670 (ABC59109), Medtr8g086730 (ACJ84248), PtCYP707A4 (EEF78708), PtCYP707A5 (EEF80493), Pt CYP707A6 (EEF77492), PpCYP707A14 (EEF08458), PpCYP707A1 (ABC66559), PpCYP707A2 (ABC66589), PpCYP707A3 (ABC66559), POPTR_0002s12770 (EEF08493), POPTR_0004s14820 (EEF73891), POPTR_0004s24360 (EEF87380), POPTR_0009s10450 (EEF77492), RCOM_0813450 (EEF43689), RCOM_0814150 (EEF43729), RCOM_1407690 (EEF38770), RCOM_1590410 (EEF52401) and Pp1s281_82V6.
**FIG. 2 Continued**
### Table 2. Comparison of amino acid identity (top) and similarity (bottom) between Glycine max CYP707A proteins

<table>
<thead>
<tr>
<th></th>
<th>A1a</th>
<th>A1b</th>
<th>A2a</th>
<th>A2b</th>
<th>A2c</th>
<th>A3a</th>
<th>A3b</th>
<th>A4a</th>
<th>A4b</th>
<th>A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>GmCYP707A1a</td>
<td>468</td>
<td>97 %</td>
<td>81 %</td>
<td>76 %</td>
<td>71 %</td>
<td>50 %</td>
<td>47 %</td>
<td>50 %</td>
<td>48 %</td>
<td>53 %</td>
</tr>
<tr>
<td>GmCYP707A1b</td>
<td>–</td>
<td>98 %</td>
<td>88 %</td>
<td>83 %</td>
<td>79 %</td>
<td>71 %</td>
<td>66 %</td>
<td>68 %</td>
<td>66 %</td>
<td>73 %</td>
</tr>
<tr>
<td>GmCYP707A2a</td>
<td>455</td>
<td>467</td>
<td>82 %</td>
<td>77 %</td>
<td>73 %</td>
<td>50 %</td>
<td>47 %</td>
<td>49 %</td>
<td>48 %</td>
<td>53 %</td>
</tr>
<tr>
<td>GmCYP707A2b</td>
<td>382</td>
<td>390</td>
<td>468</td>
<td>84 %</td>
<td>76 %</td>
<td>48 %</td>
<td>46 %</td>
<td>47 %</td>
<td>46 %</td>
<td>51 %</td>
</tr>
<tr>
<td>GmCYP707A2c</td>
<td>417</td>
<td>424</td>
<td>–</td>
<td>86 %</td>
<td>83 %</td>
<td>68 %</td>
<td>64 %</td>
<td>66 %</td>
<td>65 %</td>
<td>70 %</td>
</tr>
<tr>
<td>GmCYP707A3a</td>
<td>357</td>
<td>363</td>
<td>425</td>
<td>85 %</td>
<td>47 %</td>
<td>45 %</td>
<td>45 %</td>
<td>45 %</td>
<td>50 %</td>
<td>–</td>
</tr>
<tr>
<td>GmCYP707A3b</td>
<td>390</td>
<td>396</td>
<td>408</td>
<td>92 %</td>
<td>65 %</td>
<td>61 %</td>
<td>63 %</td>
<td>62 %</td>
<td>67 %</td>
<td>–</td>
</tr>
<tr>
<td>GmCYP707A4a</td>
<td>338</td>
<td>342</td>
<td>336</td>
<td>364</td>
<td>428</td>
<td>46 %</td>
<td>44 %</td>
<td>43 %</td>
<td>43 %</td>
<td>48 %</td>
</tr>
<tr>
<td>GmCYP707A4b</td>
<td>248</td>
<td>252</td>
<td>268</td>
<td>270</td>
<td>271</td>
<td>273</td>
<td>274</td>
<td>275</td>
<td>276</td>
<td>277</td>
</tr>
<tr>
<td>GmCYP707A5</td>
<td>349</td>
<td>353</td>
<td>367</td>
<td>371</td>
<td>375</td>
<td>380</td>
<td>385</td>
<td>390</td>
<td>395</td>
<td>400</td>
</tr>
</tbody>
</table>

The identity and similarity of amino acids between G. max CYP707A proteins were compared with GeneDoc 2.6 software ([Karl et al., 1997, http://www.nrbsc.org/gfx/genedoc/]). In each row, the numbers indicate the number of identical (top) and similar (bottom) amino acids in two proteins, and the percentage shows the percentage of identical (top) and similar (bottom) amino acids in two proteins. For instance, when GmCYP707A1b is compared with GmCYP707A1a, 455 amino acids are identical and 459 amino acids are similar in the two proteins; when GmCYP707A1a is compared with GmCYP707A1b, 97 % of amino acids are identical and 98 % of amino acids are similar in the two proteins. – indicates a comparison between one CYP707 protein and itself with respect to similar amino acids.

![Gene structures of GmCYP707A, AtCYP707A and MtCYP707A genes constructed with GSDS. Genomic DNA sequences and cDNA sequences were downloaded from Phytozome (http://www.phytozome.org). Ten soybean CYP707A members can be grouped into three subgroups: subgroup I (seven exons and six introns), subgroup II (eight exons and seven introns) and subgroup III (nine exons and eight introns).](https://academic.oup.com/aob/article-abstract/110/3/743/2769127)
Expression patterns of GmCYP707As under imbibition, dehydration–rehydration and salinity conditions

It was reported that ABA regulates seed dormancy (Jacobsen et al., 2002). Under default conditions, dry seeds maintained a high level of ABA but decreased levels upon imbibition (Kushiro et al., 2004). To verify the involvement of the ABA 8′-hydroxylase gene in soybean seed germination, we quantified mRNA levels of CYP707A genes from collected embryos and cotyledons at 0, 1, 6 and 24 h after imbibition through qRT-PCR. We found that A1a, A1b, A2a, A2b, A2c and A5 were quickly induced at imbibition and peaked at 24 h after imbibition in embryos (Fig. 4C). A1a, A1b, A2a and A2b were also induced in cotyledons at 24 h after imbibition; A2c, A3a and A5 were high at the beginning of imbibition but decreased dramatically after 24 h in cotyledons; and A3b and A4a were relatively lower and remained stable in cotyledons (Fig. 4D).

As drought increases ABA biosynthesis and alters ABA catabolism (Seiler et al., 2011), we investigated the expression levels of GmCYP707A genes in roots and leaves dried for 0, 2, 4 and 8 d, and rehydrated for 1, 6 and 24 h to uncover the responses of soybean ABA 8′-hydroxylase genes to water stress.

In soybean roots, except for A2a, expression levels of GmCYP707A genes were induced by short-term (2 d) or long-term (8 d) dehydration. Among them, A1b, A2b, A2c, A4a and A5 responded positively to 2 d of dehydration but their levels of expression decreased after 8 d of dehydration, while expression levels of A1a, A3a and A4b remained high after 8 d of dehydration. Upon 1 h of rehydration, transcripts of A1a, A3a and A4b remained high after 8 d of dehydration. The A2a gene might be rehydration-specific, given that its transcripts stayed at a low level during dehydration but peaked after 6 h of rehydration (Fig. 5A).

In soybean leaves, all GmCYP707A genes were induced by dehydration (Fig. 5B). Under dehydration conditions, the transcript level of A2c reached a maximum on day 2, mRNA

---

**Table 3. Structure of ten GmCYP707A genes**

<table>
<thead>
<tr>
<th>Proposed name</th>
<th>No. of exons</th>
<th>No. of introns</th>
<th>First exon length (bp)</th>
<th>Total exon length (bp)</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>GmCYP707A1a</td>
<td>7</td>
<td>6</td>
<td>201</td>
<td>1407</td>
<td>I</td>
</tr>
<tr>
<td>GmCYP707A1b</td>
<td>7</td>
<td>6</td>
<td>198</td>
<td>1404</td>
<td>I</td>
</tr>
<tr>
<td>GmCYP707A2a</td>
<td>7</td>
<td>6</td>
<td>204</td>
<td>1407</td>
<td>I</td>
</tr>
<tr>
<td>GmCYP707A2b</td>
<td>7</td>
<td>6</td>
<td>78</td>
<td>1278</td>
<td>I</td>
</tr>
<tr>
<td>GmCYP707A2c</td>
<td>7</td>
<td>6</td>
<td>108</td>
<td>1287</td>
<td>I</td>
</tr>
<tr>
<td>GmCYP707A3a</td>
<td>9</td>
<td>8</td>
<td>201</td>
<td>1449</td>
<td>II</td>
</tr>
<tr>
<td>GmCYP707A3b</td>
<td>9</td>
<td>8</td>
<td>321</td>
<td>1539</td>
<td>II</td>
</tr>
<tr>
<td>GmCYP707A4a</td>
<td>9</td>
<td>8</td>
<td>213</td>
<td>1491</td>
<td>II</td>
</tr>
<tr>
<td>GmCYP707A4b</td>
<td>8</td>
<td>7</td>
<td>201</td>
<td>1320</td>
<td>III</td>
</tr>
<tr>
<td>GmCYP707A5</td>
<td>8</td>
<td>7</td>
<td>207</td>
<td>1440</td>
<td>III</td>
</tr>
</tbody>
</table>

The genomic and cDNA sequences of ten soybean CYP707A genes were extracted from the Phytozome database and analysed in the GSDS website (http://gsds.cbi.pku.edu.cn/index.php).

---

Fig. 4. Expression patterns of ten GmCYP707A genes in soybean roots (A), leaves (B), embryos (C) and cotyledons (D). The name A1a represents GmCYP707A1a, and so on. The times indicated in legend of C and D are times after imbibition of soybean seeds. Soybean plants were grown in sand culture system, and relative expression level of genes tested were from four independent experiments (means ± s.e.). The soybean housekeeping gene Glyma10g38460, a calcium/calmodulin-dependent protein kinase-related gene, was used as an endogenous control to normalize the samples. The times indicated in C are times after imbibition of soybean seeds in C and D.
abundance of A2b, A4a and A4b peaked on day 4, and expressions of A1a, A1b and A2a were highest on day 8. After rehydration, expression levels of A3a, A3b and A5 were increased, but the others remained stable or decreased. Under dehydration–rehydration conditions, A1a and A1b in soybean roots and leaves displayed different patterns. In roots, A1a and A1b were highly expressed before rehydration. By contrast, A1a and A1b were induced by dehydration stress, but decreased quickly after 24 h of rehydration in leaves. A2a was induced significantly by rehydration in roots, but by dehydration in leaves. A2b showed higher level in leaves after 4 d of dehydration and then decreased. A2c showed a similar pattern between roots and leaves, responding positively to dehydration very quickly but decreasing after 4 d. The transcript abundances of A3b, A4a and A4b were low in roots before stress, in contrast to A3a and A5. A3a showed a similar pattern between roots and leaves: it was increased by dehydration and responded to rehydration very rapidly but decreased after 24 h of rehydration. A4b and A5 also responded rapidly to rehydration (Fig. 5A, B).

ABA is positively involved in salt stress responses (Nambara and Marion-Poll, 2005). Salt treatment alters ABA content in plant tissues (Yang et al., 2011). To verify the involvement of GmCYP707A genes in salt stress, we investigated their responses under short-term (0, 1 and 24 h) and long-term (8 days) salinity. All of the family members were induced by salt stress in soybean roots (Fig. 6A). A1a, A2a, A2c, and A3a responded quickly (before 24 h) whereas the remaining family

![Fig. 5. Expression analyses of the ten GmCYP707A genes in roots (A) and leaves (B) under dehydration and rehydration treatment. Total RNAs were extracted from soybean roots and leaves at the indicated time points, which had been dehydrated for 8 d and rehydrated for 24 h. A1a represents GmCYP707A1a, and so on. The soybean plants were grown in sand culture system, and relative expression levels of the genes tested were from four independent experiments (means ± s.e.). The soybean housekeeping gene Glyma10g38460 was used as an endogenous control to normalize the samples.](https://academic.oup.com/aob/article-abstract/110/3/743/2769127)
members needed a longer time to be induced. In soybean leaves, except A2c, all of the members were induced; levels of A1a, A1b, A3a, A3b, A4a, A4b and A5 peaked in the short term (24 h) while A2a and A2b took longer to peak (Fig. 6B).

**DISCUSSION**

**Verification of GmCYP707A1a as an ABA 8′-hydroxylase**

GmCYP707A1a was previously predicted to interact with GmWNK1 and might be involved in ABA catabolism (Wang et al., 2010). AtCYP707A2 was documented as a functional ABA 8′-hydroxylase and played a crucial role in determining ABA levels in seeds. An atcyp707a2 T-DNA mutant was able to maintain high levels of ABA even after 24 h of imbibition (Kushiro et al., 2004). To test whether GmCYP707A1a is a functional ABA 8′-hydroxylase, we used a T-DNA mutant of atcyp707a2 to provide a complementary analysis.

The level of ABA is maintained through its biosynthesis, catabolism and conjugation with other molecules in plant tissues (Nambara and Marion-Poll, 2005). Biosynthesis of ABA has been extensively studied at transcriptional and translational levels, and numerous genes encoding enzymes responsible for ABA generation have been cloned and studied. Moreover,
the crucial hydroxylase CYP707A responsible for ABA catabolism has attracted much recent attention. The ABA 8'-hydroxylase gene in Arabidopsis and rice has been studied in detail, but not previously in soybean. We have reported that soybean WNK1 interacts with a candidate ABA 8'-hydroxylase, GmCYP707A1a, based on a bacterial hybridization and BiFC technique (Wang et al., 2010), but it was not clear whether GmCYP707A1a acts as an ABA 8'-hydroxylase. To clarify this, we tested the functions of GmCYP707A1a in Arabidopsis. As indicated in Fig. 1, loss of function of AtCYP707A2 altered its ABA sensitivity as determined by primary root growth and lateral root formation, as well as seed germination. Accordingly, we over-expressed soybean GmCYP707A1a in a cyp707a2 T-DNA knock-down mutant, the only single mutant that showed a significant phenotype related to seed germination under ABA treatment (Kushiro et al., 2004). Germination rate analysis indicated that GmCYP707A1a possibly encodes an ABA 8'-hydroxylase due to the complementation of over-expression of GmCYP707A1a to AtCYP707A2. We postulated that knock-down of AtCYP707A2 decreases the turnover rate of endogenous ABA, but over-expression of GmCYP707A1a in atcyp707a2 rescues the turnover rate of ABA, and high levels of ABA inhibit seed germination. In addition, under normal conditions (no ABA application), compared with the wild-type, the number of lateral roots of atcyp707a2 also decreased significantly, but over-expression of GmCYP707A1a rescued lateral root formation (P < 0.05; Fig. 1D), indicating that a higher endogenous ABA level retards root formation. Hence we argue that GmCYP707A1a is an ABA 8'-hydroxylase. However, it will be necessary to provide biochemical evidence that GmCYP707A1a functionally hydroxylates ABA. ABA is involved in seed germination and crop performance under abiotic stresses such as drought, salt and cold. Accordingly, transgenic plants over-expressing ABA biosynthetic or ABA-signalling genes can improve tolerance to these stresses (Iuchi et al., 2001), but over-expression of ABA hydroxylase decreases levels of endogenous ABA and generates ABA-deficient phenotypes (Nitsch et al., 2009). ABA negatively interacts with gibberellin to inhibit seed germination (Jacobson et al., 2002; Liu et al., 2010), and hence decreasing ABA levels in seeds through up-regulating expression of ABA hydroxylase will stimulate seed germination of soybean.

Gene structure and evolutionary analysis of the soybean CYP707A family

Although the genome of Glycine max was released in 2010 (Schmutz et al., 2010), the functions of most soybean genes remain unclear. Analysing the structure, evolution and functions of the soybean gene family can be achieved through data mining, and some successful cases have been reported (Lu et al., 2010; Li and Dhaubhadel, 2011). Genome-wide BLAST searches reveals that the soybean genome harbours ten CYP707A genes (Table 1), located on seven different chromosomes and with similar ORF length and protein size (Table 3). The Arabidopsis genome contains four CYP707A genes, and rice, barley, bean and maize two, two, three and five, respectively (Vallabhpaneni and Wurtzel, 2010). However, soybean contains ten genes based on our results, implying that the regulation networks of ABA catabolism in soybean are more complicated than in other species. The relatively higher number of CYP707A family genes in soybean is consistent with the suggestion that gene duplication is universal in the soybean genome during its evolution (Schmutz et al., 2010).

Phylogenetic analysis indicates that CYP707As from plants can be divided into two subgroups, each consisting of two clades (Fig. 2A). The appearance of CYP707A from Physcomitrella paten, an older multicellular plant, implies that ABA hydroxylation in higher land plant cells evolved from a non-flowering moss. Alignment of amino acid sequences of CYP707As from plants demonstrates the higher conservatism between different CYP707As (Fig. 2B). We also found a highly conserved and important motif, PFG[NSD][G][TVIA][HR][SAM]CPG, in soybean CYP707As, which is crucial for its catalytic function (Kushiro et al., 2004), indicating this motif may be the tag for ABA 8'-hydroxylase. Further detailed analysis on the effects of mutation at this motif is promising in deciphering the mechanisms of CYP707A in soybean.

Expression patterns of soybean CYP707A family genes in seeds, roots and leaves

As the four known CYP707A genes in Arabidopsis have different expression patterns and functions (Kushiro et al., 2004), we analysed the expression patterns of ten soybean CYP707A genes to reveal their functions in soybean. A1a and A1b were found to be predominantly expressed in roots, A4a, A4b and A5 in leaves, and A2b, A2c and A3a in both roots and leaves (Fig. 4A, B), indicating that each plays a different role in ABA catabolism related to seed germination and stress responses in different tissues. Higher mRNA abundance of A1a, A1b and A3a in roots suggests they play prominent roles in root ABA metabolism, and predominant levels of A3b, A4a, A4b and A5 in leaves indicate their crucial roles in leaf ABA catabolism. Tissue-specific patterns of the CYP707A family appear to be universal. For example, ABA8ox3a in maize is predominantly expressed in leaves, ABA8ox1a in roots, ABA8ox2 and ABA8ox1a in endosperm, and ABA8ox2, ABA8ox1a and ABA8ox1b in embryos (Vallabhpaneni and Wurtzel, 2010). In Arabidopsis, AtCYP707A3 plays important roles in mediating ABA levels in vegetative tissues and is mainly involved in responses of stomata and vascular tissues to water deficit, but AtCYP707A2 is mainly expressed in seeds and plays a prominent role in seed dormancy (Kushiro et al., 2004; Umezawa et al., 2006; Okamoto et al., 2011). It is reasonable to speculate that each CYP707A in soybean has a specific role in development and stress adaptations.

Responses of soybean CYP707A family genes to imbibition, dehydration and salt stresses

It has been shown that ABA levels decrease during seed imbibition (Hable et al., 1998; Jacobson et al., 2002). Arabidopsis dry seeds have a basal level of ABA; upon imbibition, ABA level in seeds decreases sharply and reaches the basal level after 12 h (Kushiro et al., 2004), indicating
stimulated ABA catabolism during seed imbibition. AtCYP707A2 mRNA is highly abundant in dry seeds and is up-regulated immediately after imbibition, peaks at 6 h and then decreases (Kushiro et al., 2004). Consistent with their results, we found that mRNA levels of A1a, A1b, A2a, A2b, A2c and A5 were rapidly up-regulated after imbibition and reached a maximum after 24 h in soybean embryos (Fig. 4C), implying that these genes may be involved in soybean seed germination and play overlapping roles. mRNA levels of A3a, A3b, A4a and A4b were low throughout imbibition. However, we lack direct evidence for the genes involved in seed germination. We also noted that A1a, A1b, A2a and A2b were induced rapidly both in embryos and cotyledons (Fig. 4C, D), implying that they may be involved in ABA catabolism during early seed germination. Furthermore, A2c, A3a and A5 decreased in soybean cotyledons during imbibition, highlighting the complexity of ABA metabolism (Fig. 4D).

The ABA contents in Arabidopsis increased in response to dehydration and there was a sharp decrease of ABA levels upon rehydration (Umezawa et al., 2006). We found that dehydration greatly stimulated expression of GmCYP707A2a, A2b, A3b, A4a and A4b in soybean roots. We thus envisaged that upon rehydration ABA level is decreased via stimulated oxidation. Transcripts of only GmCYP707A5 in leaves were quickly induced by 1 h of rehydration (Fig. 5B). As A5 is not expressed in roots, is present at relatively low level in leaves under normal conditions and is induced rapidly by rehydration in leaves, we postulate that A5 is the major player in down-regulating ABA content in leaves under rehydration.

Expression levels of all AtCYP707A genes were increased under 300 mm NaCl stress (Saito et al., 2004). Similarly, we noted rapid induction of A2c and A4b by 300 mm NaCl treatment at 1 h, but not in leaves (Fig. 6). Meanwhile there was a rapid decline of A2c transcripts in leaves under salinity conditions. A3a, A4a, A4b and A5 were also induced under salt stress in roots in which transcript levels remained low before treatment (Fig. 6A). Whether the changes of CYP707A genes are related to an ABA increase in leaves is thus worthy of further study.

On the other hand, high humidity (90 % relative humidity) treatment decreases Arabidopsis ABA contents and increases levels of PA, DPA and catabolites of ABA 8′-hydroxylation. Consistent with that, the transcript of AtCYP707A3 is significantly increased by high humidity (Okamoto et al., 2009). OsABA8ox1, encoding ABA 8′-hydroxylase, is induced by ethylene under submergence and is responsible for the decline of endogenous ABA (Saika et al., 2007). In addition, the transcript of tomato CYP707A1 is regulated by the phytohormones gibberellin and auxin (Nitsch et al., 2009). The above studies have also demonstrated that CYP707A genes are regulated by other developmental and environmental factors. Interestingly, recent studies have identified four CYP707A genes encoding ABA 9′-hydroxylase in Arabidopsis, although their activities are relatively weak (Okamoto et al., 2011). We are also interested in verifying whether soybean CYP707A5 act as ABA 9′-hydroxylases.

As expressions of soybean CYP707A genes were regulated by imbibition, drought and salt stress, we analysed the cis-elements in the promoter region of soybean ABA 8′-hydroxylases and found the existence of ABA-, salt- and dehydration-responsive elements (Supplementary Data Table S2). Therefore, we postulated that imbibition, water and salt stress signalling leads to changes of mRNA or protein levels of ABA-related transcription factors, which bind to the promoter of ABA 8′-hydroxylase to regulate their expression, ultimately resulting in changes of endogenous ABA levels. Different expression patterns of ten ABA 8′-hydroxylase genes indicate the complexity of ABA hydroxylation in soybean.

Together, the transgenic study data presented here provide the first biological evidence for GmCYP707A1a as an ABA 8′-hydroxylase, and we have explored the expression patterns of all GmCYP707A genes in soybean under drought and salt stress conditions in details. Further detailed studies, such as analysis of promoter activity in soybean combined with transgenic studies, and extensive biochemical analysis, will reveal the specific role of soybean ABA 8′-hydroxylase in ABA turnover in the near future.

SUPPLEMENTARY DATA
Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: list of quantitative real-time PCR primers. Table S2: cis-element analysis of promoters of GmCYP707A genes.

ACKNOWLEDGEMENTS
We thank the Arabidopsis Seed Resource Center (ABRC) for providing the T-DNA insertional line of atcyp707a2, and Dr Guohui Zhu for providing the over-expression line of AtCYP707A2. pOx vector was a gift from Dr Yaoguang Liu (South China Agriculture University). This study was supported by the National Natural Science Foundation of China (No. 31071848) and National Key Basic Research Special Funds of China (2011CB100301).

LITERATURE CITED


