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ORIGINAL PAPER

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Genomic characterization of the S-adenosylmethionine decarboxylase genes from soybean

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Abstract A full-length gene *GmSAMDC1*, encoding the S-adenosylmethionine decarboxylase (SAMDC), a key enzyme involved in polyamine biosynthesis, was identified from soybean expressed sequence tags and was characterized. *GmSAMDC1* encoded a peptide of 355 amino acids. When compared with other plant SAMDCs, the GmSAMDC1 protein had several highly conserved regions including a putative pro-enzyme cleavage site and a PEST sequence. The 5′ leader sequence of the the *GmSAMDC1* mRNA contained two additional open reading frames (ORFs), which may regulate the translational process. The genomic sequence of the *GmSAMDC*1 gene contained three introns in the 5′ leader sequence, but no intron in the 3′-UTR or the main pro-enzyme ORF. A simple sequence repeat (SSR) was found in intron 2, and the *GmSAMDC1* gene was mapped to linkage group D1 using this SSR. The genomic organization of the *GmSAMDC1* gene in the subgenus *Glycine* and the subgenus *Soja* was found to be different by Southern-blot and PCR analysis. A pseudogene, *GmSAMDC2*, was also identified. This gene contained no intron and lost its two uORFs. Northern-blot analysis showed that the *GmSAMDC1* gene expression was induced by salt, drought and cold, but not induced by wounding; suggesting that the gene was implicated in response to multiple-stress conditions.

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Introduction

In plants, as in animals, yeast and bacteria, polyamines (PAs) such as spermidine, spermine and their precusor putrescine, seem to be related to cell division, embryogenesis, floral and fruit development, root formation and stress response (Evans and Malmberg 1989; Walden et al. 1997). The polyamine biosynthetic pathways have been well-characterized. In plants, putrescine is either synthesized from ornithine via ornithine decarboxylase (ODC) or from arginine via arginine decarboxylase (ADC). The formation of the tri-amine spermidine from the diamine putrescine, and the formation of the tetra-amine spermine from spermidine, are mediated by the enzyme S-adenosylmethionine decarboxylase (SAMDC). The substrate for the enzyme is S-adenosylmethionine (SAM) and the product is the decarboxylated SAM (dcSAM) which provides the aminopropyl moiety for spermidine and spermine biosynthesis. The activity of SAMDC has been shown to be rate-limiting in the biosynthesis of these polyamines (Slocum 1991)

Up to now several genes encoding SAMDC had been cloned and characterized from human (Pajunen et al. 1988), yeast (Kashiwagi et al. 1990), bacteria (Tabor and Tabor 1987), and plant species such as potato (MadArif et al. 1994), spinach (Bolle et al. 1995), Tritordeum (Dresselhaus et al. 1996), carnation (Lee et al. 1997) and *Arabidopsis thaliana* (Franceschetti et al. 2001). There is little sequence similarity between the SAMDC from *Escherichia coli* and those from eukaryotic resources. But the similarity among the plant SAMDCs is relatively high. The *SAMDC* genes from rice and wheat have also been cloned and characterized in our Laboratory (Li and Chen 2000a, b). In the present study, a soybean (*Glycine max* (L.) *Merr*) *GmSAMDC1* gene was isolated. Its genomic structure, the linkage location and

the expression pattern were characterized. The polymorphism between the different subgenus *Glycine* and the subgenus *Soja* were investigated. Another *SAMDC* gene, *GmSAMDC2*, was also identified from the subgenus *Soja*. The genomic structure of the two genes was compared and the possibility that *GmSAMDC2* was a pseudogene was discussed.

Materials and methods

Plant material, growth conditions and stress treatments

Seeds of the soybean (*Glycine max* (L.) Merr) cultivar Kefeng 1 and the other 23 accessions from 11 species (*Glycine argyrea*, *Glycine canescens*, *Glycine clandestine*, *Glycine cyrtoloba*, *Glycine falcate*, *Glycine latifolia*, *Glycine tabacina*, *Glycine tomentella*, *Glycine soja*, *Glycine gracillis* and *Glycine max*) of the two subgenera of *Glycine* and *Soja* (Wu et al. 2001a) were grown in pots filled with vermiculite. Two-week-old seedlings were harvested and stored at −70°C for DNA isolation. The two-week-old seedlings of Kefeng 1 were used for the treatment with salt, drought, cold and wounding. For salt-stress treatment, the seedlings were immersed with the roots, in a solution of 0.8% NaCl for different times. For wounding treatment, leaves on the seedlings were cut and collected at 0, 2, 6 and 12 h after the initiation of the cutting. For drought treatment, the seedlings were withheld from water for 4, 5 and 6 days, and collected. The seedlings that were already subjected to drought treatment for 6 days were re-watered and collected after 1 day. For cold treatment, the seedlings were transfered to a chamber of 4°C for different times, and the leaves were sampled. Leaves from all the treatments were harvested at the indicated times and stored at −70°C for RNA isolation.

Cloning of the cDNA and the genomic sequence corresponding to the *GmSAMDC1* gene

A *SAMDC* gene was identified from soybean cultivar Kefeng 1 by EST analysis and designated as *GmSAMDC1*. The full-length sequence of *GmSAMDC1* has been deposited in GenBank under the accession No. AF488307. In order to obtain the genomic sequence corresponding to the *GmSAMDC1* gene, two pairs of primers were designed and used in PCR analysis. The first pair (the sense primer 5′-ACGAGCTTTCTGATTGCCTT-3′ and the anti-sense primer 5′-ACCGCCATGGCCATGTAACA-3′) was used to amplify the 5′ end of the gene, and the second pair (the sense primer 5′-TGTTACATGGCCATGGCGGT-3′ and the anti-sense primer 5′-GCACGCAAACAAAGTGATTT-3′) was used to amplify the 3′ end of the gene. The PCR reaction was performed with 200 ng of genomic DNA, 0.5 μM of each primer, 0.2 mM of dNTPs and 1 unit of La *Taq* polymerase (TaKaRa), with 1×PCR buffer in a total volume of 25 μl. An initial denaturation at 94°C for 4 min was followed by 35 cycles of 1 min at 94°C, 2 min at 54°C and 3 min at 72°C. The amplified fragments were purified and cloned into the pGEM-T easy vector (Promega) for sequencing.

Southern hybridization and PCR analysis

Genomic DNA extraction and Southern-blot analysis were performed as described previously (Chen et al. 1991). Ten micrograms of the genomic DNA were digested with *Taq*I, and separated on a 0.8% agarose gel and transferred onto Hybond N+ nylon membranes. Hybridization was carried out for 16 h at 65°C using the α-^{32}P-dCTP-labeled full-length *GmSAMDC1* cDNA as a probe. The filters were washed with 2×, 1×, 0.5×SSC/0.1% SDS for 15 min at 65°C, respectively, and exposed to FUJI Medical X-ray film at −70°C.

To compare the genomic difference among various accessions of soybean, two primers (the sense primer 5′-ACGAGCTTTCTGATTGCCTT-3′ and the antisense primer 5′-ACCGCCATGGCCATGTAACA-3′) were used to amplify the 5′ end of the *GmSAMDC1* genome sequence from the genomic DNAs.

Northern hybridization analysis

Total RNA was isolated from different plant materials by the procedure of Zhang et al. (1995). Thirty micrograms of the total RNA for each lane were denatured and fractionated on a 1.2% agarose gel containing formaldehyde, and blotted onto Hybond N+ nylon membranes in 20×SSC. Northern hybridization was carried out at 65°C using the α-^{32}P-dCTP-labeled full-length *GmSAMDC1* cDNA as a probe. The filters were washed with 2×, 1×, 0.5×SSC/0.1% SDS for 15 min at 42°C and exposed to FUJI Medical X-ray film at −70°C. After stripping the probes, the same blots were re-hybridized with the 18S rRNA gene to verify the equal loading.

Gene mapping

A mapping population of a soybean recombinant inbred line NJRIKY, which was derived from a cross between Kefeng 1 and Nannong 1,138-2 (He et al. 2001; Wu et al. 2001b), was used to map the gene. The SSR primers were designed according to the sequence near the SSR and used to amplify with the genomic DNAs from the parents and the individuals of the mapping population. The amplified products were separated on a 3% agarose gel and the segregation result was analyzed using the software of Mapmaker (version 3.0).

Results

Structural analysis of the *GmSAMDC1* gene

During a soybean EST sequencing project, about 30,000 ESTs from soybean cultivar Kefeng 1 were sequenced and analyzed. One *SAMDC*-like gene was identified and studied further. The corresponding gene was designated *GmSAMDC1*. The full-length cDNA of *GmSAMDC*1 was 1,824 bp in length, consisting of 556 bp of the 5′ leader sequence and 200 bp of the 3′ untranslated region (UTR). There was a putative polyadenylation signal in the 3′-UTR (AATAAA, 1,784 bp–1,789 bp). The complete open reading frame of 1,068 bp encoded a SAMDC pro-enzyme of 355 residues with a calculated molecular weight of 38.99 kDa. The deduced polypeptide sequence of *GmSAMDC1* was compared with known SAMDCs from plants, mammals and yeast (Fig. 1A). The alignment indicated the presence of several highly conserved regions in GmSAMDC1. One region included the sequence of LSESSLF from residues 67 to 74 in GmSAMDC1, representing a putative pro-enzyme cleavage site. The cleavage of the SAMDC pro-enzyme would result in the formation of a small β-chain (N-terminal part) and a larger α-chain (c-terminal) (Pajunen et al. 1988; Kashiwagi et al. 1990; MadArif et al. 1994; Da'dara et al. 1996). Another conserved region was the PEST sequence of TIHVTPEDGFSYASFE from residues 245 to 260. This sequence was probably associated with the rapid turnover of the SAMDC protein (Rogers et al. 1986) (Fig. 1A). An aspartic residue in the human

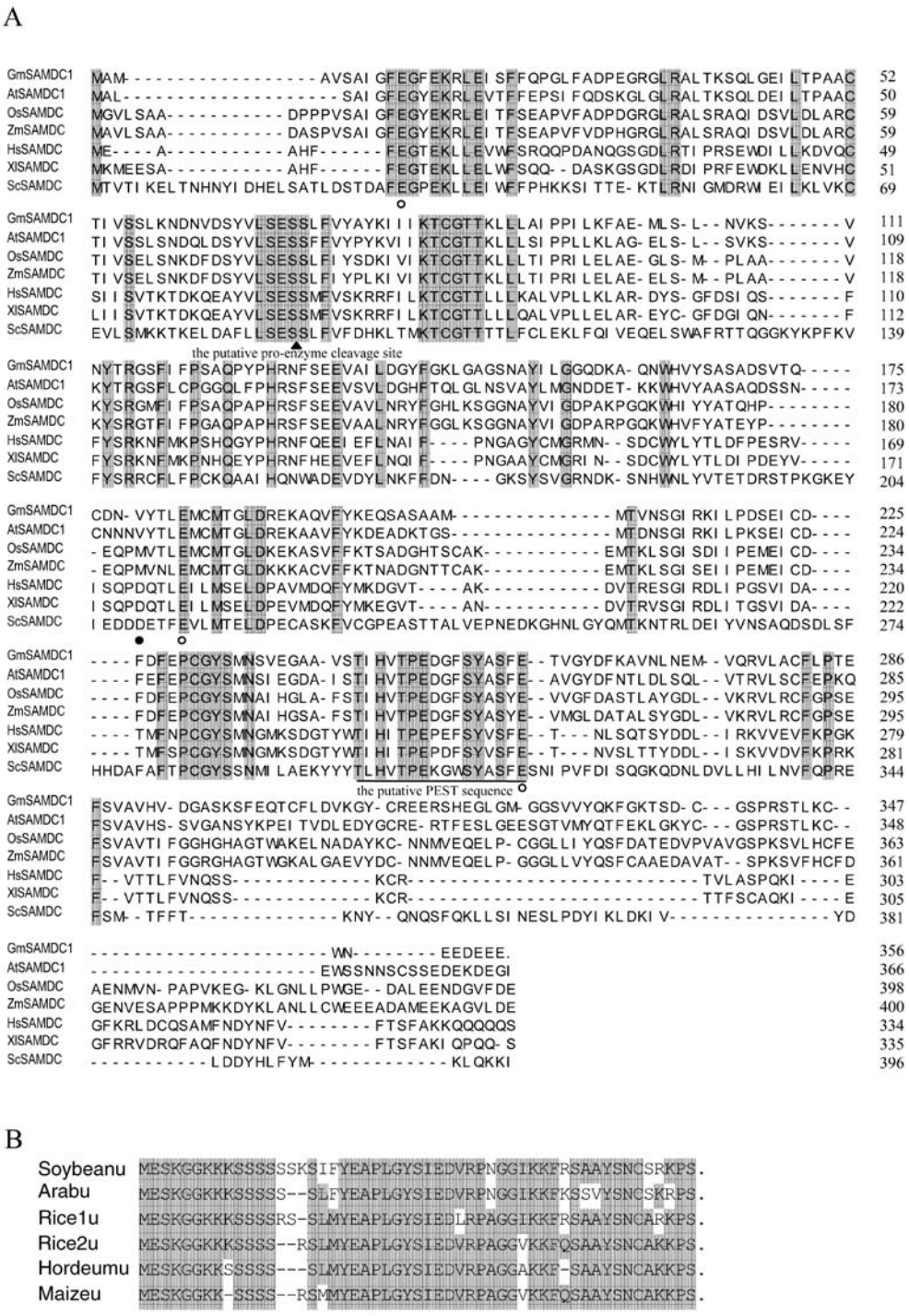

Fig. 1 A Comparison of the deduced amino acid sequences of the GmSAMDC1 with the AtSAMDC (*A. thaliana*, Y07765), OsSAMDC (*Oryza sativa*, AF067194), ZmSAMDC (*Zea mays*, Y07767), HsSAMDC (*Homo sapiens*, M21154), XlSAMDC (*Xenopus laevis*, S82621) and ScSAMDC (*Saccharomyces cerevisiae*, M38434). Gaps are introduced to maximize similarity. The *triangle* indicates a putative cleavage site of the SAMDC pro-enzyme and the putative PEST sequence is *underlined*. Conserved glutamate residues necessary for the stimulation of pro-enzyme processing of the human AdoMetDC by putrescine are indicated by *open circles*. The aspartic residue necessary for the stimulation of processing in the human enzyme by putrescine is indicated by a *filled circle*. *Shading* indicates the identity of amino acids. **B** Alignment of the deduced amino acid sequences of the small uORF in the 5′ leader sequences of plant *SAMDC* mRNA. Six sequences of Soybeanu (*G. max*, AF488307), Arabu (*A. thaliana*, Y07765), Rice1u (*O. sativa*, AJ251899), Rice2u (*O. sativa*, Y07766), Hordeumu (*Hordeum chilense*×*Triticum durum*, X83881) and Maizeu (*Z. mays*, Y07767) were compared

SAMDC (Asp-174) identified by Xiong (Xiong et al. 1997) as being necessary for stimulation of the processing of the human pro-enzyme by putrescine was absent from GmSAMDC1 and the other plant SAMDCs. Whereas the other three glutamic residues (Glu-11, Glu-178 and Glu-256), necessary for stimulation of the processing of the human pro-enzyme by putrescine, were all conserved in GmSAMDC1 and the other plant and animal SAMDCs. The phylogenetic analysis showed that the GmSAMDC1 clustered with the SAMDC from *Arabidopsis*, but not with others; and exhibited 62.6% similarity with the SAMDC from *Arabidopsis*. The similarity between plant SAMDCs and those from yeast or mammals was less than 30%.

In the 5′ leader sequence of *GmSAMDC1*, a small upstream ORF (uORF) was identified, and encoded a peptide of 53 amino acid residues. Similar uORFs were also found in the 5′ leader sequence of the other plant

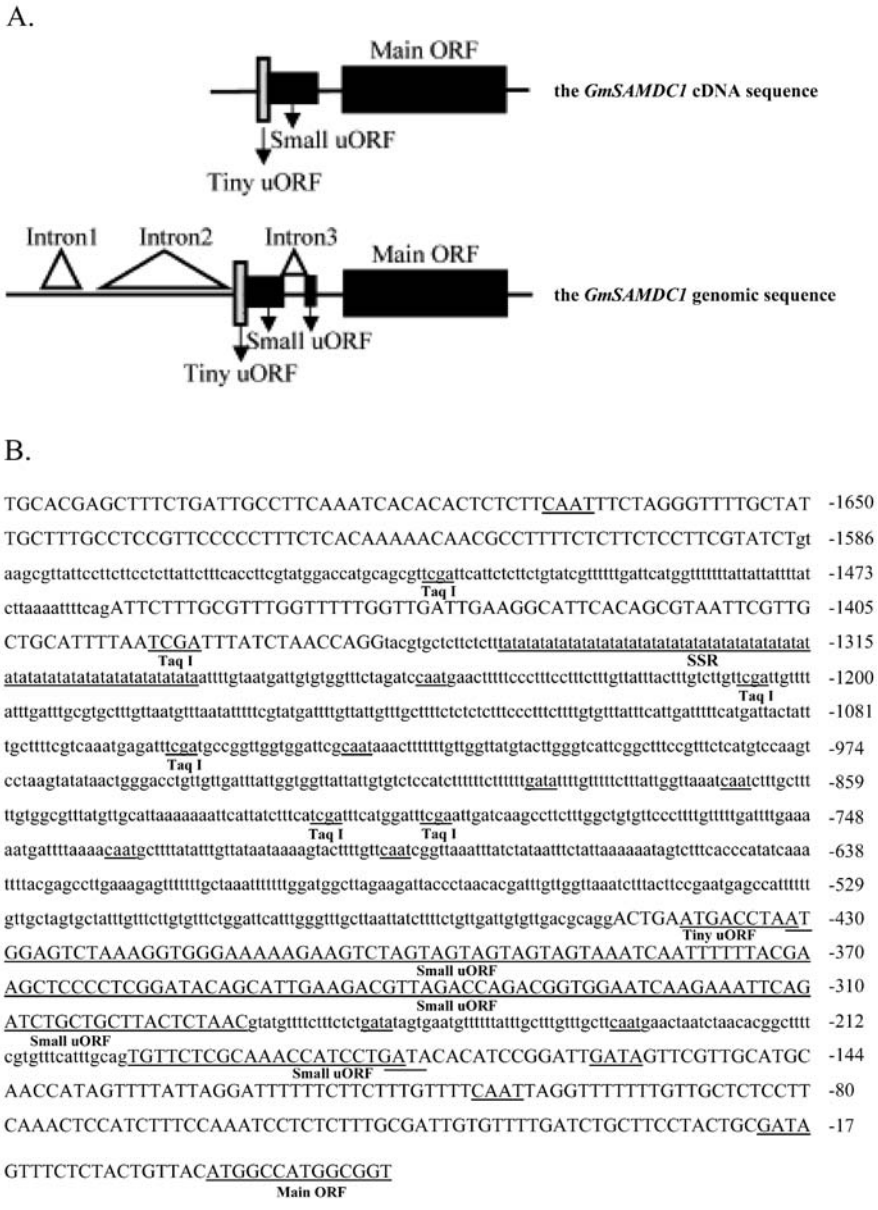

Fig. 2 A Schematic representation of the structure of the *GmSAMDC1* cDNA and the *GmSAMDC1* genomic sequence. The relative positions of the introns and overlapping tiny and small uORFs are depicted. **B** The 5′ end of the *GmSMADC1* genomic sequence. The two uORFs and the SSR sequences were *underlined*. The three introns were shown by *lowercase letters*. The predicted protein binding sites CAAT and GATA were *underlined*. The cutting sites of *Taq*I were also *underlined*

SAMDC genes (Franceschetti et al. 2001). The amino acid sequence of these peptides was highly conserved (Fig. 1B), with only one region of variation towards the N-terminus where the variation was due primarily to varying numbers of successive serine residues. The small uORF may play roles of the translational repression in response to polyamines (Franceschetti et al. 2001). Furthermore a tiny uORF upstream of, and overlapping the small uORF, was also found (Fig. 2A). This tiny uORF encoded a peptide of only two residues (MT). The last nucleotide (A) of the tiny uORF stop codon was the first nucleotide of the initiating codon (ATG) of the small uORF, suggesting that if the ATG of the tiny uORF was identified by the translational machinery, it was unlikely that the ATG of the small uORF would be recognized (Franceschetti et al. 2001).

Genomic sequence and mapping of the *GmSAMDC1* gene

The genomic sequence corresponding to the *GmSAMDC1* cDNA was amplified from Kefeng 1 genomic DNA and cloned. The result of sequencing showed that the gene contained no intron in the 3′ UTR or the main pro-enzyme ORF. However, three introns (129 bp, 931 bp and 95 bp) were found in the 5′ leader sequence of the genomic sequence of the *GmSAMDC1* gene, and splicing followed the GT/AG rule (Fig. 2A, B).

The sequences of the three introns were further analyzed, and a simple sequence repeat (SSR) (TA repeat) was identified in intron 2 (Fig. 2B). SSR has been extensively used as molecular markers in the construction of the linkage map (Dib et al. 1996). Thus we cloned and sequenced the genomic region corresponding to the 5′ leader sequence of the *GmSAMDC1* cDNA

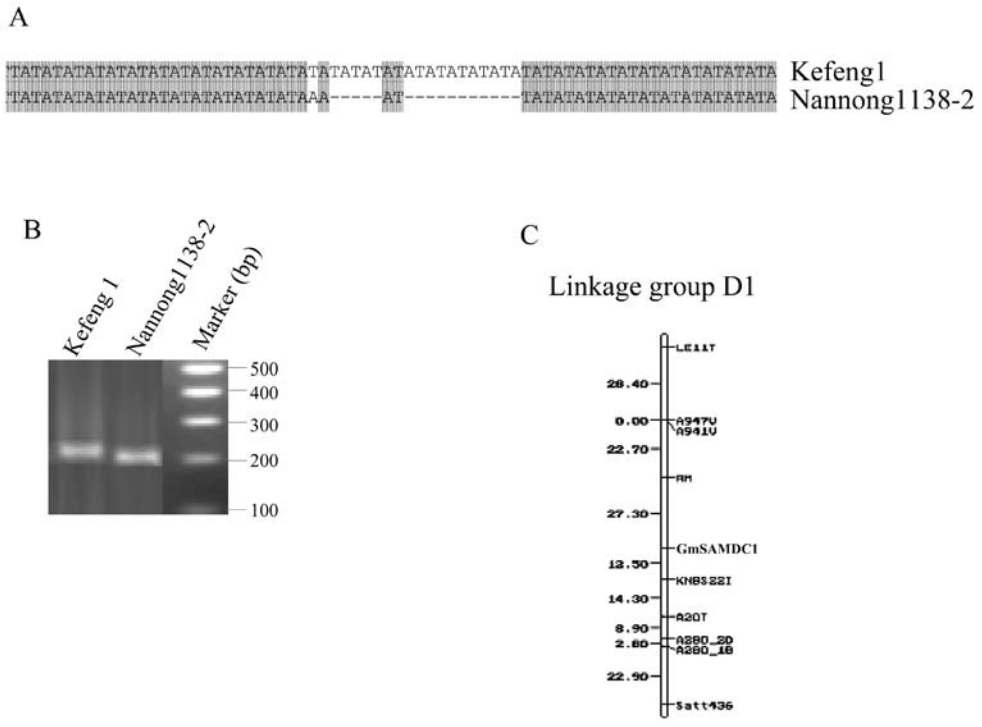

Fig. 3 A The sequence of SSRs in the intron 2 of the *GmSAMDC1* genomic sequences from the two parents of the mapping population: Kefeng1 and Nannong 1,138-2. **B** PCR amplification of the SSR sequence from the parents, Kefeng1 and Nannong 1,138-2. The amplified fragments were separated on a 3% agarose gel. The length of the fragments was 240 bp and 224 bp in Kefeng 1 and Nannong 1,138-2, respectively. **C** Mapping of the *GmSAMDC1* gene on soybean linkage group D1

from another soybean cultivar, Nannong 1,138-2. A similar SSR (TA repeat) was found. However, the length of this SSR was shorter than that from Kefeng 1 (Fig. 3A), indicating the presence of a polymorphism between the two SSRs. Therefore, the mapping population of a soybean recombinant inbred line NJRIKY, derived from a cross between Kefeng 1 and Nannong 1,138-2, was used to map the *GmSMADC*1 gene. A pair of primers (the sense primer: 5′-TCGTTGCTGCATTTTAATCG-3′, and the antisense primer: 5′-TAAACATTAACAAAGCACGC-3′) was designed according to the sequence near the SSR, and used to amplify different fragments from the parents (Fig. 3B) and the individuals of the mapping population. The length of the fragments from Kefeng 1 and Nannong 1,138-2 was 240 bp and 224 bp, respectively. The segregation of the two polymorphic fragments was analyzed using the software of Mapmaker (version 3.0). As a result, the *GmSAMDC1* gene was mapped to the linkage group D1 (Fig. 3C).

Comparison of the *SAMDC* genomic organization among different accessions

Since sequence variation was found between two cultivars Kefeng 1 and Nannong 1,138-2, we further compared the genomic organization of the *SAMDC* gene among different accessions from two subgenera, *Glycine* and *Soja*. The genomic DNAs from 24 accessions of 11 species were digested with *Taq*I and hybridized with the α-^{32}P-dCTP-labeled full-length *GmSAMDC1* cDNA. The result was presented in Fig. 4A. In the subgenus *Soja*, all the accessions had at least five apparent hybridized bands and the pattern was the same, indicating that the organization of the *SAMDC* gene(s) was identical or very similar. We further examined the genomic sequence of the *GmSAMDC1* gene from Kefeng 1 and found ten sites for *Taq*I (whereas, six sites in the 5′ leader sequence was shown in Fig. 2B). The expected sizes of the DNA fragments were 15 bp, 137 bp, 150 bp, 151 bp, 183 bp, 243 bp, 441 bp, 588 bp and 838 bp, plus two flanking sequences of unknown length containing 182 bp and 51 bp of the 5′-end and 3′-end sequences, respectively. Considering the presence of introns and the use of *GmSAMDC1* cDNA as probes for hybridization, the hybridized bands should include fragments of 838 bp, 588 bp, 441 bp, 150 bp and 137 bp. From Fig. 4A, the hybridized pattern was substantially consistent with the predictions. The stronger intensity of the 588 bp band may imply the presence of another *SAMDC* gene in the soybean genome. In the subgenus *Glycine*, the pattern was different. The largest band (838 bp) in subgenus *Soja* was lost in subgenus *Glycine*, indicating the occurrence of genomic alteration in this subgenus. Since the 838-bp fragment contained the regulatory sequences of the *GmSAMDC1* gene, the loss of it may affect the translation efficiency of *GmSAMDC1*. Variations in other small fragments were also observed in subgenus *Glycine* (Fig. 4A).

To further examine the genomic difference among different accessions, PCR analysis was performed to amplify the 5′ end of the *GmSAMDC1* genomic sequence (Fig. 4B). The total cDNAs from Kefeng 1 and the cloned *GmSAMDC1* cDNA were used as templates for controls. It can be seen that one fragment, identical to the predicted length of 567 bp, was obtained from both the Kefeng 1 cDNA and the cloned *GmSAMDC*1 cDNA. In other *Soja*

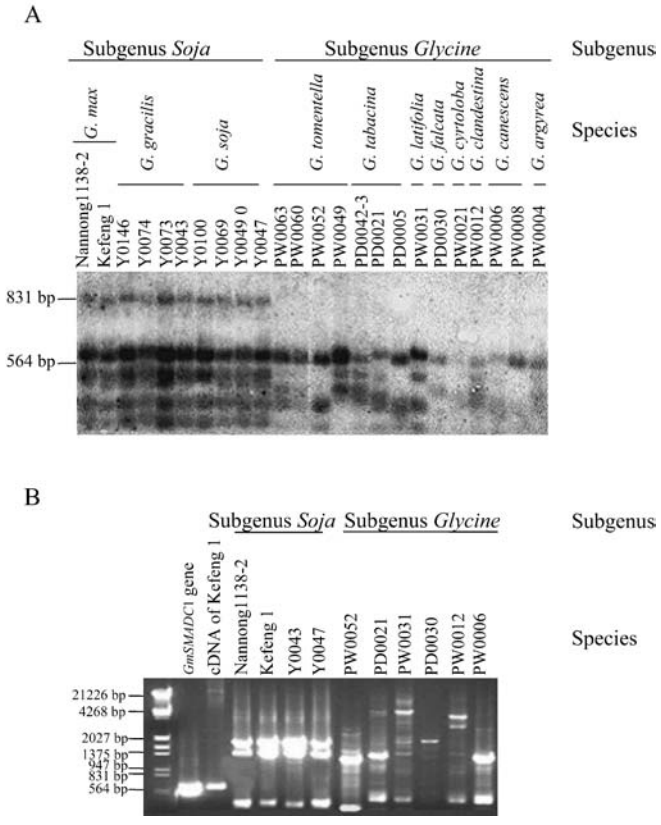

Fig. 4 Analysis of the genomic organization of the *GmSAMDC* gene in the two subgenera of *Glycine* and *Soja*. **A** Southern hybridization analysis of the *GmSAMDC* gene. Ten micrograms of genomic DNA from 24 accessions of 11 species were digested with *Taq*I. After electrophoresis on a 0.8% agarose gel, DNAs were transferred onto Hybond N^+ nylon membrane and hybridized with the labeled full-length *GmSAMDC1* cDNA probe. **B** Amplification of the 5′ leader sequence of the *GmSAMDC* gene from ten accessions. The total cDNAs of Kefeng 1 and the cloned *GmSAMDC1* cDNA was used as PCR templates

accessions, three fragments were obtained. The three fragments from Kefeng 1 were further cloned, sequenced and compared. We found that the middle-sized fragment was not related to the *GmSAMDC1* gene, whereas the other two bands were related. The sequence of the large fragment (1,722 bp) was at the 5′ end of the *GmSAMDC1* genomic sequence (Fig. 2B, Fig. 5). The sequence of the small fragment showed similarity to the 5′ end sequence of the *GmSAMDC1* genomic sequence, and its corresponding gene was named *GmSAMDC2* (Fig. 5). The striking difference between the 5′ end of *GmSAMDC2* and of the *GmSAMDC1* genomic sequence, was that the *GmSAMDC2* lost the uORFs and the three introns (Fig. 5). Because only one fragment was amplified from the cDNAs of Kefeng 1 (Fig. 4B, the third lane from the left), and this fragment corresponded to the sequence of the *GmSAMDC1* cDNA, it was very likely that *GmSAMDC2* represented a pseudo-gene for soybean SAMDC and wasn't transcribed.

PCR amplification for the 5′ end sequence of *SAMDC* was also performed for the accessions from subgenus *Glycine* (Fig. 4B). Various patterns were observed, indicating the divergence of the regulatory regions of different *SAMDC* genes.

The expression of the *GmSAMDC1* gene in response to salt, drought, cold and wounding stresses

Effects of various environmental stresses on expression of the *GmSAMDC1* gene were investigated by Northern-blot analysis. Upon salt treatment, a gradual increase of *GmSAMDC1* expression was observed (Fig. 6A). Drought stress also induced accumulation of the *GmSAMDC1* transcript, and its level reached a peak after 5 days (Fig. 6B). Re-watering of the drought-stressed plants

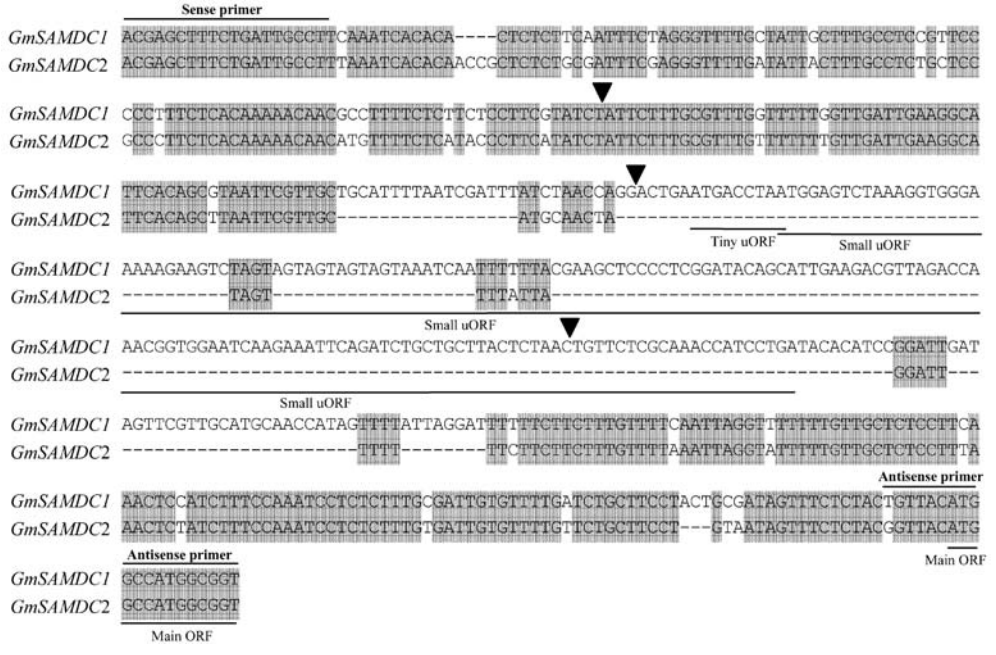

Fig. 5 Comparison of the 5′ end genomic sequences of the *GmSAMDC1* and *GmSAMDC2* genes from Kefeng 1. The tiny uORF, the small uORF and the part of the main ORF of the *GmSAMDC1* gene were *underlined*. The position of three introns in the sequence of the *GmSAMDC1* genomic sequence was marked by the *three triangles*, but the sequence of the three introns was not shown here in order to indicate the comparison more clearly. The primers used to amplify the genomic DNA were also indicated

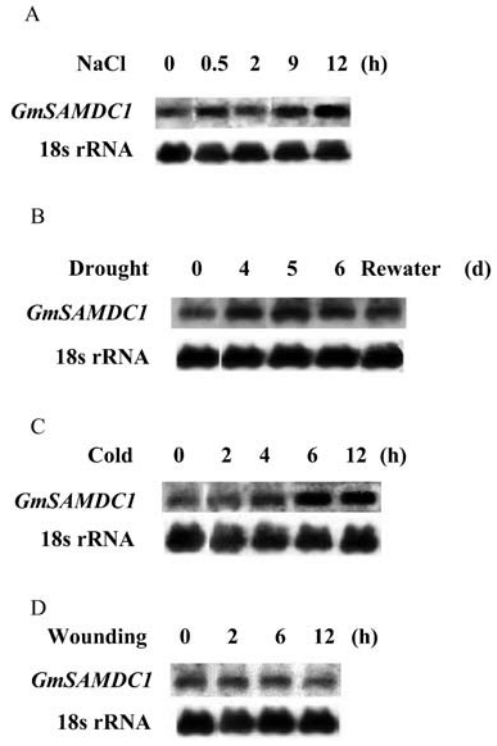

Fig. 6 Expression of the *GmSAMDC1* gene in response to NaCl, drought, cold and wounding. Total RNA was extracted from 2-week-old soybean seedlings treated with 0.8% NaCl (**A**), drought (**B**), cold (4°C) (**C**) and wounding (**D**) for different times. Hybridization was carried out using the radio-labeled full-length *GmSAMDC1* cDNA as the probe. The same blots were stripped of probes and re-hybrized with the 18S rRNA gene to verify the loading

reduced the mRNA level. Upon cold treatment, the expression of the *GmSAMDC1* was steadily increased and reached a significant level at 6 h after the initiation of the experiment (Fig. 6C). Wounding didn't cause a significant change of the *GmSAMDC1* gene expression (Fig. 6D).

Discussion

In the present study, a *GmSAMDC1* cDNA was isolated from the soybean (*G. max L.*) cultivar Kefeng 1 by EST analysis. The deduced amino acid sequence of GmSAMDC1 shared a moderate level of sequence similarity to the plant SAMDCs, and a low similarity to those from yeast and mammals. The GmSAMDC1 had several highly conserved regions, including a putative pro-enzyme cleavage site and a PEST sequence.

The *SAMDC* genes of human, rat, mouse and *Drosophila* were interrupted by introns throughout the main ORF, and there were no introns in the 5′ leader sequence (Maric et al. 1992; Pulkka et al. 1993; Larsson and Rasmuson-Lestander 1997; Nishimura et al. 1999). In contrast, the soybean *GmSAMDC1* gene was devoid of introns in the 3′-UTR and the main ORF, but has three introns in the 5′ leader sequence. This structural feature was similar to the *SAMDC* genes in *A. thaliana* (U63633) and *Ipomoea nil* (U64927), which also had three introns in the 5′ leader sequence. However, some *SAMDC* genes had less introns in the 5′ leader sequence, e.g. the second gene (AJ251915) from *A. thaliana* had two introns, one rice gene (Y07766) had two introns and one potato gene (S74514) had one intron. The intron in the potato *SAMDC* gene was located four nucleotides upstream of the tiny uORF (MadArif et al. 1994). A similar position (five nucleotides upstream of the tiny uORF) was found for intron 2 in the current *GmSAMDC*1 genomic sequence. Furthermore, the 5′ leader-sequence in the potato *SAMDC* gene has a putative TATA box (MadArif et al. 1994). Many putative protein binding sites were identified in the 5′ leader sequence of the *GmSAMDC1* gene, e.g. the Homeo domain factor Pbx-1 binding site (CAAT) and the GATA binding site (GATA) (Fig. 2B), which was predicted by the program of MatInspector (http://www.genomatix.de/software_servi-ces/software/MatInspector/matinspector.html) (Quandt et al. 1995). Therefore the transcriptional regulation may be present in the soybean *SAMDC* gene.

The *GmSAMDC1* gene had a long 5′ leader sequence, which had limited similarity to those from animals. The long transcript leaders of the *SAMDC* mRNAs were highly conserved among different species of plants and contained a small uORF, encoding 50 amino acid residues, and a tiny uORF, encoding 2 amino acid residues. In mammalian *SAMDC* mRNA, the leader sequence had a shorter uORF encoding a hexapeptide MAGDIS. It has been reported that translational regulation of mammalian *SAMDC* was mediated through the small uORF encoding the peptide MAGDIS (Hill and Morris 1992; Ruan et al. 1994). The 5′ end of the mRNA, including the MAGDIS uORF, was also responsible for the translational regulation of the *SAMDC* gene in response to polyamine levels (Suzuki et al. 1993; Shantz et al. 1994). Similar results have been reported for *Catharanthus roseus SAMDC* (Schröder and Schröder 1995). In the *GmSAMDC1* gene, the role of the tiny uORF and the small uORF in the translational regulation remained to be demonstrated.

Another genomic sequence was also identified from Kefeng 1 and corresponded to the *GmSAMDC2* gene. However, unlike the *GmSAMDC1*, there were no introns, and the uORF in the 5′ end of the *GmSAMDC2* gene. Considering the fact that only one expected fragment corresponding to the 5′ end of the *GmSAMDC1* mRNA was amplified from the cDNAs of Kefeng 1, and no expected fragment corresponding to the 5′ end of the *GmSAMDC2* was amplified from the same cDNAs; it was highly likely that the *GmSAMDC*2 gene was not functional and thus seemed to be a pseudogene. Two uORFs and/or introns may be necessary for the transcription of the *SAMDC* gene in plants. The loss of the two uORFs and/or introns during evolution may result in the formation of *GmSAMDC*2.

The organization of the *SAMDC* gene in the genome of different accessions from two subgenera of *Glycine* and *Soja* was revealed by Southern-blot and PCR analysis. We found that the hybridized pattern and the PCR-amplified pattern were consistent and identical in the subgenus *Soja*. In the subgenus *Glycine*, however, the patterns were different, indicating the divergence of the *SAMDC* gene. It is interesting to note that, in the Southern-blot analysis, the 838 bp fragment was not detected in the subgenus *Glycine*. This observation may indicate the presence of variation in the regulatory region of the *SAMDC* genes, since the 838 bp fragment was located at the 5′ end of the *SAMDC* gene and contained the two uORFs.

Previous studies suggested that SAMDC was probably the rate-limiting enzyme in the biosynthesis of polyamines, because the level of dcSAM in the living organisms is very low and the SAMDC protein has a relatively short half-life of about 1–2 h (Tabor and Tabor 1984). The accumulation of PAs in response to environmental stresses, such as acid stress (Yong and Galston 1983), salt stress (Basu and Ghosh 1991) and osmotic stress (Flores and Galston 1982), could be the consequences of the induction of the polyamine biosynthesis enzyme-encoding genes or an increase of these enzyme activities. Li and Chen (2000a) has found that the salt-tolerant rice responded more quickly in *SAMDC* expression than the salt-sensitive rice did. Results from the present experiments showed that the *GmSAMDC1* transcript was induced by salt, drought and cold, suggesting that the regulation at transcriptional level was very important in the activation of the *GmSAMDC1* gene under stress conditions.

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