Multiple untranslated exons and introns might be related to differences of expression in barley S-adenosylmethionine synthetase genes

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Absrtact

Barley S-adenosylmethionine synthetase (*Hv*SAMS) catalyses the biosynthesis of S-adenosylmethionine (SAM) from ATP and L-methionine. *HvSAMS 1*, *2*, *3*, and *4* genes and their 5' upstream regions were isolated and analyzed. The genomic DNA sequences of *HvSAMS 1*, *2*, *3*, and *4* genes did not contain intron. However, two introns and two exons were present on the 5' upstream regions of each *HvSAMS 1*, *2*, *3*, and *4*. They were present within 1787 bp (*HvSAMS 1*), 1698 bp (*HvSAMS 2*), 1916 bp (*HvSAMS 3*), *and* 1537 bp (*HvSAMS 4*) from the translated initiation site.

Although high sequence homology was found among the four *HvSAMS* genes and first exon on the 5' upstream region, numerous deletions and insertions were found in the second exon from the 5' upstream region and two introns. The second exon from the 5' upstream region among *HvSAMS 1*, *2*, *3*, and *4* showed high structural variation. In order to identify proposed alternative splicing occurred in 5' upstream region, RT-PCR with exons and introns of promoter specific primers were conducted using barley grains of different developmental stages. Presence of different transcript sequences derived from the 5' upstream region might indicate different upstream sequence use of *HvSAMS* genes.

METHODS

-Plant material : early maturity barley germplasm “GSHO 2504”
Arabidopsis thaliana (Col-0)
-Promoter isolation : Marathon cDNA amplification kit (Clontech)
Universal GenomeWalker (Clontech)
-Transient expression assay : deletion vector series (Fig. 3)
Agrobacterium tumefaciens (GV3101)
-Analysis : Blast alignment, PLACE web signal scan, and PlantCARE
RT-PCR
GUS activity assay
Histochemical staining

RESULTS

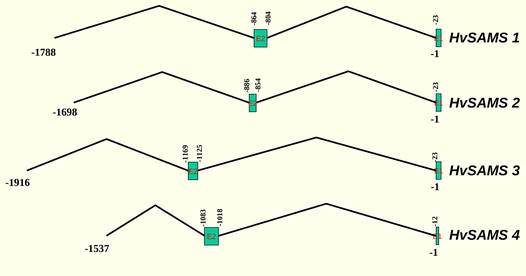

Fig. 1 Schematic representation of the 5´ UTRs of the *HvSAMS 1*, *2*, *3*, and *4*. Solid bars represented exons (E1 and E2), whereas introns were indicated by the straight/”Λ” lines. The nucleotide of the ATG translation initiation codon was assigned as position 1 in the nucleotide sequence, and the nucleotide positions upstream of position 1 were presented as negative numbers.

Table 1 Analysis of the *HvSAMS 1*, *2*, *3*, and *4* of 5’ untranslated regions. The PlantCare was used for each *cis*-elements.

Gene	cDNA[a]		genomic DNA[b]		Conserved sequence	Function
	cis-element	Location	cis-element	Location		
HvSAMS 1	AAGAA-motif	(+)78	AAGAA-motif	(-)601 (+)1825	GAAAGAA	-
	CAAT-box	(+)37	CAAT-box	(-)1553 (-)920 (-)1718 (+)1176 (-)1562	CAAT	common cis-acting element in promoter and enhancer regions
	EIRE	(+)5	EIRE	(+)959	TTCGACC	elicitor-responsive element
	LTR	(-)14	LTR	(-)968	CCGAAA	cis-acting element involved in low-temperature responsiveness
	MBS	(+)51	MBS	(-)480 (+)1005	CAACTG	MYB binding site involved in drought-inducibility
	SARE	(+)5	SARE	(+)959	TTCGACCTCCTT	cis-acting element involved in salicylic acid responsiveness
	circadian	(+)37			CAANNNNATC	cis-acting regulatory element involved in circadian control
	-		A-box	(-)515	CCGTCC	cis-acting regulatory element
	-		C-box	(+)1367	CTGACGTCAG	cis-acting regulatory element involved in light responsiveness
	-		G-box	(+)570 (+)891 (+)675 (+)1756	CACGTT	cis-acting regulatory element involved in light responsiveness
	-		TCA-element	(+)453	CCATCTTTTT	cis-acting element involved in salicylic acid responsiveness
	-		TGACG-motif	(+)898 (+)1519 (+)1368	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness
HvSAMS 2	CAAT-box	(+)9	CAAT-box	(+)831 (-)1585 (+)469 (+)1064 (-)589 (+)1494 (-)758 (-)289	CAAT	common cis-acting element in promoter and enhancer regions
	GAG-motif	(-)37			AGAGATG	light responsive element
	MBS	(+)23	MBS	(+)845	CAACTG	MYB binding site involved in drought-inducibility
	circadian	(+)9	circadian	(-)583 (+)831	CAANNNNATC	cis-acting regulatory element involved in circadian control
	-		A-box	(-)1311 (-)1626 (-)1562	CCGTCC	cis-acting regulatory element
	-		ABRE	(+)928	TACGTG	cis-acting element involved in the abscisic acid responsiveness
	-		CGTCA-motif	(+)1179 (+)1472	CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness
	-		G-Box	(-)928	CACGTA	cis-acting regulatory element involved in light responsiveness
	-		GCN4_motif	(-)628	TGAGTCA	cis-regulatory element involved in endosperm expression
	-		LTR	(-)808	CCGAAA	cis-acting element involved in low-temperature responsiveness
HvSAMS 3	-		Skn-1_motif	(+)448 (-)461	GTCAT	cis-acting regulatory element required for endosperm expression
	-		TGA-element	(-)1300	AACGAC	auxin-responsive element
	-		TGACG-motif	(-)1179 (-)1472	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness
	CAAT-box	(+)29	CAAT-box	(+)1594 (-)1894 (+)781 (+)1147 (+)453	CAAT	common cis-acting element in promoter and enhancer regions
	circadian	(+)43			CAANNNNATC	cis-acting regulatory element involved in circadian control
	-		ABRE	(+)876 (-)1139	TACGTG	cis-acting element involved in the abscisic acid responsiveness
	-		AuxRR-core	(+)1802	GGTCCAT	cis-acting regulatory element involved in auxin responsiveness
	-		CGTCA-motif	(+)854	CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness
	-		G-box	(+)678 (-)1430 (+)1141 (+)1868 (+)876 (-)1491	CACGTA	cis-acting regulatory element involved in light responsiveness
	-		MBS	(-)982	CGGTCA	MYB Binding Site
	-		Skn-1_motif	(+)855 (+)1857	GTCAT	cis-acting regulatory element required for endosperm expression
	-		TCA-element	(+)549	GAGAAGAATA	cis-acting element involved in salicylic acid responsiveness
	-		TGACG-motif	(-)854	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness
HvSAMS 4	CGTCA-motif	(+)23	CGTCA-motif	(-)316	CGTCA	cis-acting regulatory element involved in the MeJA-responsiveness
	Skn-1-motif	(+)24	Skn-1-motif	(-)1364	GTCAT	cis-acting regulatory element required for endosperm expression
	circadian	(+)20	circadian	(-)1054	CAANNNNATC	cis-acting regulatory element involved in circadian control
	-		ABRE	(-)220 (-)305	TACGTG	cis-acting element involved in the abscisic acid responsiveness
	-		AuxRR-core	(+)951	GGTCCAT	cis-acting regulatory element involved in auxin responsiveness
	-		CAAT-box	(-)216 (+)981 (-)987 (-)1288 (+)1350	CAAT	common cis-acting element in promoter and enhancer regions
	-		ERE	(+)1465	ATTTCAAA	ethylene-responsive element
	-		G-Box	(+)220 (+)1538 (-)270	CACGTA	cis-acting regulatory element involved in light responsiveness
	-		GCN4_motif	(+)96	TGAGTCA	cis-regulatory element involved in endosperm expression
	-		LTR	(+)157	CCGAAA	cis-acting element involved in low-temperature responsiveness
	-		MBS	(+)1384	CAACTG	MYB binding site involved in drought-inducibility
	-		TCA-element	(+)1162 (-)1500	GAGAAGAATA	cis-acting element involved in salicylic acid responsiveness
	-		TGA-element	(+)297	AACGAC	auxin-responsive element
	-		TGACG-motif	(+)316	TGACG	cis-acting regulatory element involved in the MeJA-responsiveness

[a] **The column was isolated from 5’ RACE clones.**
[b] **The column was isolated from genome walker.**

Table 2 List of the forward primers used for the RT PCR analysis. The used reverse primers were *HvSAMS 1*, *2*, *3*, and *4* specific primers. Intron 1s were indicated intron regions of the 5´ UTR between E1 and E2.

Genes	PCR target	Primer sequence (5' → 3')
HvSAMS 1	Exon1	AGAGCATCACTACCACGAAAG
	Intron1	CTGCTGTATGGCCGGGTAGA
	Exon2	TACATTCGACCTCTTTCGGTTC
HvSAMS 2	Exon1	AGAGCATCTCTACCACCAAAG
	Intron1	CGTCCTGATCTCATGTTTCGA
	Exon2	CTCCTGAACAATAGCATCAGC
HvSAMS 3	Exon1	AGAGCATCACTAGCACGAAAG
	Intron1	GGGTGACCGCGTCTTCTCGTTC
	Exon2	CTTCCCTTCGGTTCCTTGGC
HvSAMS 4	Exon1	AGGCCAAAGAAGATGGCTGAAG
	Intron1	CCTTTCGAGATTGGGTGTACTG
	Exon2	CCGTGCTGTGCTCGAGTGTC

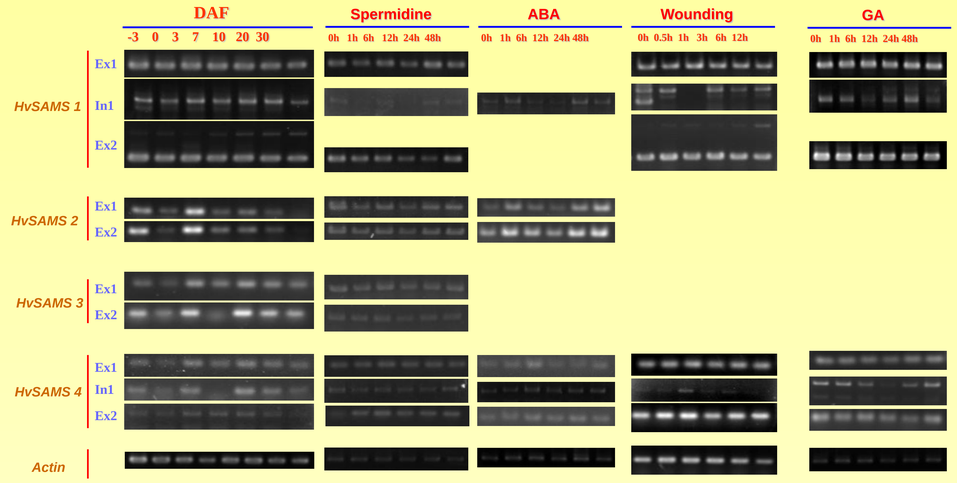

Fig. 2 Transcript accumulation profiles of *HvSAMS 1*, *2*, *3*, AND *4* expressed in plant growth hormones and abiotic stress treatment using RT-PCR analysis. Plant material was 4 week-old leaves of barley treated with a solution of 100 μM each of ABA, GA3, spermidin.

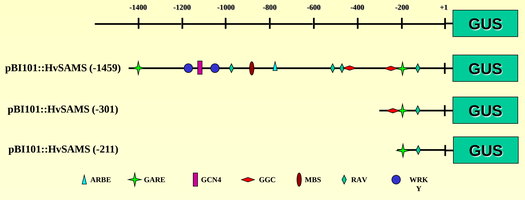

Fig. 3 Schematic diagram of different *HvSAMS 2* promoter::GUS fusions. The different length promoter fragments were inserted into pBI 121, which was removed CaMV 35S. The putative *cis*-acting elements were represented by symbols.

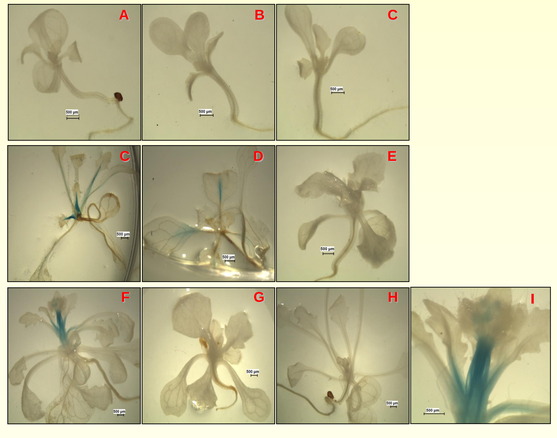

Fig. 4 Histochemical localization of GUS activity in transgenic *Arabidopsis* seedlings. *Arabidopsis* Columbia was transformed with transformation vectors. A, C, F, and I, pBI101::HvSAMS (-1459)::GUS fusion vector; B, D, and G, pBI101::HvSAMS (-301)::GUS fusion vector; C, E, and H, pBI101::HvSAMS (-211)::GUS fusion vector. A, B, and C, 1 week-old transgenic plants (T_2); D, E, and F, 2 week-old transgenic plants (T_2); F, G, and H, 3 week-old transgenic plants (T_2); I, 3 times magnified image for F

Acknowledgement

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