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Isolation and Characterization of *HvSAMS* Gene in Early Maturity Barley

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극조숙 보리 종실에서 *HvSAMS* 유전자의 분리 및 검정

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Objective

The purpose of this study was to isolate and characterize a noble gene, which was differentially expressed gene in seed development of early maturity barley.

Materials and methods

Plant material : The grain of early maturity barley, K800, (GSHO 1732 GS96, *eam10*)

Different Display : DH (Differential Hybridization)

Southern Blot : Dig-labeled detection

Northern Blot : Southern Star™ detection kit

Results and Discussion

To study seed development in early maturity barley (possessing *eam10*), we constructed a cDNA library (Final titer : 6.94×10^9 pfu/ml). Differential Hybridization (DH) method was performed to isolate genes differentially expressed. Sixty six clones that were related to grain maturity and seed development were selected (Table 1). As screening cDNA library, we identified a noble gene, *HvSAMS* (*Hordeum vulgare* S-AdenosylMethionine Syntase). Sequence analysis revealed that *HvSAMS* was 1182 bp in length encoding a single polypeptide of 394 amino acid residues. Southern blotting analysis of barley DNA performed with a DIG labeled full-length cDNA probe of *HvSAMS* revealed two bands if restricted with *EcoRI*, *XbaI*, *XhoI*. Three bands could be detected if the DNA was restricted with *HindIII*(Fig. 1). *HvSAMS* transcripts were most abundant in stem in northern analysis (Fig. 2). Overexpression in the Arabidopsis using a pCAMBIA 1301 is underway.

Acknowledgement : This work was financially grant from BG21, RDA, Rep of Korea

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Table 1 Differentially expressed clones in grain of K800 (GSHO 1732 GS96, *eam 10*) at DAF 7.

Clone No.	Putative Identification	Clone No.	Putative Identification
EA3	methionine synthase protein	EB92	calnexin - maize
EA4	putative transketolase	EB100	copper chaperone (CCH) -related [Arabidopsis thaliana]
EA5	AT-hook protein 1	EC13	OJ1081_B12.5
EA6	UVB-resistance protein-like	ED4	RUBISCO SUBUNIT BINDING -PROTEIN BETA SUBUNIT
EA13	S-adenosylmethionine synthetase 1	ED14	asparaginyl endopeptidase REP-2
EA16	T-complex protein 1	EB65	expressed protein
EA18	Histone H2A.2.1	EB99	lipoxygenase (EC 1.13.11.12) 2
EA19	vacuolar proton-inorganic pyrophosphatase	EE100	putative 60S ribosomal protein
EA20	major facilitator superfamily antiporter	EF2	All non-redundant GenBank CDS
EA24	Putative RING-H2 finger protein RHB1a	EF3	glycosyltransferase family 8
EA28	putative PKC ζ -interacting protein	EF20	glutamate permease
EA30	contains EST C93475(C51754) ~syntaxin-like protein	EF29	putative XS domain containing protein
EA31	succinyl-CoA ligase beta subunit	EF34	putative thiolase
EA32	hypothetical protein [Oryza sativa]	EF33	Cysteine proteinase 1 precursor
EA33	Superoxide dismutase	EF43	pyruvate dehydrogenase E1 beta subunit isoform 2
EA35	putative cinnamoyl-CoA reductase	EF44	TRIOSEPHOSPHATE ISOMERASE, CYTOSOLIC
EA36	unknown protein	EF45	probable 26S proteasome non-ATPase chain S5a [imported]
EA41	CITRATE SYNTHASE, GLYOXYSOMAL PRECURSOR	EF49	LeOPT1
EA42	putative peptide deformylase	EF51	senescence-associated protein-like protein
EA43	cytoplasmic malate dehydrogenase	EF52	At2g16920~unknown protein [Oryza sativa]
EA44	S-adenosylmethionine decarboxylase proenzyme	EF54	putative protein kinase Xa21, receptor type precursor
EA45	cell-autonomous heat shock cognate protein 70	EF66	chlorophyll a/b-binding apoprotein CP24 precursor
EA46	putative cystathionine gamma synthase	EF67	putative mannose-6-phosphate isomerase
EA47	T10024.24	EF68	alpha-L-arabinofuranosidase/ beta-D-xylosidase isoenzyme ARA-I
EB5	OJ000223_09.1	EF69	expressed protein
EB25	putative zinc protease	EF72	expressed protein
EB30	histone H2B153	EF82	Fructose-bisphosphate aldolase
EB43	lipid transfer protein	EF84	Carbonic anhydrase
EB58	elongation factor 1 alpha	EF88	60S ribosomal protein L6 (RPL6C)
EB61	translation elongation factor eEF-1 alpha chain	EF89	Putative AP2 domain containing protein
EB62	P0698A10.17	EF98	putative glycine-rich protein
EB76	putative polypyrimidine tract-binding protein	EF100	putative ADP-ribosylation factor
EB77	60S ribosomal protein L10		
EB82	allene oxide synthase		

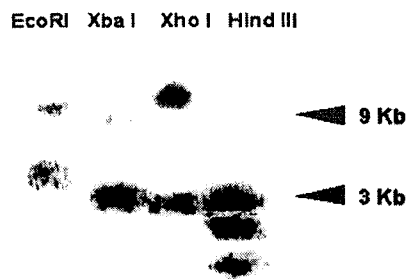


Fig. 1. Southern blot analysis of the genomic DNA of K800. The DNA was digested with *EcoRI*, *XbaI*, *XhoI*, and *HindIII*.

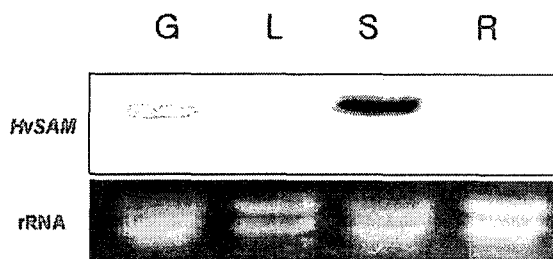


Fig. 2. Northern blot hybridization of the *HvSAMS* gene in different plant tissues. G: grain, L: leaf, S: stem, and R: Root.