Molecular Characterization of a cDNA encoding putative integral membrane protein, *HvSec61*, expressed during early kernel development in barely

Jang CS, Lee TG, and Seo YW
Division of Biotechnology and Genetic Engineering, Korea University

Purpose

In order to study molecular events that occur during kernel development, SSH method was performed using grains from developmental stages (14 DAF and 5 DAF) in barely. Differentially expressed one clone showed high similarity cDNAs encoding Sec61α in the wheat, however, didn't have similarity with any gene in the barley. This gene was designated to *HvSec61*α (*Hordeum vulgare* Sec61α subunit). The purpose of this study is to characterize expression and putative function of the *HvSec61*α gene.

Materials & methods

Plant materials : cv. Karl (*Hordeum vulgare* L.)
Methods :
- Expression assay: SSH method, Northern blot, *in situ* hybridization
- Functional assay: Overexpression in Arabiodopsis, abiotic stresses, Tris-Tricine gel

Results and discussion

The cDNA encoding *HvSec61*α (accession no. AAK94784) contained 1,425-bp open reading frame (ORF) that encoded a putative Sec61α subunit precursor of 475 amino acids. The transcript levels of *HvSec61*α were low in grains at 5 DAF and increased to 8 until 11 DAF. However, expression of the *HvSec61*α gene dramatically decreased from 14 to 20 DAF. The transcripts of *HvSec61*α were highest expressed in grains but scarcely expressed in pericarps, stems, and leaves. Transcript levels of the *HvSec61*α gene did not show in the grains at 5 DAF, however, expressed in vascular bundles of the lodicle. At 8 DAF kernels, the *HvSec61*α gene was expressed in the scutellum near embryo and in vascular tissues of the glume. In the 14 DAF kernels, transcripts of the *HvSec61*α gene were slightly expressed in the scutellum and in vascular tissues. The *HvSec61*α gene was induced to exogenous plant growth hormones such as ABA, 6BAP, and MeJA, but not GA. Expression of the *HvSec61*α gene was not dramatically changed by drought treatment, however, was increased by NaCl or cold treatment. The two lines of transgenic plants showed vigorous growth than control plants in drought treatment. The accumulation of storage protein in the transgenic plants was similar with that of control plants.

Corresponding author : Seo, Yong Weon E-mail: seoag@korea.ac.kr Tel: 02-3290-3005

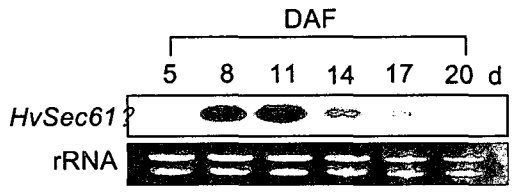

Fig. 1. Northern blot hybridization of *HvSec61a* gene in grain of the barley during development. The grain materials were harvested from the kernels of 5, 8, 11, 14, 17, and 20 day after fertilization (DAF). d: days.

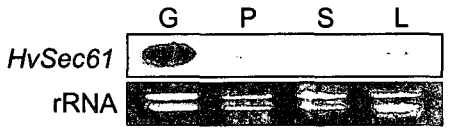

Fig. 2. Northern blot hybridization of *HvSec61a* gene in different tissues. Total RNA of four tissues from the barley in DAF 14 day was fractionated on a 1% denaturing agarose gel. G: grain, P: Pericarp, S: stem, and L: Leaf.

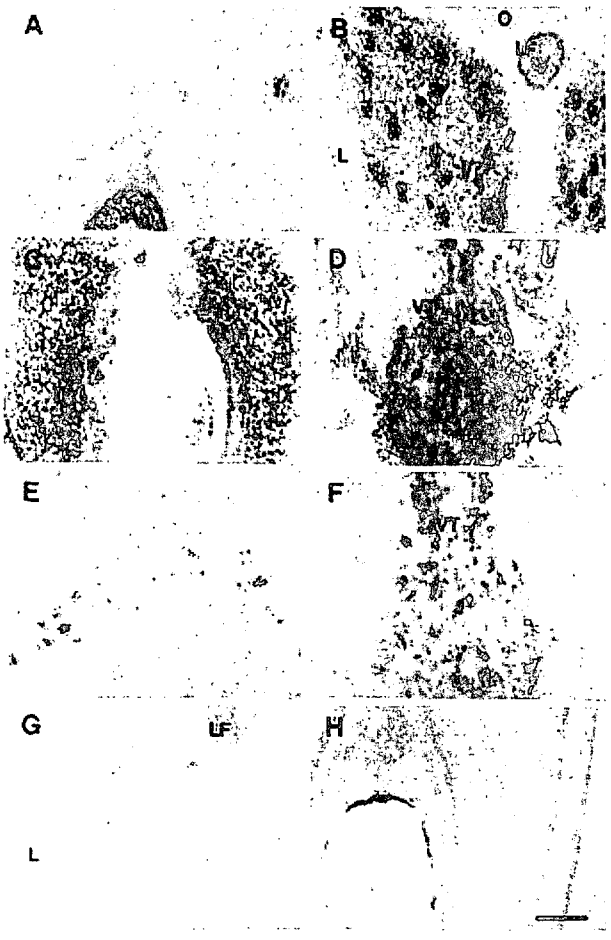

Fig. 3. In situ hybridization of *HvSec61a* mRNA in various barley grain during development. A bule-purple color indicates a positive signal. A and B: Transverse section of the grain of DAF 5. C and D: Transverse section of the grain of DAF 8. E and F: Transverse section of the grain of DAF 14. G: Transverse section of DAF 5. H: Transverse section of DAF 8. G and H: Negative signal (hybridized without *HvSec61a*) L: lodicle, LF: lodicle filament, VT: vascular tissue. Scale bar = 500 um.