P055

Isolation and Molecular characterization of genes expressed during early stage of kernel development in Barley (*Hordeum vulgare* L.)

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Objective

The objective of this study was to characterize hordoindoline a and b genes that were expressed during early stage of seed development in barley cv. 'Karl'.

Material & Methods

Plant materials: barley cv. 'Karl'

Methods: SSH (Suppression Subtractive Hybridization) and DH (Differential Hybridization) methods for isolation of novel genes induced during seed development.
Northern blotting and in situ hybridization for molecular characterization of *hordoindoline a, b*

Results

Two cDNAs, Hordeum vulgare Indoline a and b (HvID-a and HvID-b), encoding hordoindolines known to be related to grain hardness were isolated using two differentialscreening methods- suppression subtractive hybridization (SSH) and differential hybridization (DH). The HvID-a and HvID-b contained a 450 bp and a 444 bp open reading frame (ORF) that encoded the putative hordoindoline-a and -b precursors consisting of 150 and 148 amino acids, respectively. The HvID-a gene was highly expressed at 5 DAF, reached a peak at 8 DAF and declined slightly to 20 DAF (Fig. 1). While the expression of HvID-b began to be detectable at 8 DAF when it's expression was higher than other developmental stages and declined slightly to 20 DAF (Fig. 1). The HvID-a and HvID-b genes were predominantly detected in the aleurone cell layers in late kernel development, e.g. at 20 DAF (Fig. 2). The HvID-a and HvID-b transcripts were predominantly detective in lodicule and aleurone layer of developing seed, but not in embryo (Fig. 2).

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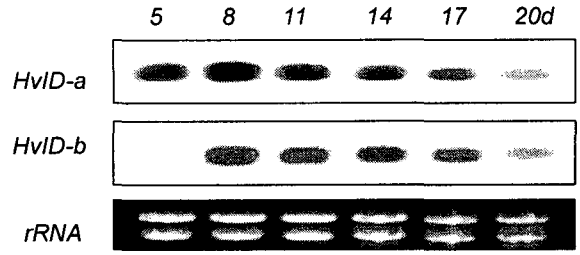

Fig 1. Northern blot hybridization of HvDI -a and HvID-b genes in grain of the barley during development. Total RNA (20 ug per sample) of grain was fractionated on a 1% denaturing agarose gel. The grain materials were harvested during development of DAF 5, 8, 11, 14, 17, and 20. d : days after fertilization. cv. Karl

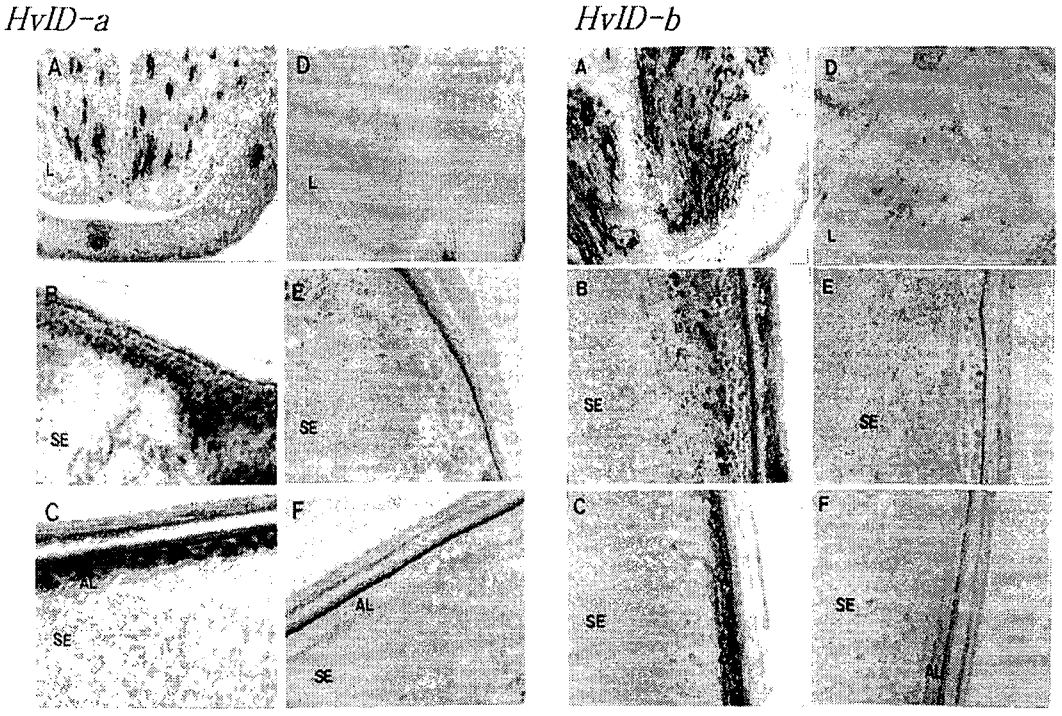

Fig. 2. Localization of HvID-a, HvID-b, genes expression in development grain. A and D were grain of DAF 5. B and E were grain of DAF 17. C and F were grain of DAF 20. A, B, and C were antisense RNA probe. D, E, and F were sense RNA probe. L: Lodicule, SE: Starch endosperm, AL: Aleurone layer.