P85. Hordein Subunit Variation during Endosperm Protein Accumulation Detected by Immunological Methods

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Objectives

This paper describes a quantitative examination of the accumulation of hordein proteins and the presence or absence of changes in the protein polypeptides pattern during grain development.

Materials and Methods

Plant Materials: "Olbori" and "Seodunchalbori" were field-grown at Deokso during 1999~2000. Spikes were harvested 8, 12, 18, 24, 30, 36, 42, and 47 days after fertilization (DAF).

Hordein Extraction: The extraction was done on endosperm isolated as follows; lemma, glume, palea, and embryo were removed with tweezers. Hordeins extracted from 40 mg flour with 1 ml 55% (v/v) aqueous isopropanol at 60℃ for 30 min in a sonication bath.

Antibody Production: New Zealand white rabbits were injected with hordein emulsion for producing anti-hordein polyclonal antibody (AHPab). Rabbits were bled out when optimum specificity was found.

ELISA: The hordein concentrations of each stage were determined as 0.9 $\mu g/\mu \ell$ using bradford assay. Samples (0.9 $\mu g/\mu \ell$) in triplicate were applied to each well in microtitrate plate. A wavelength of 405 nm was used for reading protein concentration.

Electrophoresis and Immunoblotting assay: Hordeins of the same concentration (0.9 $\mu g/\mu \ell$) were separated by 1D SDS-PAGE in two gel formats. One gel was silver-stained and the other gel was used for immunoblotting assay.

Results and Discussion

◈ As both "Olbori" and "Seodunchalbori" hordeins were increased DAF, optical density (O·D) of ELISA also was increased (Fig. 1, Table 1).

◈ 1D SDS-PAGE and immunoblotting assay showed that there were not hordein subunit fractions both 8 and 12 DAF but hordein subunit density gradually increased from 18 to 47 DAF (Fig. 2. A and B).

◈ Especially, the results of immunoblotting assay showed that C hordein was apparently separated 3 bands with molecular weight of 68.7 kDa and 2 bands with 55.7 kDa (Fig. 2. B).

◈ ELISA using AHPab would provide early generation screening for high or low endosperm protein because this system required only few nanograms of hordeins.

◈ Produced AHPab will provide additional information which was ambiguous when using 1D SDS-PAGE solely.

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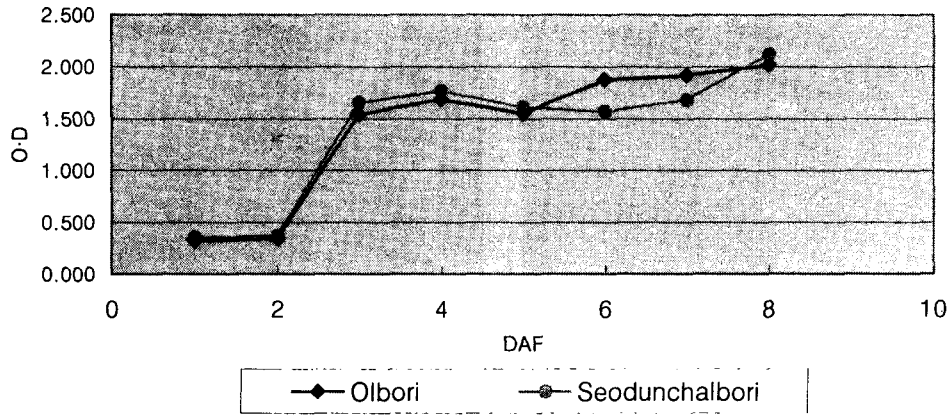

Fig 1. Changes of hordein during endosperm protein accumulation in Olbori and Seodunchalbori using ELISA (1~8: 8~47 DAF, respectively).

Table 1. Optical densities of Olbori and Seodunchalbori hordein.

	Days After Fertilization (DAF)							
O·D	8	12	18	24	30	36	42	47
Olbori	0.324	0.340	1.544	1.686	1.555	1.878	1.919	2.015
Seodunchalbori	0.352	0.370	1.654	1.768	1.610	1.568	1.683	2.125

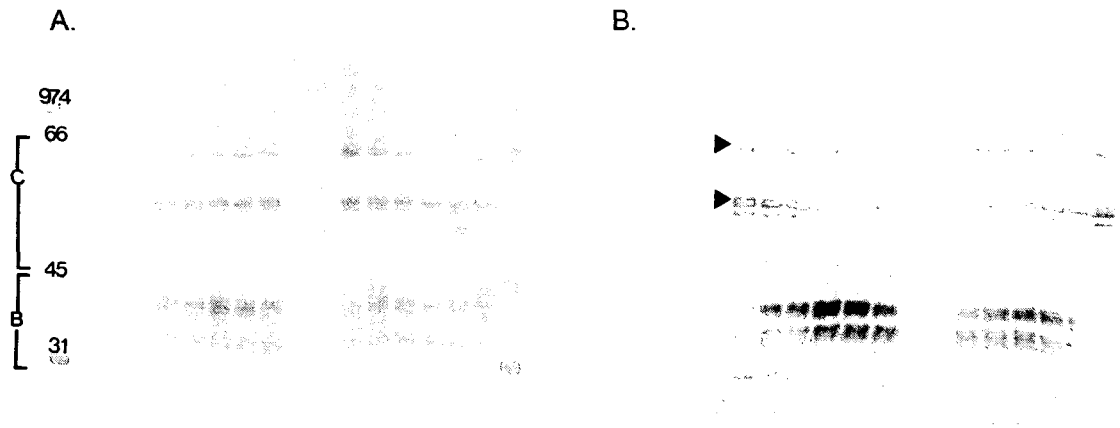

Fig 2. A. 1D SDS-PAGE of unreduced hordein fractions. Lane1~8: Olbori hordeins, lane 9~16: Seodunchalbori hordeins from material 8, 12, 18, 24, 30, 36, 42, and 47 DAF, respectively (M: molecular size marker). B. Immunoblotting of unreduced hordein. Lane unmbers and sources are same as Fig 2. A. The D, C, and B designate the range of each hordein. The arrows indicate separat bands in the C hordein.