OB7. Identification of wheat lemma proteins separated by two-dimensional gel electrophoresis: towards Fusarium Head Blight (FHB) proteome analysis

Woo Sun Hee*, Kimura Makoto, Higa Arisa, Kim Hong Sig [1], Park Sang Il[1], Son Seok Yong[1], Yamaguchi Isamu and Jong Seung Keun[1]

Lab. of Remediation Research, PSC. RIKEN, 2-1 Hirosawa, Wako, 351-0198 Japan

[1] Dept. of Agronomy, Chungbuk National University, Cheongju, 360-763 Korea

Objectives

This study builds on a preliminary report on how knowledge of the proteome can complement studies at the gene level.

Materials and Methods

1 DAP lamma sample, two-dimensional electrophoresis, *N*-terminal amino acid sequence analysis, internal amino acid analysis, Reversed-phase (RP)-high-pressue liquid chromatography (HPLC), homology search of amino acid sequence and identification of cDNA encording the sequenced protein were used for this analysis.

Results and Discussion

Two-dimensional polyacrylamide gel electrophoresis protein mapping analysis, the pattern of gene expression in specific tissues at a specific stage can be displayed and characterized. A total of 500 wheat spike (lemma) proteins were detected by 2-DE. Over 500 lemma proteins in the p*I* range of 3.5-10 and molecular mass range of 14.4-97 kDa were reproducibly resolved after Coomassie blue (CBB) staining, representing about 50% of the estimated total genomic output of wheat. Most lemma proteins had an isoelectric point between 5.0 and 7.0. When we used silver staining for protein detection, more than 700 spots could be visualized on a single gel. These proteins were electroblotted from the 2-DE gels onto polyninylidene diffluoride (PVDF) membrane blots. Among them, 70 proteins were subjected to the *N*-terminal sequencing, and 47 proteins could be determined the *N*-terminally blocked proteins by the improved Reversed-phase (RP)-high-pressue liquid chromatography (HPLC) and Cleveland peptide mapping method. The amino acid sequences of these proteins were compared with those of the SWISS-PROT and NCBI databases and the sequences of 6 proteins were found identical of those of proteins already reported in wheat. Among 70 proteins sequenced, 33 showed sequence homology with proteins from the other plants such as barley, maize, rice, *Arabidopsis* and pea. There are a lot of proteins without known functions. These proteins are important as target for functional analysis in the wheat proteome analysis. Therefore, we needed the sequence information for precise identification of the proteins. At present, the improved peptide mapping is considered to be useful in the wheat and other plants proteome analysis. Using the publicly available wheat expressed sequence tag (EST) database at the National Centre for Biotechnology Information (NCBI), a further 44 protein spots were mached to ESTs.

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*Corresponding author : Tel 043-261-2510 E-mail woosh322@hotmail.com