Functional proteome analysis of wheat: systematic classification of abiotic stress-responsive proteins

Abu Hena Mostafa Kamal\textsuperscript{A}, Ki-Hyun Kim\textsuperscript{A}, Kwang-Hyun Shin\textsuperscript{A}, Dong-Hoon Shin\textsuperscript{A}, Hyung-Seok Seo\textsuperscript{A}, Dae-Seong Park\textsuperscript{A}, Sun-Hee Woo\textsuperscript{B} Moon-Soon Lee\textsuperscript{B} and Keun-Yook Chung\textsuperscript{C,D}

\textsuperscript{A}Dept. of Crop Science, Chungbuk National University, Cheongju 361-763, Korea.
\textsuperscript{B}Department of Industrial Plant, Chungbuk National University, Cheongju 361-763, Korea.
\textsuperscript{C}Dept. of Agricultural Chemistry, Chungbuk National University, Cheongju 361-763, Korea.
\textsuperscript{D}Corresponding author. Email kychung@cbnu.ac.kr

Abstract
Crucial functions of the plant cell are to take action against environmental stresses for self-defense.
Evaluation of two-dimensional electrophoresis gels revealed several proteins to be differentially expressed as a result of abiotic stress among cultivars. 217 protein spots of interest were, after an in-gel tryptic digestion, identified using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry.
Ten percent of abiotic stress responsive proteins were identified in cv. Keumgang followed by 7% in cv. Jinpum and cv. China-108, 12% in cv. Yeennon-78, 31% in cv. Norin-61 and 33% in cv. Kantou-107 in our experiment. Of the total number of 575 identified proteins, 345 proteins were recognized as abiotic stress responses unique proteins with isoforms, of which 34% are induced by heat, 27% by drought, 15% by salt, 13% by cold and 11% by other environmental stress. Furthermore, elucidating the function of proteins expressed by genes in stress tolerant and susceptible plants will not only advance our understanding of plant adaptation and tolerance to environmental stresses, but also may provide important information for designing new strategies for crop improvement.

Key Words
Abiotic stress, matrix-assisted laser desorption/ionization-time of flight, proteomics, wheat.

Introduction
Any living organism has to survive with conditions of stress. Specifically for plants, the possibilities to escape from stress are limited because plants are motionless (Kuiper 1998). As a general rule, emblematic response to environmental stress conditions is established by the induction of a set of stress proteins that protects the organism from cellular damage. Abiotic stresses such as heat, cold, drought, salinity, nutrient, ozone, heavy metals, ultra-violet light, visible light, chemical toxicity and oxidative stress are serious intimidation to agriculture. In this study, we determined specific proteins induced by each abiotic stress, particular emphasis will be placed on the heat shock, drought, cold, salt and others environmental stress by using proteomic approaches, which is fruitful information for improving stress tolerance cultivars.

Materials and methods
Plant materials
Six genotype of wheat seeds (two Korean: Keumgang, Jinpum; two Chinese: China-108, Yennon-78 and two Japanese: Norin-61, Kantou-107) were used in this study for identification of biotic stress responses proteins by proteomics analysis.

Sample preparation by KCl solubility method
Osborne's (1924) solubility method that we routinely use to fractionate wheat endosperm proteins takes advantage of the solubility properties of wheat endosperm proteins in KCl, SDS, and acetone with some modifications (Hurkman and Tanaka 2007).

Two-dimensional gel electrophoresis (2-DE)
Soluble proteins of whole seed storage were examined by two-dimensional gel electrophoresis (Kamal et al 2009a,b). Protein spots in 2-DE gels were visualized by Coomassie Brilliant Blue (CBB) R-250 staining (Woo et al. 2002). Each sample was run three times and the best visualized gels were selected.

In-gel digestion and mass spectrometry analysis
Selected protein spots were excised from preparative loaded gels, stained with Coomassie brilliant blue (R-
Bioinformatics analysis
The proteins were identified by searching NCBI nr, SWISSPORT, MASCOT database using the MASCOT program (http://www.matrixscience.com, Matrixscience, UK). When more than one peptide sequence was assigned to a spectrum with a significant score, the spectra were manually examined. Sequence length, gene name and also protein functions were identified by searching Swiss Prot / TrEMBL database using UniProtKB (http://www.uniprot.org).

Results and discussion
We observed heat increases or decreases in proteins by heat stress using proteomic technique in wheat grain. These proteins include heat shock proteins (1,000-91,000), heat stress transcription factor (27,000-54,000), granule bound starch synthesis (58,000-60,000), GTP binding proteins (22,000-24,000), beta-amylase (9,000-24,000), eucaryotic initiation factor (12,000-46,000), elongation factor (24,000-50,000), ribulose biphosphate (13,000-52,000) related proteins and so on, which are found in mature seeds. These results confirmed the results previously by Majoul et al. 2004, demonstrating that the synthesis of HSPs occurs in the full range of wheat tissues including developing grains (Giornini and Galili 1991). These drought adaptive changes rely largely on alterations in gene expression. We identified different abscisic acid responsive proteins (10,000-41,000), LEA protein (12,000) such as dehydrins (16,000-30,000), chaperonin (10,000), cyste peroxideroxidase (23,000-24,000), ethylene response (19,000-28,000), and elongation factor TU (50,000) in six wheat cultivars, which is responsible for drought stress.

The plants show sorts of adaptation in any specific temperature. Plants face high and low-temperature stress. In our experiment, we revealed some cold stress related proteins such as cold acclimation proteins (9,000-22,000), cold shock proteins (16,000-38,000), ABA inducible proteins (10,000-41,000), cyclophilin (13,000-18,000), low temperature regulated proteins (7,000-14,000), kinase like protein (6,000-74,000), mitogen activated (40,000-80,000), transcriptional adaptor (7,000-29,000), and translation initiation (12,000-17,000) in six wheat cultivars. These results would confirm previous work describing to determine the genetic nature of these mechanisms. Several cold-responsive genes of unknown function were identified from cold-acclimated wheat (Breton et al. 2003). The most common plant stress in soils is salinity. Some aspects of salt stress responses are intimately related to drought and cold stress responses (Zhu 2001). In our experiment, we identified some salt stress responsive proteins such as salt stress protein (8,000-26,000), ABA inducible (10,000-41,000), aquaporin (21,000-31,000), peroxiredoxin (23,000-24,000), bowman-brick type proteinase inhibitor (5,000), calcineurin B like protein (25,000-29,000), cyclophilin (1,000-22,000), zinc finer protein (3,000-43,000), potassium channel (81,000-93,000), calcium channel (8,000), and RNA binding proteins (16,000-20,000) in six wheat. Two-dimensional electrophoresis was used to reveal changes in protein expression of rice; they identified several salt responsive proteins including salt stress protein and ABA (Dooki et al. 2006). We identified heavy metals such as cadmium (18,000), copper (14,000-19,000), aluminium (49,000), manganese (19,000), metallothion like (4,000-42,000), molybdenum (20,000), Rab GTP (guanine nucleotide-binding proteins) binding (2,000), Rac/Ras like GTP binding (23,000-24,000),
germin like (23,000-24,000) wall associated kinase (57,000-88,000), and some unclear abiotic stress responsive proteins in our experiments.

**Conclusion**
Using two-dimensional electrophoresis, this study identified proteins involved in heat, drought, cold, salt and some others abiotic stress responses in wheat. Our findings reveal a proteomic profile of abiotic stress in wheat, which may provide benefits in two major areas, in the better understanding of abiotic stress proteins including their functions, and the understanding of stress related physiology in wheat grain.

**Acknowledgements**
BioGreen 21 Program (20070301034043), RDA, Korea.

**References**