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

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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. Yeonnon-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-

250), then washed with 100 µl distilled water couple to tryptic digestion (Kamal *et al.* 2009a,b). In MALDI-TOF/MS (AXIMA CFR$^+$ Plus, Shimadzu, Japan) analysis, proteins separated by 2-DE were digested in gels according to the method described by Fukuda *et al.* (2003).

Bioinformatics analysis

The proteins were identified by searching NCBInr, SWISSPORT, MASCOT database using the MASCOT program (http://www.matrixscience.com,Matrixscienc,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 initaition 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), cys peroxiredoxin (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.

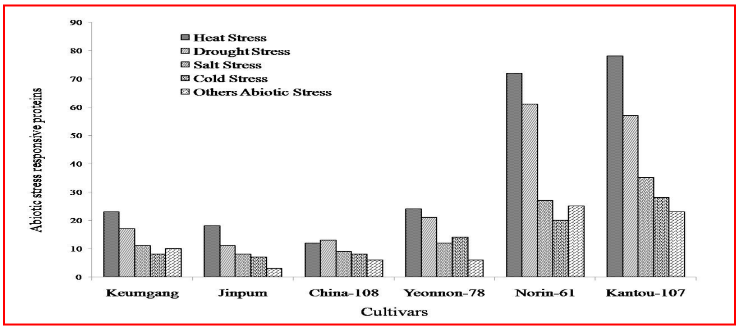

Figure 1. Functional distribution of the total identified abiotic stress responsive proteins among wheat cultivars.

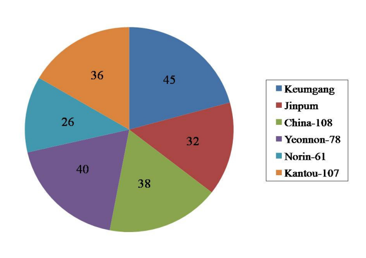

Figure 2. Distribution of total detected protein spots by two-dimensional electrophoresis.

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-brik 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.

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