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Abiotic stress responsive proteins of wheat grain determined using proteomics technique

Abu Hena Mostafa Kamal[1], Ki-Hyun Kim[1], Kwang-Hyun Shin[1], Jong-Soon Choi[2], Byung-Kee Baik[3], Hisashi Tsujimoto[4], Hwa Young Heo[5], Chul–Soo Park[6], Sun–Hee Woo[1*]

[1]Dept. of Crop Science, Chungbuk National University, Cheong-ju, 361-763, Korea
[2]Proteome Research Team, Korea Basic Science Institute, Daejeon 305-333, Korea
[3]Dept. of Crop Soil and Sciences, Washington State University, Pullman, WA 99164-6420, USA
[4]Laboratory of Plant Genetics and Breeding Science, Tottori University, Tottori 680-8553, Japan
[5] Breeding Resource Developments, National Institute of Crop Science, Suwon, 441-857, Korea
[6] Winter Crop & Upland Crop, National Institute of Crop Science, Iksan 570-080, Korea

***Corresponding author: shwoo@chungbuk.ac.kr**

Abstract

The analysis of stress-responsiveness in plants is an important route to the discovery of genes conferring stress tolerance. Evaluation of two-dimensional electrophoresis gels revealed many proteins to be differentially expressed as a result of abiotic stress among cultivars. More than 140 protein spots were detected by 2-DE, and some selected protein spots analyzed by MALDI-TOF mass spectrometry. 22.58 % of abiotic stress responsive proteins were identified in cv. China-108, 32.25 % in cv. Yeonnon-78, 70.96 % in cv. Norin-61 and 69.35 % in cv. Kantou-107. Of the total identified proteins, 124 proteins were recognized as abiotic stress responsive unique proteins, of which 31.56 % are induced by heat, 26.61 % by drought, 23.38 % by salt, 21.77 % by cold and 22.58 % 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 but also tolerance to environmental stresses. Genes that have been identified by proteomics can be used for marker-assisted breeding or gene transformation programs to improve the architecture of crop plants and resistance or tolerance to abiotic stresses.

Key words: Abiotic stress; Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF); Proteomics; Two-dimensional electrophoresis (2-DE); Wheat grain

Introduction

Any living organism has to survive conditions. 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 deficiency, ozone, heavy metals, UV-B radiation, visible light, chemical toxicity and oxidative stress are serious intimidation to agriculture. Abiotic stresses usually cause protein dysfunction. The yield and quality of cereals are severely affected by heat stress in many countries (Treglia *et al.,* 1999). Heat stress affects the grain yield and quality of wheat through affecting sink strength and source capacity. Wheat genotypes express a differential response to chronic heat as well as a heat shock (Yang *et al.,* 2002). Drought and soil salinity significantly affect plant growth, development and productivity, thus posing a severe threat to agriculture throughout the world. It is projected that up to 50% of agricultural yield will be lost due environmental stresses such as heat, cold, drought, salinity, nutrient, heavy metals and so on, compared to about 10 to 20% crop loss caused by biotic stress such as pathogens and diseases (Kreps *et al.,* 2002). Water is the most wide-ranging difficulty among abiotic stresses for production of wheat in the world environment. Strategy is to obtain plants with higher performance under water stress conditions by identifying and modifying the molecular mechanisms that take place when the water availability becomes limiting. Drought and heat are the two major abiotic constraints affecting the yield and quality of wheat. Plants, as sessile organisms, rely on proteomic plasticity to remodel themselves during periods of developmental change, to endure varying environmental conditions, and to respond to biotic and abiotic stresses. To investigate these facts, more comprehensive approaches that include quantitative and qualitative analyses of gene expression products are necessary at the transcriptome, proteome, and metabolome levels. Environmental stresses that result in cellular dehydration, such as freezing, salt and water stress, often lead to similar changes in plant gene expression and metabolism (Cook *et al.,* 2004; Kreps *et al.,* 2002). The proteome is not a static entity, being it affected by multiple modifications such as cell cycle, changes of external conditions, kind of tissue examined, and fastidious physiological states. Proteomics is becoming a powerful tool to analyze biochemical pathways and the complex response of plants to environmental stimuli. In

particular, comparative proteomic investigations of plants before and after specific or interactive stresses allow us to obtain information on how defensive mechanisms are adopted from plants. Proteomics also makes an essential bridge between the transcriptome and metabolome (Wang *et al.,* 2004; Gray and Heath, 2005), complementing genomics research. Only by grouping all this information together is it possible to achieve a comprehensive and exhaustive analysis of the mechanism of plant defense against abiotic and biotic stress. Upon several stress responses protein, protein-protein interaction and post-translation modification have been also identified (Salekdeh *et al.,* 2002). In the last decade, methodological improvements have allowed comparative proteomic investigations of plants under stress which have allowed us to analyses biochemical pathways and the complex response of plants to environmental stimuli (Qureshi *et al.,* 2007). 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 proteomic approaches.

Materials and methods

Plant Materials

Wheat grain of four cultivars (two Chinese cvs. China-108, Yennon-78 and two Japanese cvs. Norin-61, Kantou-107) were used in this study for identification of abiotic stress responsive proteins by proteomic analysis. Wheat were grown in field at the National Institute of Crop Science, Suwon, Korea. Wheat were grown in field under low temperature (-20° ~ -10° C) for four months, then slowly increase temperature and naturally exposed up to 28° C until harvesting. The harvested grains were stored at -20° C until used.

Sample preparation by KCl solubility method

Osborne's (1924) solubility method was used to fractionate wheat endosperm with some modifications (Hurkman and Tanaka, 2007; Kamal *et al.,* 2009**)**. Wheat flour (50 mg) was suspended in 200 µl of cold (4 °C) KCl buffer (50 mM Tris-HCl, 100 mM KCl, 5 mM ethylene-diaminetetraacetic acid (EDTA) (pH 7.8). The suspension was incubated on ice for 5 min with intermittent mixing by vortex including sonication (Sonics and Materials Inc., USA) and centrifuged at 16,000 × g for 15 min at 4 °C (Hanil Science Industrial Co. Ltd. Korea). The pellet was suspended in 800 µl of SDS buffer (2% SDS, 10% glycerol, 50 mM DL-dithiothreitol (DTT), 40 mM Tris–Cl, pH 6.8), incubated for 1 hr at room temperature, and insoluble material removed by centrifugation at 16,000 × g for 10 min at room temperature. The proteins were precipitated by the addition of 4 vol. of cold (-20 °C) acetone and incubation overnight at -20 °C. Following centrifugation, the pellet was rinsed with cold acetone, then dried by vacuum centrifugation (BIOTRON Inc., Korea) and solublized in urea buffer (9 M urea, 4% Triton X-114, 1% DTT, and 2% ampholytes) at 250 µl.

Gel electrophoresis

Soluble proteins of whole seed were examined by two-dimensional gel electrophoresis according to the protocol of O`Farrell (1975). Sample solutions (200 µg proteins) were loaded on to the acidic side of the IEF (Iso-electric focusing) gel with pH range of 3-10 for the first dimension. SDS-PAGE in the second dimension (Nihon Eido, Tokyo, Japan) was performed with 12% separation and 5% stacking gels. 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, and then washed with 100 µl distilled water. Each gel piece was dehydrated by 25 mM ammonium bicarbonate (ABC) / 50% acetonitrile (ACN) and washed with 10 mM DTT /0.1 M ABC. Gel pieces were dried under vacuum centrifugation, rehydrated with 55 mM iodoacetamide (IAA) / 0.1 M ABC for 30 min in dark. After removing the solution, the gels pieces were vortexed with 100 mM ammonium bicarbonate for 5 min and soaked in ACN for dehydration. The gel pieces were then dried under vacuum centrifugation. Trypsin solution (4µl) was added in rehydrated gel particles and incubated for 45 min at 4^0 C and overlaid with 30 µL of 25 mM ABC (pH 8.0) to keep them immersed. The gel pieces were then incubated overnight at 37ºC. After incubation, the solution was spin down and transferred to a 500 µl siliconized tube. The gel particles were suspended in 40 µl ACN / DDW / TFA (660 µl:330 µl:10 µl) three times and 100% ACN, then vortexed for 30 min, respectively. The supernatant was dried under vacuum centrifugation for 2 hr. The digests were desalted with C_{18} Zip Tip (Millipore, Boston) and subjected to the analysis by MALDI-TOF Mass spectrometry.

Bioinformatics Analysis

According to Kamal *et al.* (2009), by using peptide fragmentation methods, the proteins were identified by searching NCBI non-redundant database using the MASCOT program (http://www.matrixscience.com). The search parameters allowed for modifications of acetyl (K), carbamidomethyl (C), oxidation (M), propionamide (C) with peptide tolerance (±100 ppm). For MS/MS searches, the fragmentation of a selected peptide molecular ion peak is used to identify with a probability of less than 5%. When more than one peptide sequence was assigned to a spectrum with significant score, the spectra were manually examined. Sequence length, gene name and also protein functions (categorized such as heat, drought, salt, cold and other environmental stress) were identified by searching Swiss-Prot / TrEMBL database using UniProtKB (http://www.uniprot.org).

Results and Discussion

2D-PAGE based protein comparison among four wheat cultivars

We identified more than 140 protein spots among four cultivars using the gels of pH 3-10. There were analyzed by MALDI-TOF/MS about 38 protein spots in China-108, 40 in Yeonnon-78, 26 in Norin-61 and 36 in Kantou-107. The protein spots patterns were highly reproducible for at least three self-determining protein extractions. Using 2-DE gels of pH 3-10, we observed qualitative variations of 19 protein spots in four wheat cultivars.

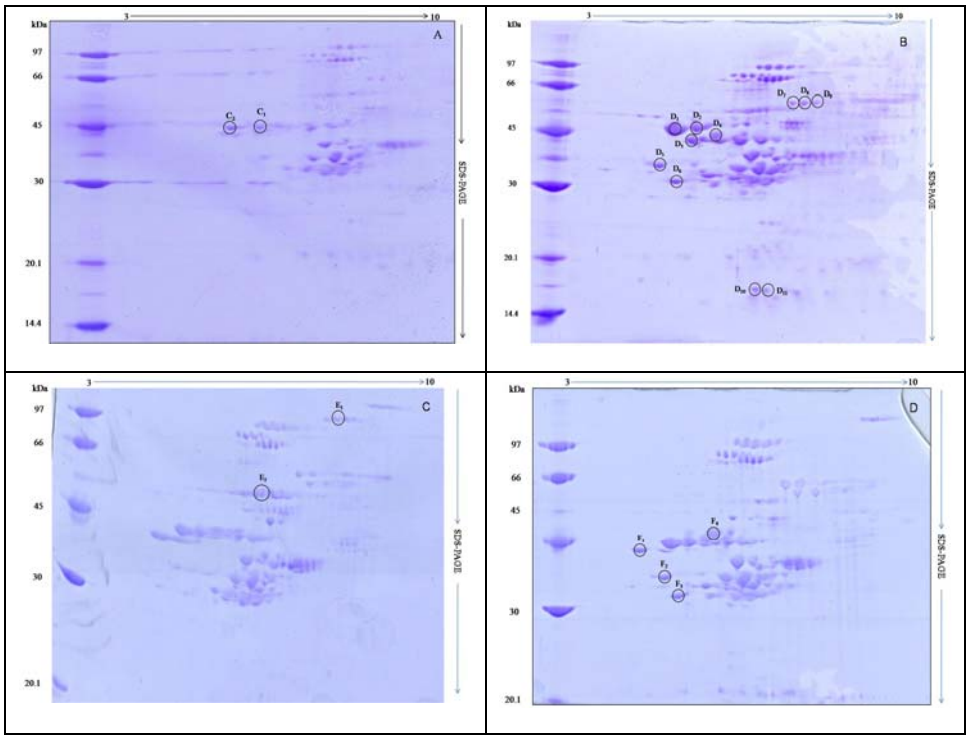

Fig 1. (A-D) 2D gel analyses of proteins extracted from mature wheat seeds (A; China-108, B; Yeonnon-78, C; Norin-61, D; Kantou-107). First dimension was performed on IEF with pH 3-10. In the second dimension gels were used and proteins were visualized using Coomassie Brilliant Blue (R-250). Circle shows that dissimilar protein spots among wheat cultivars

Among them, the protein spots C_{1-2} were only found in China-108 (Fig. 1A), D_{1-11} in Yeonnon-67 (Fig. 1B), E_{1-2} in Norin-61(Fig. 1C) and F_{1-4} in Kantou-107 (Fig. 1D). These results strongly indicate that these identified proteins are cultivar specific and show the difference among these cultivars (Yahata *et al.,* 2005; Kamal *et al.,* 2009).

Abiotic stress responsive proteins identified by mass spectrometry

Heat stress responsive proteins

Out of 39 heat stress responsive proteins, 8 proteins were identified in China- 108, 18 in Yeonnon-78, 31 in Norin-61, 27 in Kantou-107 (Fig 2). We observed heat increases or decreases in proteins by heat stress using proteomic technique in wheat grain. These proteins include heat shock proteins , heat stress transcription factor, granule bound starch synthesis, GTP binding proteins, beta-amylase, eucaryotic initaition factor, elongation factor, ribulose biphosphate related proteins and so on (Supplementary table I). 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) and mature grain (Blumenthal *et al.,* 1990), and heat stress transcription factor to be up regulated by heat(Qin *et al.,* 2007). We also found some granule bound starch synthesis, as reported Mojoul *et al.* (2004). The adverse effect of temperature on starch synthesis is responsible for the reduction in grain weight and hence for the decreased in yield (Wallwork *et al.,* 1998). We also identified GTP-binding proteins, which are involved in signal transduction mechanism in plant systems, and these proteins regulate a flow of kinases that play a vital role in environmental stress signal transduction, and also high temperature seem to up-regulate the synthesis of GTP-binding proteins resulting in increased kinase activity(Grover *et al.,* 2003). Vacuolar ATP synthesis proteins are induced by heat (Golldack and Dietz, 2001). Some others identified proteins were the ribulose biphosphate, elongation factors and eucaryotic translation initiation factors. Ribulose biphosphate (Rubisco) is the enzyme that converts CO_2 to plant biomass. Rubisco electrovated temaperature inhibits photosynthetic CO_2 fixation in plant species. The temperature-induced decreased in the activation of Rubisco due to this inhibition resulting increased the temperature in chloroplast (Kobza and Edwards, 1987). Besides, translational activity was involved in the stress response. Some heat up regulated proteins showed the similarities to elongation factors (EF) and eucaryotic translation initiation factors (eIFs). Heat shock involves changes in the expression patterns of the eIFs, the EF 1-beta, EF 1-alpha, EF Tu and eIF (4A, 4B, 4E, 4E-1, 4E-2, 5A-1, 5A-2, SUII), as were investigated in wheat leaves as a heat shock (Gallie *et al.,* 1998). Nevertheless, we identified chaperone, ferritin, annexin, calcium-binding proteins, thioredoxin and ascorbate peroxidase and tubulin in four

wheat cultivars (Supplementary Table I). Heat shock proteins (HSPs) function as molecular chaperones in maintaining homeostasis of protein folding and are related to the acquisition of thermotolerance (Wang *et al.,* 2004) and ferritin encoding heat regulated (Qin *et al.,* 2008). Calcium is a universal molecule in both animals and plants, and the transient increase in Ca^{2+} level during heat stress is well documented in plant. Heat shock triggers cytosolic Ca^{2+} bursts, which is transducted by Ca^{2+} bindig proteins(CBP) such as calmodin (CaM),calcineurin (CBL), annexin and then up regulates the expression of HSPs (Liu *et al.,* 2003). Thioredoxin and ascorbate peroxidase increase more during short-term heat shock than during long-term heat treatments (Qin *et al.,* 2008). Tubulin proteins are coupled to GTP binding proteins, which play a role in heat resistance in plant (Segal and Feldman, 1996). We are also identified serine carboxypeptidase, glucose-1-phosphate, glucose-6-phosphate and S-adenosyl-methionone synthetase proteins (Supplementary table I).

Drought stress responsive proteins

In response to water deficit, plants have developed various strategies to cope with stress conditions through a combination of metabolic, physiological and morphological changes. These drought adaptive changes rely largely on alterations in gene expression. Out of 33 drought stress responsive, 9 proteins were identified in China-108, 15 in Yeonnon-78, 20 in Norin-61, 26 in Kantou-107 (Fig. 2). We identified different abscisic acid responsive proteins, LEA protein such as chaperonin, cys peroxiredoxin, ethylene response, and elongation factor TU in four wheat cultivars, which is responsible for drought stress (Supplementary Table I). We also observed cyclin-dependent kinase like, zinc finger, transcription factor like MYB, lipid transfer proteins and WRKY in this study (Supplementary table I). Water stress quickly reduced the mitotic activity of mesophyll cells in the meristematic zone and reduced the zone of cell division; the early decline in the Cdc2-like kinase activity indicates that the activation of the enzyme was directly affected by stress (Schuppler *et al.,* 1998). AtMYB2 and AtMYC2 (rd22BP1) are MYBand bHLH-related transcription factors that bind to MYBRS and MYCRS, respectively (Abe *et al.,* 2003). All these transcription factors function as transcriptional activators in the expression of stressinducible genes. Although understanding of the down-regulation of gene expression under stress conditions is also important for understanding molecular responses to abiotic stresses, little is known about cis- and trans-acting factors involved in repression of stress-down-regulated genes. Zinc finger or zinc finger motif was highly enhanced under drought, cold and salinity stress in *Arabidopsis* (Sakamoto *et al.,* 2004). Two genes (TaLTP1 and TaLTP2) encoding lipid transfer proteins (LTPs) were isolated from wheat-rye near-isogenic line (NIL). High levels of expression of TaLTPs in the tissue layers between the vascular bundles might play a role in the drought tolerance response of the wheat crown (Jang *et al.,* 2002). WRKY transcriptional proteins are implicated in responses to the abiotic stresses of wounding (Cheong *et al.,* 2002), the combination of drought and heat (Rizhsky *et al.,* 2002), and cold (Huang and Duman, 2002). We also detected triticin in four wheat cultivars (Supplementary table I). The complement triticin of globulin storage proteins is very complex and

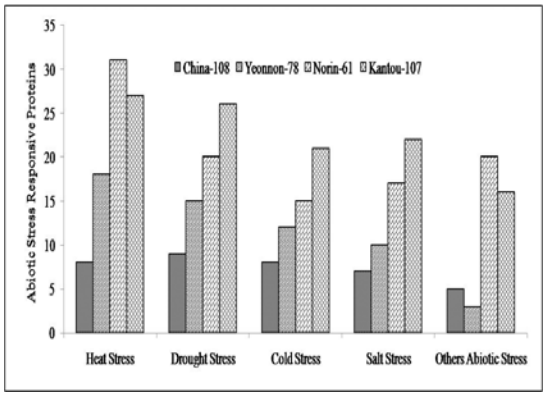

Fig 2. Functional distribution of the total identified abiotic stress responsive proteins among four wheat cultivars

warrants further analysis, particularly since some of these proteins increase in response to high temperature (Dupont *et al.,* 2006).

Cold stress responsive proteins

Out of 27 cold stress responsive proteins, 8 proteins were identified in China-108, 12 in Yeonnon-78, 15 in Norin-61, 21 in Kantou-107 (Fig. 2). We revealed some cold stress related proteins such as cold shock proteins, ABA inducible proteins, cyclophilin, low temperature regulated proteins, mitogen activated, and translation initiation in four wheat cultivars (Supplementary table I). Several cold-responsive genes of unknown function were identified from cold-acclimated wheat (Breton *et al.,* 2003). WCSP1 (cold shock protein 1) induction was cold-specific because neither drought and salinity, nor heat stress induced WCSP1 expression (Karlson *et al.,* 2002). Some major protein as cold shock protein, ABA inducible proteins and so on, synthesized in vivo and in vitro during cold acclimation (Houde *et al.,* 1992). Protein synthesis is necessary for the development of freezing tolerance, and several distinct proteins accumulate during acclimation to cold, as a result of change in gene expression (Guy, 1999). ABA appears to have an important role in inducing freezing tolerance in wheat, which is influenced by water shortage, increasing their freezing tolerance (Xu *et al.,* 2006). Cyclophilin, aquaporin, chitinase plays important role in cold (up regulated) stress of wheat (Houde *et al.,* 2006). The effects of dehydration, cold-temperature treatment, and osmotic and salt stress on the expression of an abscisic acid-responsive protein kinase mRNA (PKABA1) was determined in wheat seedlings (Holappa and Simmons, 1995). Mitogen have three protein kinases, MAPKKK, MAPK and a ribosomal kinase homologue, increased markedly and simultaneously when plants were treated with low temperature (Zhang *et al.,* 2006). The progressive increase in AP1 transcription was consistent with the progressive effect of vernalization on flowering time (Yan *et al.,* 2003). Initiation Factor 1 levels increased two to threefold upon cold shock , and some mutations in the *infA* gene coding for IF1 result in cold sensitivity, further strengthening the possible role of S1 domain proteins in cold acclimation (Giuliodori *et al.,* 2004).

Salt stress responsive proteins

Out of 29 salt stress responsive proteins, 7 proteins were identified in China-108, 10 in Yeonnon-78, 17 in Norin-61, 22 in Kantou-107 (Fig. 2). We identified some salt stress responsive proteins such as salt stress protein, ABA inducible, aquaporin, bowman-brik type proteinase inhibitor, calcineurin B like protein, cyclophilin, potassium channel, and RNA binding proteins in four wheat cultivars (Supplementary table I). Dooki *et al.* (2006) identified several salt responsive proteins including salt stress protein and ABA using 2-DE. Aquaporins-like proteins transport water across cellular membranes and play vital roles in all organisms under salt stress (Forrest and Bhave, 2008). Peroxiredoxin (2-Cys) used as antioxidant enzyme in roots and shoots of salt stressed seedling (Uniprot). This peptide encoded by WRS15 (myb-transcription factor) contains a Bowman-Birk domain sharing a high level of sequence, the expressin level of WRS15 was increased in SR3 wheat roots exposed to salt stress (Shan *et al.,* 2008). Calcineurin is a Ca^{2+}- and calmodulin-dependent serine/threonine phosphatase, and notably, the expression of the mouse calcineurin gene in rice resulted in its higher salt stress tolerance than the non-transgenic rice (Ma *et al.,* 2005). Cyclophilins is up-regulated under salt stress (Godoy *et al.,* 2000). The physiological role of their homologues with putative zinc finger motif remains unclear. Zinc finger proteins play important role in growth and development in plants, and were characterized as salt stressed proteins in *Arabidopsis* (Xu *et al.,* 2006). NaCl is the most plentiful salt encountered by plants under salinity stress, both Ca^{2+} and K^{2+} affect intracellular Na^{+} concentrations (Zhong and Lauchli, 1994), finally calcium enhances K^{+}/Na^{+} selectivity and increases salt tolerance (Liu and Zhu, 1997). RNA binding proteins have probably functioned in salt stress (Sha Valli Khan *et al.,* 2007).

Other stress responsive proteins

Out of 28 others abiotic stress responsive proteins, 5 proteins were identified in China-108, 3 in Yeonnon-78, 20 in Norin-61, 16 in Kantou-107 (Fig. 2).We identified heavy metals such as cadmium, copper, aluminium, manganese, metallothion like, molybdenum Rac/Ras like GTP binding, germin like, wall associated kinase, and some unclear abiotic stress responsive proteins in our experiments (Supplementary table I). Metallothionein-like protein, putative wall-associated protein kinase, and the putative small GTP-binding protein Rab2, were up-regulated by Cu stress (Zhang *et al.,* 2009). The level of the molybdenum cofactor (MoCo) increased by the source and concentration of nitrogen was studied in annual ryegrass resulting facing this plant in salt stress (Sagi *et al.,* 1997). Rac like GTP binding protein plays an important role for GTPase in oxidative stress response (Baxter- Burrell *et al.,* 2002). GLPs (glucagon-like peptide) function primarily as superoxide dismutase (SOD) to protect plants from the effects of oxidative stress (Khuri *et al.,* 2001). Aluminium-activated malate transporters play an important role in plant Al tolerance (Liu *et al.,* 2009). In plants, the role of SOD has received much attention, since reactive oxygen species have been found to be produced during many adverse conditions such as drought, salinity, chilling stress (Martinez *et al.,* 2001).

In 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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References

Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. Plant Cell. 15:63–78

Baxter-Burrell A, Yang Z, Springer PS, Bailey-Serres J (2002) RopGAP4-dependent Rop GTPase rheostat control of *Arabidopsis* oxygen deprivation tolerance. Science. 296: 2026-2028

Blumenthal CS, Barlow EWR, Wrigley CW (1993) Growth environment and wheat quality: the effect of heat stress on dough properties and gluten proteins. Cereal Sci. 18:3–21

Breton G, Danyluk J, Charron JB, Sarhan F (2003) Expression profiling and bioinformatic analyses of a novel stress-regulated multispanning transmembrane protein family from cereals and *Arabidopsis*. Plant Physiol. 132:64-74

Cheong YH, Chang HS, Gupta R, Wang X, Zhu T, Luan S (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. Plant Physiol. 129: 661-677

Cook D, Fowler S, Fiehn O, Thomashow MF (2004) A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of *Arabidopsis*. Proc Natl Acad Sci USA. 101:15243-15248

Dooki AD, Mayer-Posner FJ, Askari H, Zaiee AA, Salekdeh GH (2006) Proteomic responses of rice young panicles to salinity. Proteomics. 6: 6498–6507

DuPont FM, Hurkman WJ, Vensel WH, Chan R, Lopez R, Tanaka CK, Altenbach SB (2006) Differential accumulation of sulfur-rich and sulfur-poor wheat flour proteins is affected by temperature and mineral nutrition during grain development. Cereal Sci. 44: 101–112

Forrest KL, Bhave M (2008) The PIP and TIP aquaporins in wheat form a large and diverse family with unique gene structures and functionally important features. Funct Integr Genomics. 8: 115-133

Gallie DR, Le H, Caldwell C, Browning KS (1998) Analysis of translation elongation factors from wheat during development and following heat shock. Biochem. Biophys Res Commun. 245: 295–300

Giornini S, Galili G (1991) Characterization of HSP-70 cognate proteins from wheat. Theor Appl Genet. 82: 615-620

Giuliodori AM, Brandi A, Gualerzi CO, Pon CL (2004) Preferential translation of cold-shock mRNAs during cold adaptation. RNA 10: 265-276

Godoy AV, Lazzaro AS, Casalongue CA, Segundo BS (2000) Expression of a Solanum tuberosum cyclophilin gene is regulated by fungal infection and abiotic stress conditions. Plant Sci. 152:123–134

Golldack D, Dietz KJ (2001) Salt-induced expression of the vacuolar H^+-ATPase in the common ice plant is developmentally controlled and tissue specific. Plant Physiol. 4: 1643–1654

Gray GR, Heath D (2005) A global reorganization of the metabolome in Arabidopsis during cold acclimation is revealed by metabolome fingerprinting. Plant Physiol 124: 236–248

Grover A, Kapoor A, Lakshmi OS, Agarwal S (2001) Understanding molecular alphabets of the plant abiotic stress responses. Plant Mol Biol. 80:206–216

Guy CL (1999) Molecular responses of plants to cold shock and cold acclimation. J Mol Microbiol Biotechnol. 1: 231–242

Holappa LD, Simmons MKW (1995) The Wheat Abscisic Acid-Responsive Protein Kinase mRNA, PKABA1, Is Up-Regulated by Dehydration, Cold Temperature, and Osmotic Stress. Plant Physiol 108: 1203–1210

Houde M, Belcaid M, Ouellet F, Danyluk J, Monroy AF, Dryanova A, Gulick P, Bergeron A, Laroche A, Links MG, MacCarthy L, Crosby WL, Sarhan F (2006) Wheat EST resources for functional genomics of abiotic stress. BMC Genomics. 7:149

Houde M, Danyluk J, Laliberte JF, Rassart E, Dhindsa RS, Sarhan F (1992) Cloning, characterization and expression of a cDNA encoding a 50-kiladalton protein specifically induced by cold acclimation in wheat. Plant Physiol. 99: 1381-1387

Huang T, Duman JG (2002) Cloning and characterization of a thermal hysteresis (antifreeze) protein with DNA-binding activity from winter bittersweet nightshade, *Solanum dulcamara*. Plant Mol Biol. 48:339-350

Hurkman WJ, Tanaka CK (2007) Extraction of wheat endosperm proteins for proteome analysis. Chromatography 15:344-350

Jang CS, Kim DS, Bu SY, Kim JB, Lee SS, Kim JY, Johnson JW, Seo YW (2002) Isolation and characterization of lipid transfer protein (LTP) genes from a wheat-rye translocation line. Plant cell reports. 20:961-966

Kamal AHM, Kim KH, Shin DH, Seo HS, Shin KH, Park CS, Heo HY, Woo SH (2009) Profile of pre-harvest sprouting wheat by using MALDI-TOF Mass Spectrometry. Plant Omics J. 2:110-119

Karlson D, Nakaminami K, Toyomasu T, Imai R (2002) A cold-regulated nucleic acid-binding protein of winter wheat shares a domain with bacterial cold shock proteins. J Biol Chem. 277: 35248-35256

Khuri S, Bakker FT, Dunwell JM (2001) Phylogeny, function, and evolution of the cupins, a structurally conserved, functionally diverse superfamily of proteins. Mol Biol Evol. 18: 593-605

Kobza J, Edwards GE (1987) Influences of Leaf Temperature on Photosynthetic Carbon Metabolism in Wheat. Plant Physiol. 83:69–74

Kreps JA, Wu Y, Chang HS, Zhu T, Wang X, Harper JF (2002) Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress. Plant Physiol 130:2129–41

Kuiper PJC, (1998) Adaptation mechanism of green plants to environmental stress. The role of plant sterols and the phosphatidyl linolenoyl cascade in the functioning of plants and the response of plants to global climate change. pp 209-215 in: Csermely, P. (Ed.), Stress of Life. From Molecules to Man. Annals of the New York Academy of Sciences. New York.

Liu HT, Li B, Shang ZL, Li XZ, Mu RL, Sun DY, Zhou RG (2003) Calmodulin is involved in heat shock signal transduction in wheat. Plant Physiol. 132: 1186-1195

Liu J, Magalhaes JV, Shaff J, Kochian LV (2009) Aluminum-activated citrate and malate transporters from the MATE and ALMT families function independently to confer *Arabidopsis* aluminum tolerance. Plant J. 57: 389–399

Liu JP, Zhu JK (1997) An Arabidopsis mutant that requires increased calcium for potassium nutrition and salt tolerance. Proc Natl Acad Sci USA. 94: 14960–14964

Ma X, Qian Q, Zhu D (2005) Expression of a calcineurin gene improves salt stress tolerance in transgenic rice. Plant Mol Biol. 58: 483-495

Majoul T, Bancel E, Triboi E, Hamida JB, Branlard G (2004) Proteomic analysis of the effect of heat stress on hexaploid wheat grain: Characterization of heat-responsive proteins from non-prolamins fraction. Proteomics. 4: 505–513

Martinez CA, Loureiro ME, Oliva MA, Moacyr Maestri M (2001) Differential responses of superoxide dismutase in freezing resistant *Solanum curtilobum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. Plant Sci. 160:505–515

O`Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem. 250: 4007-4021

Osborne TB (1924) The Vegetable Proteins. London: Longmans Green 1-151

Qin D, Wu H, Peng H, Yao Y, Ni1 Z, Li Z, Zhou C, Sun Q (2008) Heat stress-responsive transcriptome analysis in heat susceptible and tolerant wheat (*Triticum aestivum* L.) by using Wheat Genome Array. BMC Genomics. 9:432

Qin F, Kakimoto M, Sakuma Y, Maruyama K, Osakabe Y, Tran LS, Shinozaki K, Yamaguchi-Shinozaki K (2007) Regulation and functional analysis of ZmDREB2A in response to drought and heat stresses in *Zea mays* L. Plant J. 50:54-69

Qureshi M I, Qadir S, Zolla L (2007) Proteomics-based dissection of stress-responsive in plants. Plant Physiol. 164: 1239–60

Rizhsky L, Liang H, Mittler R (2002) The combined effect of drought stress and heat shock on gene expression in tobacco. Plant Physiol. 130: 1143-1151

Sagi M, Savidov NA, L'vov NP, Lips SH (1997) Nitrate reductase and molybdenum cofactor as affected by salinity and nitrogen source. Plant Physiol. 99: 546–553

Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Arabidopsis Cys2/His2-Type Zinc-Finger Proteins Function as Transcription Repressors under Drought, cold, and High-Salinity Stress Conditions. Plant Physiol. 136: 2734–2746

Salekdeh GH, Siopongco J, Wade LJ, Ghareyazie B, Bennett J (2002) Proteomic analysis of rice leaves during drought stress and recovery. Proteomics. 2: 1131–1145

Schuppler U, He PH, John PCL, Munns R (1998) Effect of Water Stress on Cell Division and Cell-Division-Cycle 2-Like Cell-Cycle Kinase Activity in Wheat Leaves. Plant Physiol. 117: 667–678

Segal G, Feldman M (1996) Tubb1, a beta-tubulin cDNA from common wheat. EMBL/GenBank/DDBJ databases

Sha Valli Khan PS, Hoffmann L, Renaut J, Hausman JF (2007) Current initiatives in proteomics for the analysis of plant salt tolerance, Current Science. 93: 6-11

Shan L, LI C, Chen F, Zhao S, Xia G (2008) A Bowman-Birk type protease inhibitor is involved in the tolerance to salt stress in wheat. Plant Cell Envir. 31: 1128 – 1137

Treglia A, Spano G, Rampino P, Giangrande E, Nocco G, Mita G, Di Fonzo N, Peppotta C (1999) Identification by in vitro translation and Northern blot analysis of heat shock mRNAs isolated from wheat seeds exposed to different temperatures during ripening. Cereal Sci. 30: 33–38

Wallwork MAB, Logue SJ, MacLeod LC, Jenner CF (1998) Effect of high temperature during grain filling on starch synthesis in the developing barley grain. Aust J Plant Physiol. 25: 173–181

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9:244–52

Woo SH, Fukuda M, Islam N, Takaoka M, Kawasaki H, Hirano H (2002) Efficient peptide mapping and its application to identification of embryo proteins in the rice proteome analysis. Electrophoresis 23: 647-654

Xu S, Wang X, Chen J (2006) Zinc finger protein 1 (ThZF1) from salt cress (*Thellungiella halophila*) is a Cys-2/His-2-type transcription factor involved in drought and salt stress. Plant Cell Rep. 26: 497-506

Yahata E, Funatsuki WM, Nishio Z, Tabiki T, Takata K, Yamamoto Y, Tanida M, Saruyama H (2005) Wheat cultivar-specific proteins in grain revealed by 2-DE and their application to cultivar identification of flour. Proteomics. 5: 3942–3953

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene VRN1. Proc Natl Acad Sci USA. 100: 6263-6268

Yang J, Sears RG, Gill BS, Paulsen GM (2002) Genotypic differences in utilization of assimilate sources during maturation of wheat under chronic heat and heat shock stresses. Euphytica. 125: 179-188

Zhang H, Lian C, Shen Z (2009) Proteomic identification of small, copper-responsive proteins in germinating embryos of *Oryza sativa*. Ann Bot. 103: 923–930

Zhang T, Liu Y, Yang T, Zhang L, Xu L, An L (2006) Diverse signals converge at MAPK cascades in plants. Plant Physiol Biochem. 44: 274–83

Zhong H, Lauchli A (1994) Spacial distribution of solutes, K, Na, Ca and their deposition rates in the growth zone of primary cotton roots: Effects of NaCl and CaCl2. Planta. 194: 34–41

Supplementary Table 1. List of identified abiotic stress-responsive proteins in four wheat cultivars including name of gene

Functions	MW	p*I*	Species	Accession Number	Gene Name	Cultivar Specific SC (%)			
						C^a	C^b	J^a	J^b
17.6 kDa class I heat shock protein [H]	17590	5.36	*Arabidopsis thaliana*	HSP12_ARATH	HSP17.6	-	17	22	-
17.8 kDa class II heat shock protein [H]	17786	5.33	*Zea mays*	HSP22_MAIZE	-	-	-	11	24
18.2 kDa class I heat shock protein [H]	18123	6.77	*Arabidopsis thaliana*	HSP13_ARATH	HSP18.2	-	20	13	-
1-Cys peroxiredoxin [S,D]	23875	6.30	*Triticum turgidum* subsp. durum	gi|12247762	PER1	-	35	21	-
200 kDa cold-induced protein [C]	2721	6.75	*Triticum aestivum*	Q9S8M5_WHEAT	-	-	-	-	36
23.5kDa heat-shock protein [H]	23443	4.89	*Triticum monococcum*	gi|147225062	-	-	-	-	21
2-Cys peroxiredoxin BAS1, chloroplastic [S]	23314	5.71	*Triticum aestivum*	BAS1_WHEAT	TSA	-	-	25	22
ABA response element binding factor [C]	41700	5.83	*Triticum aestivum*	Q8LK78_WHEAT	ABFB	7		37	19
ABA-inducible protein WRAB1 [D,S,C]	18267	8.63	*Triticum aestivum*	gi|4929080	Wrab19	-	25	64	36
Abscisic acid insensitive protein 3 [D,S,C]	27534	4.10	*Arabidopsis thaliana*	gi|149207533	ABI3	-	9	-	17
Alpha-tubulin [H]	5581	5.55	*Triticum turgidum* subsp. durum	gi|82174009	atu3	-	21	-	49
Aluminum-activated malate transporter [O]	49460	6.64	*Secale cereale*	gi|77166842	-	-	-	-	13
Annexin D4 [H]	36198	6.88	*Arabidopsis thaliana*	ANXD4_ARATH	ANN4	-	-	18	-
Aquaporin [D,S,C]	21140	9.14	*Triticum aestivum*	gi|161897630	PIP1-8	-	-	17	11
Ascorbate peroxidase [H]	15360	4.70	*Triticum aestivum*	Q8LLM6_WHEAT	-	-	-	44	-
Beta-amylase 1 [H]	9615	6.10	*Triticum monococcum*	gi|148529650	BAMY1	-	-	21	14
Biostress-resistance-related protein [O]	33992	8.93	*Triticum aestivum*	Q84VJ1_WHEAT	-	-	-	28	-
Blue copper-binding protein-like [O]	19196	8.77	*Oryza sativa*	Q7F1H3_ORYSA	-	-	-	27	-
Bowman-Birk type proteinase inhibitor II-4 [D,S]	5941	8.45	*Triticum aestivum*	IBB2_WHEAT	-	26	42	56	23
Cadmium-induced protein AS8 [O]	18290	9.30	*Arabidopsis thaliana*	CDI8_ARATH	At4g19070	-	-	29	31
Calcineurin B-like protein 10 [H,S]	29895	4.88	*Oryza sativa* subsp. japonica	CNBLA_ORYSJ	CBL10	-	-	28	10

Calcium-dependent protein kinase [D,S,C]	59722	6.20	*Triticum aestivum*	gi|164472660	CPK1C	18	-	39	27
Calmodulin [H]	16838	4.10	*Triticum aestivum*	CALM_WHEAT	-	-	-	35	100
Calnexin homolog 2 [H]	60451	4.74	*Arabidopsis thaliana*	CALX2_ARATH	At5g07340	-	-	11	-
Casein kinase I [S]	53569	9.86	*Arabidopsis thaliana*	Q9FFH8_ARATH	-	-	-	12	19
Catalase I [H]	56440	6.58	*Triticum aestivum*	CATA2_WHEAT	CATA	-	-	5	-
CBFIVd-D9 [C]	28770	8.76	*Triticum aestivum*	gi|117653943	-	-	-	12	-
Chaperone protein dnaJ 13 [D,H]	59191	8.89	*Arabidopsis thaliana*	DNJ13_ARATH	ATJ13	-	-	16	36
Cold shock domain protein 3 [C]	21520	5.73	*Triticum aestivum*	Q75QN8_WHEAT	WCSP3	-	24	-	22
Cold shock protein-1 [C]	21334	5.74	*Triticum aestivum*	gi|21322752	WCSP1	-	-	6	16
Cold-regulated protein [C]	16812	9.93	*Brassica rapa* subsp. chinensis	gi|82659775	ICE1	42	-	36	22
Copper transporter-like protein COPT3 [O]	14395	9.39	*Arabidopsis thaliana*	gi|18496854	-	-	-	-	28
Cyclic nucleotide-gated ion channel 1[D]	17126	4	*Arabidopsis thaliana*	CNGC1_ARATH	CNGC1	-	-	4	-
Cyclin delta-3 [D]	36883	5.65	*Arabidopsis thaliana*	Q94JY0_ARATH	-	-	-	17	-
Cyclin dependent protein kinase [D]	25361	8.84	*Triticum aestivum*	gi|86438780	cdc2-1A	23	23	-	16
Cyclin-B2-2 [D]	47473	5.69	*Oryza sativa* subsp. indica	CCB22_ORYSI	CYCB2-2	-	-	36	24
Cyclophilin [D,C]	13584	9.19	*Triticum aestivum*	gi|82547214	CYP23-d	27	22	77	39
Cytokinin dehydrogenase [O]	1703	8.59	*Triticum aestivum*	CKX_WHEAT	-	86	80	23	60
Defensin Tm-AMP-D1.2 [O]	5694	8.51	*Triticum monococcum*	DEF12_TRIMO	-	7	-	37	65
Dehydrin Xero 2 [D]	20897	9.38	*Arabidopsis thaliana*	XERO2_ARATH	XERO2	-	-	24	44
Delta 1-pyrroline-5-carboxylate synthetase [D,S]	77652	6.14	*Triticum aestivum*	Q58QF6_WHEAT	-	34	18	-	-
DEP1 [O]	31664	8.29	*Triticum aestivum*	gi|208293840	-	-	-	13	-
Dof-type zinc finger protein [D,S]	3459	11.71	*Triticum aestivum*	gi|192898656	-	-	-	26	100
Drought-induced protein 1 [D]	9932	9.44	*Glycine latifolia*	Q6XPS8_9FABA	-	-	-	-	23
Drought-induced S-like ribonuclease [D]	28380	5.25	*Oryza sativa*	Q69JX7_ORYSA	P0569E	-	13	21	13

					11.38				
Elongation factor 1-alpha [H]	49135	9.20	*Triticum aestivum*	EF1A_WHEAT	TEF1	12	4	-	32
Em protein [C]	9953	5.55	*Triticum aestivum*	EM1_WHEAT	EM	5	16	19	-
Ethylene receptor-like protein [O]	28536	7.44	*Triticum aestivum*	gi|29465662	-	-	-	24	28
Eukaryotic initiation factor 4B [H]	14845	5.18	*Triticum aestivum*	gi|6739520	Eif4B	21	16	42	67
Ferritin 2A [H]	9021	5.47	*Triticum aestivum*	gi|210061145	-	43	-	23	11
Germin-like protein 1-3 [O]	23615	8.45	*Oryza sativa* subsp. japonica	GL13_ORYSJ	GER8	-	-	11	16
Glucose-1-phosphate adenylyltransferase [H]	33236	5.13	*Triticum aestivum*	S 05078	AGA.1	16	31	8	23
Granule-bound starch synthase GBSSII [H]	66008	6.38	*Triticum aestivum*	Q9SQ58_WHEAT	-	-	22	14	26
GTP binding protein [H]	23041	6.96	*Zea mays*	gi|163838698	-	-	41	57	17
Heat shock protein 1 [H]	8401	8.26	*Glycine max*	HSP12_SOYBN	HSP683 4-A	-	-	39	44
Heat shock protein 16.9 [H]	2263	8.09	*Triticum aestivum*	Q42417_WHEAT	hsp16.9-17LC3	76	88	71	71
Heat stress transcription factor C-1b [H]	27209	8.93	*Oryza sativa* subsp. japonica	HFC1B_ORYSJ	HSFC1 B	-	21	25	29
Initiation factor 2 alpha kinase-like [H]	59704	5.18	*Oryza sativa*	Q6Z658_ORYSA	-	-	-	8	-
L-ascorbate peroxidase 1, cytosolic [S]	27086	5.31	*Oryza sativa* subsp. indica	APX1_ORYSI	APX1	-	-	52	-
LEA D-11 dehydrin [D,H]	12803	7.21	*Triticum aestivum*	gi|21624242	Wdhn13	-	-	45	21
Lipid transfer protein precursor [D]	9827	8.89	*Triticum turgidum* subsp. durum	Q9FEK9_TRITU	ltp7.1	7	-	70	18
Low-temperature regulated protein BN26 [C]	14456	6.37	*Brassica anpus*	JQ2281	-	-	-	-	31
Manganese superoxide dismutase [O]	19302	6.77	*Triticum aestivum*	gi|125663927	-	-	-	-	14
Metal tolerance protein B [O]	42316	6.14	*Arabidopsis thaliana*	MTPB_ARATH	MTPB	-	-	-	22
Metallothionein-like protein 1 [O]	7378	4.44	*Triticum aestivum*	MT1_WHEAT	ALI1	54	62	62	24
Mitogen-activated protein kinase kinase 4 [C,S]	40091	9.46	*Arabidopsis thaliana*	M2K4_ARATH	MKK4	-	-	-	18
Molybdenum cofactor synthesis protein 2B [O]	20944	5.68	*Oryza sativa* subsp. japonica	MOC2B_ORYSJ	MOCS2	-	-	10	-
Mutant granule bound starch synthase I [H]	58894	5.60	*Triticum aestivum*	Q8W2G8_WHEAT	waxy	-	19	-	-

MYB transcription factor TaMYB1 [D,S,C]	31894	8.93	*Triticum aestivum*	Q27W75_WHEAT	-	-	-	31	23
NADH dehydrogenase subunit J [O]	14825	6.11	*Triticum monococcum*	gi|164453470	ndhJ	-	-	100	28
Ozone-responsive stress-related protein-like [O]	8780	8.85	*Oryza sativa*	Q9LWT2_ORYSA	-	-	-	35	-
Plastidic alpha 1,4-glucan phosphorylase [O]	74852	5.41	*Triticum aestivum*	gi|34485589	-	-	-	-	-
Potassium channel AKT2/3 [S]	91253	6.09	*Arabidopsis thaliana*	AKT2_ARATH	AKT2	-	-	12	6
Probable calcium-binding protein CML30 [D,S,C]	22682	4.20	*Arabidopsis thaliana*	CML30_ARATH	CML30	-	-	-	20
Probable elongation factor 1-gamma 2 [H]	46374	5.55	*Arabidopsis thaliana*	EF1G2_ARATH	At1g57720	-	-	13	-
Probable RAS type GTP-binding protein [H]	24346	5.26	*Arabidopsis thaliana*	G84723	-	-	-	24	-
Probable WRKY transcription factor 24 [D]	20791	8.39	*Arabidopsis thaliana*	WRK24_ARATH	WRKY 24	-	-	32	15
Protein dehydration-induced 19 homolog 5 [D]	24536	4.73	*Arabidopsis thaliana*	DI195_ARATH	DI19-5	-	-	21	14
Protein kinase [D,S,C]	60937	9.14	*Triticum aestivum*	gi|110341792	wpk4	22	-	26	75
Putative calmodulin-domain protein kinase, 5' partial [H]	27779	4.97	*Arabidopsis thaliana*	Q9SR20_ARATH	-	-	-	-	11
Putative chaperonin 21 [D]	26308	7.71	*Oryza sativa*	Q69Y99_ORYSA	-	-	-	8	-
Putative cold shock protein-1 [C]	18689	6.28	*Oryza sativa*	Q84UR8_ORYSA	-	-	-	55	-
Putative cysteine proteinase inhibitor 9 [D,C]	12383	5.04	*Oryza sativa* subsp. japonica	CYT9_ORYSJ	Os03g0210000	-	-	-	24
Putative ethylene-responsive protein [O]	18729	5.59	*Arabidopsis thaliana*	Q8LGG8_ARATH	-	-	-	-	18
Putative germin-like protein 2-2 [O]	23645	6.49	*Oryza sativa* subsp. japonica	GL22_ORYSJ	Os02g0491700	-	-	4	-
Putative GTP-binding protein [H]	44308	6.30	*Oryza sativa*	Q6Z1J6_ORYSA	-	-	-	24	42
Putative heat shock protein 21 [H]	25337	8.48	*Arabidopsis thaliana*	gi|39104609	-	-	-	22	24
Putative low temperature and salt responsive protein [C,S]	7821	5.58	*Triticum aestivum*	Q8H1Z1_WHEAT	-	28	-	-	-
Putative mitogen activated protein kinase kinase [C,S]	80153	5.02	*Arabidopsis thaliana*	Q9CAV6_ARATH	-	-	-	-	21
Putative protein kinase [D,S,C]	22724	9.46	*Triticum monococcum*	gi|207174006	-	28	37	-	36

Putative RING protein; putative VWA protein [O]	23035	8.16	*Triticum aestivum*	gi|40644800		-	-	51	-
Putative RNA-binding protein [S]	16287	8.20	*Oryza sativa* sub sp. japonica	Q6AU49_ORYSA	-	-	-	-	4
Putative rubisco activase [H]	5599	4.65	*T. turgidum* subsp. durum	gi|62176924	rba1	-	88	-	-
Putative salt-inducible protein [S]	94223	6.72	*Oryza sativa*	gi|14488297	-	-	-	-	10
Putative stress responsive protein [S]	5627	4.33	*Arabidopsis thaliana*	Q8GWH0_ARATH	At4g30660	-	52	-	-
Putative thioredoxin [H]	29754	8.60	*Oryza sativa* subsp. japonica	Q6Z4N3_ORYSA	-	-	-	-	26
Putative zinc-binding protein [D,S]	28239	9.30	*Oryza sativa* subsp. japonica	Q6Z8A4_ORYSA	-	-	-	-	15
R2R3MYB-domain protein [D,S,C]	4931	8.79	*Zea mays*	gi|6165732	-	-	-	-	72
Rac-like GTP-binding protein 4 [O]	24063	9.53	*Oryza sativa* subsp. japonica	RAC4_ORYSJ	RAC4	-	-	57	23
Ribulose bisphosphate carboxylase large chain [H]	52817	6.22	*Triticum aestivum*	RBL_WHEAT	rbcL	-	19	-	8
Ribulose bisphosphate carboxylase small chain clone 512 [H]	13045	5.84	*Triticum aestivum*	RBS3_WHEAT	-	35	47	53	18
RING-H2 finger protein ATL1E [O]	33476	8.26	*Arabidopsis thaliana*	ATL1E_ARATH	ATL1E	-	-	16	16
S-adenosyl methionine synthetase 1 [H]	42793	5.61	*Triticum monococcum*	METK1_TRIMO	SAMS1	-	24	35	29
Salt and low temperature response protein [S,C]	8139	4.42	*Brassica rapa* subsp.pekinensis	Q6GYJ6_BRARP	-	-	35	35	35
Salt stress root protein RS1 [S]	21782	4.92	*Oryza sativa* subsp. indica	SRS1_ORYSI	OsI_001009	-	33	22	26
Salt tolerant protein [S]	17058	4.71	*Triticum aestivum*	gi|63021412	SI	23	17	5	24
Senescence-associated protein-related [O]	29623	5.85	*Arabidopsis thaliana*	gi|22331260	-	-	-	14	
Serine carboxypeptidase 3 [H]	55296	5.88	*Triticum aestivum*	CBP3_WHEAT	CBP3	-	16	16	21
Shaggy-like kinase [S]	45573	8.95	*Triticum aestivum*	gi|117646987	-	-	-	31	-
Small GTP-binding protein [O]	22941	6.90	*Triticum aestivum*	gi|57547575	-	-	-	49	-
Small heat shock protein, chloroplastic [H]	26579	9.64	*Triticum aestivum*	HS21C_WHEAT	HSP21	8	17	18	-
Stress-induced protein KIN2 [C]	6547	9.11	*Arabidopsis thaliana*	KIN2_ARATH	KIN2	-	50	-	-
Stress-responsive protein, putative [O]	21573	8.86	*Oryza sativa*	Q8LN75_ORYSA	OSJNBb01N21.	-	53	-	-

					27				
Thioredoxin H-type [H]	13515	5.12	*Triticum aestivum*	TRXH_WHEAT	-	40	40	34	45
Thylakoid-bound ascorbate peroxidase [O]	41305	5.48	*Triticum aestivum*	Q8GZC1_WHEAT	-	23	-	-	-
Translation initiation factor IF-1, chloroplastic [C]	13104	9.45	*Triticum aestivum*	IF1C_WHEAT	infA	-	-	38	67
Triticin [D]	23536	6.48	*Triticum aestivum*	gi|171027863	-	100	95	-	13
Truncated cold acclimation protein COR413-TM1 [C]	18722	11.8	*Zea mays*	Q84LB3_MAIZE	-	-	-	16	-
Tubulin beta-1 chain [H]	50254	4.73	*Oryza sativa subsp.* japonica	TBB1_ORYSJ	TUBB1	-	-	14	31
Ubiquitin [D]	8520	6.56	*Triticum aestivum*	UBIQ_WHEAT	-	23	16	48	48
Universal stress protein / early nodulin ENOD18-like [O]	17867	8.74	*Oryza sativa*	Q6ZHE6_ORYSA	-	-	-	25	47
Wall-associated kinase 4 [O]	57611	6.61	*Triticum aestivum*	Q4U3Z7_WHEAT	WAK4	8	-	11	24
WRKY family transcription factor [D]	39741	6.03	*Arabidopsis thaliana*	gi|18417879	-	-	-	24	22
Zinc finger A20 and AN1 domain-containing stress-associated protein [D]	18898	8.44	*Arabidopsis thaliana*	SAP9_ARATH	SAP9	-	42	44	27

Criteria: MW: Molecular weight; p*I*: Iso-electric Point; C^a: China-108; C^b: Yeonnon-78; J^a: Norin-61; J^b: Kantou-107; D: Drought stress; H: Heat stress; S: Salt stress; C: Cold stress; O: Other abiotic stress responses, SC: Sequence Coverage