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ORIGINAL RESEARCH

Wild Relatives of the Wheat Grain Proteome

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Abstract We applied proteomics analysis to generate a map of the wild relatives of wheat grain proteins. These differentially expressed proteins are potentially involved in metabolism, stress responses, and other biological activities. Using two-dimensional electrophoresis, we detected 119, 134, and 193 reproducible spots on gels loaded with protein samples extracted from the A, B, and D genomes, respectively, of the mature grain. In all, 89, 53, and 54 distinct proteins, respectively, were found among these genomes through MALDI-TOF mass spectrometry. Of these, 26% (*n*=52) proteins were considered distinct. They included 18.89% (*n*=17) in the A, 28.30% (*n*=15) in the B, and 37.04% (*n*=20) in the D genome, all functioning in disease and defense roles. For example, the ABA-inducible protein PHVA1 can be induced by drought, cold, heat, and salinity, while the basic endochitinase confers protection against chitin-containing fungal pathogens. The diverse functional categories found here suggest different biological processes, such as disease/defense, energy metabolism, protein synthesis and storage, cellular organization, signal transduction, transcription, and the facilitation of transport. Our findings demonstrate that these functional proteins have important roles in stress tolerance and the maintenance of quality in mature grains. The interacting effects of genetics and environment on differential protein production may be partially mediated by a regulatory mechanism in those grains.

Keywords MALDI-TOF mass spectrometry · Proteomics · *Triticum* · Two-dimensional electrophoresis (2-DE) · Wild-type wheat

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Introduction

Diploid species that are related to a wild plant can be reproductively isolated from one another. Therefore, they may comprise populations with marked differences in their morphology, tolerances to climatic fluctuations, and adaptability to diverse habitats. Because they vary genetically in their responses to biotic stress (disease, parasites, and other pests and competitors) and abiotic stress (cold, drought, heat, herbicides, and salinity; Farooq et al., 1996), as well as in their potential for advancement of quality, e.g., gluten

content in wheat (*Triticum*; Sofalian and Valizadeh 2009), these wild relatives are of great interest as genetics resources. Wheat productivity has risen spectacularly in many countries over the last half century. Average global yields have improved from about 1 t ha^{-1} in the 1950s to nearly 3 t ha^{-1} by 2000 (USDA 2010). The effects of climate change will increase the need for cultivars with tolerance to cold, heat, and flooding, while also influencing pest and disease pressures in diverse ways. Although breeding gains have been tangible, albeit incremental, solving those problems still requires a genetics approach. Thus, the current challenge is to create more efficient methods and tools for rapidly accessing the best sources of genetic variation for specific target traits and then utilizing such beneficial variations in breeding programs (Crouch et al. 2009). Gene transfer has already proven successful in conferring resistance to various pathogens and environmental stresses, and nutritionally and technologically useful characteristics from wild diploid relatives have been introduced into the genome of polyploid wheat as agronomically important traits (Appels and Lagudah 1990). For example, Bliffeld et al. (1999) have reported that constitutive overexpression of an antifungal chitinase gene in transgenic wheat leads to enhanced fungus resistance. *Aegilops speltoides* is believed to have hybridized with primitive forms of *Triticum* spp. to produce more modern wheat species as a valuable ancestor in breeding programs (Huxley 1992). Leaf or brown rust, caused by the fungal pathogen *Puccinia triticina*, is one of the most common diseases affecting wheat production worldwide. *Aegilops tauschii* (goat grass) is the progenitor of the D genome in hexaploid bread wheat (*T. aestivum*) and is avirulent on the leaf rust resistance gene *Lr1* (Ling et al. 2004).

The need for greater natural tolerance/resistance to biotic and abiotic stresses has never been more critical and will most likely be found through evaluation and elucidation of biochemical mechanisms already present in certain plant species and varieties. Proteomics approaches are being taken to ascertain target enzymes and proteins from resistant lines that could be utilized to enhance the natural tolerance of agronomically desirable varieties (Donnelly et al. 2005). Such an analysis of endosperm proteins has been conducted to evaluate end-product quality of wheat. Storage proteins in the seed are responsible for dough substance and, hence, have been evaluated extensively in an attempt to elucidate their biochemical properties, yielding better dough and higher viscoelasticity. Hybridization can be used to transfer both HMW-GS and LMW-GS from wild relatives into cultivated species (Kamal et al. 2009b). Yan et al. (2003) have identified 42 HMW-GS alleles from *A. tauschii*, among which are several alleles not presently detected in bread wheat. Consequently, *A. tauschii* is potentially a valuable genetics resource.

Starch synthesis and accumulation, which aid in dough quality, occur during grain filling. Factors affecting that process are controlled by the amyloplasts, leucoplasts found in the endosperm. Proteomics analysis of wheat amyloplasts has provided further insights into associated biochemical mechanisms (Andon et al. 2002; Dupont 2008). A proteomics evaluation of wheat grain filling has demonstrated the downstream negative effects of heat stress on dough consistency (Majoul et al. 2003). Similar investigations have demonstrated how chromosome deletion influences protein expression in wheat seeds (Islam et al. 2003a), and diploid, tetraploid, and hexaploid wheat flour proteomes have been examined to elucidate the effects of genome interaction on wheat proteins (Islam et al. 2003b). Other related research has been conducted on wheat seeds to identify target compounds and pathways for the enrichment of value-added products, but not for improvement of the agronomic properties in the plant itself.

Compared with proteomics analyses of prokaryotes, yeast, and humans, plant proteomics is a young science, partly because of the lack of complete genomic or cDNA sequences from plants (van Wijk 2001). In fact, the hexaploid species of wheat has not yet been sequenced, and we are not aware of any such studies of its wild-type relatives. Here, our main objective was to investigate the proteome assortment in mature grains from wild relatives of the wheat genome, using 2-DE and MALDI-TOF mass spectrometry. The information gained will enable future wheat researchers to use marker-assisted breeding and gene transfer technologies to target proteins whose addition or deletion can improve stress tolerance, yield quality, and other essential agronomic traits.

Materials and Methods

Seed Origins

For proteomics analysis, we selected three diploid species for wild relatives of wheat, all of which may have been involved in the evolutionary process toward bread wheat. Seeds for our tests were obtained from the National Agro-biodiversity Center, RDA, South Korea. *Triticum urartu* (A genome; PI 574468) originated in Romania, where the climate is temperate and continental and the annual average temperature ranges from 11°C in the South to 8°C in the North. *A. speltoides* (B genome; PI 487235) came from Turkey, with a continental climate. The maximum winter temperature there is −1°C but can be as low as −30°C~40°C, while summer is very dry and hot (generally >40°C). *A. tauschii* (D genome; PI 361881) was collected from Afghanistan, where the climate is typically arid or semiarid and temperatures depend upon elevation (average range of 0°C~30°C).

Chemical Reagents

All chemicals are commercially available and required no further purification. Dithiothreitol (DTT), trifluoroacetic acid (TFA), acetonitrile (ACN), ammonium bicarbonate (ABC), ammonium persulfate (APS), tetramethylethylenediamine (TEMED), and iodoacetamide (IAA) were purchased from Sigma (St. Louis, MO, USA). Ultrapure water from a Puris Ultrapure Water System (Mirae Co., Ltd., Korea) was used to prepare all solutions.

Protein Preparation

Mature seeds were ground in liquid nitrogen to a fine flour with a ceramic mortar and pestle. Samples (100 mg) were taken for fractionating the endosperm proteins to determine their solubility and precipitation properties, using methods modified from those of Damerval et al. (1986) and Donnelly et al. (2005). Briefly, the resulting powder (100 mg) was suspended in chilled (−20°C) 10% TCA in acetone containing 0.07% β-mercaptoethanol and 1% plant protease inhibitor cocktail (P9599; Sigma-Aldrich, USA). This mixture was incubated at −20°C for at least 1 h and then centrifuged at 15,000 rpm for 30 min. The pellet was washed three times (1 mL) with chilled (−20°C) acetone containing 0.07% ME and 1% plant protease inhibitor cocktail, with centrifugation for 30 min at 15,000 rpm between rinses. The supernatant was removed and the pellet was dried slowly under speed vacuum. If the dried powder was not solubilized immediately, it was stored at −80°C.

The seed proteins in the dried powder were placed in solubilization buffer (7 M urea, 2 M thiourea, 4% CHAPS, 2% DTT, 0.4% protease inhibitor cocktail, and 1% carrier ampholytes, pH 3 to 10) and then incubated at 37°C for 1 h, with vortexing every 5 min. This was followed by ultrasonication six times (30 s each) with a microtip at 30% (Sonics and Materials Inc., Newtown, MA, USA) and then a final incubation at ambient temperature for 1 h. The mixture was centrifuged at 15,000 rpm for 30 min before the supernatant was harvested. We determined the amount of protein per sample according to the method of Bradford (1976), using a Bio-Rad protein assay kit (Bio-Rad Laboratory) and BSA as a standard.

2-D Gel Electrophoresis

After protein quantification, a total of 300 μg of solubilized proteins was used for 2-DE, as previously described (Kamal et al. 2009b). The first dimension was performed with a prepared isoelectric focusing (IEF) tube gel solution consisting of a 30% acrylamide solution that contained 7 M urea, 10% NP-40, 0.5% carrier ampholytes (pH 3 to 10), 10% APS, and TEMED. Electrophoresis was conducted at 200 V for 1 h, 400 V for 1 h, and 600 V for 16 h. Afterward, the gels were equilibrated three times (30 min each) in sodium dodecyl sulfate (SDS) sample buffer (Kamal et al. 2009a). SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension was done on a 12% separation gel and 5% stacking gel at 80 mA for 3 to 4 h, until the dye line reached the end of the gel. Protein separation was verified by SDS-PAGE analysis of the TCA-precipitated sample, using a 12% separation gel and 5% stacking gel. This was followed by staining (Supplementary Fig. 1) with Coomassie Brilliant Blue (PhastGel™ Blue R; Amersham Pharmacia AB, Uppsala, Sweden). The gels were then subjected to image capturing on a high-resolution scanner (HP Scanjet G4010). Three replicates from three independent biological extracts were used for analysis.

In-Gel Digestion and Mass Spectrometry

Using a protocol modified from that reported by Kamal et al. (2009b), we excised selected protein spots from preparative gels, stained with Coomassie Brilliant Blue (R-250). These had been washed with 100 μL of distilled water and incubated three times (10 min each) at 37°C for 10 min. Each piece of gel containing protein was dehydrated three times (10 min each) by 50% ABC/50% ACN at 37°C. After the solution was removed, the pieces were soaked for 15 min in 100% ACN at 37°C and then dried under speed vacuum and alkylated with 10 mM DTT/0.1 M ABC. The gel particles were rehydrated for 30 min under darkness with 55 mM IAA/0.1 M ABC. The solution was removed and the gel pieces were vortexed with 100 mM ABC for 5 min before being soaked in ACN for dehydration. This caused the pieces to shrink and turn an opaque white. After they were dried under vacuum centrifugation, trypsin solution (20 μL in a volume of 12.5 ng μL^{-1}) was added. The rehydrated gel particles were incubated for 20 min at 0°C and overlaid with 30 μL of 50 mM ABC (pH 8.0) to keep them immersed throughout digestion. The pieces were then incubated overnight at 37° C. Afterward, the solution was spun down and transferred to a 500-μL siliconized tube. The particles were resuspended three times in a 40-μL mixture of ACN/DDW/TFA (660:330:10 μL), resuspended in 100% ACN, and vortexed for 30 min. The supernatant was dried under vacuum centrifugation for 2 h.

For analysis via MALDI-TOF mass spectrometry (AXIMA CFR^+ Plus, Shimadzu, Japan), proteins that had been separated by 2-DE were digested by trypsin according to the method of Kamal et al. (2009b). The machine was run in a reflector/time-lag-focusing mode controlled by Launchpad software ver. 2.4.0. A saturated solution of α-cyano-4-hydroxycinnamic acid was prepared in 1:1 ACN/water with 0.1% TFA serving as a matrix. Each tryptic

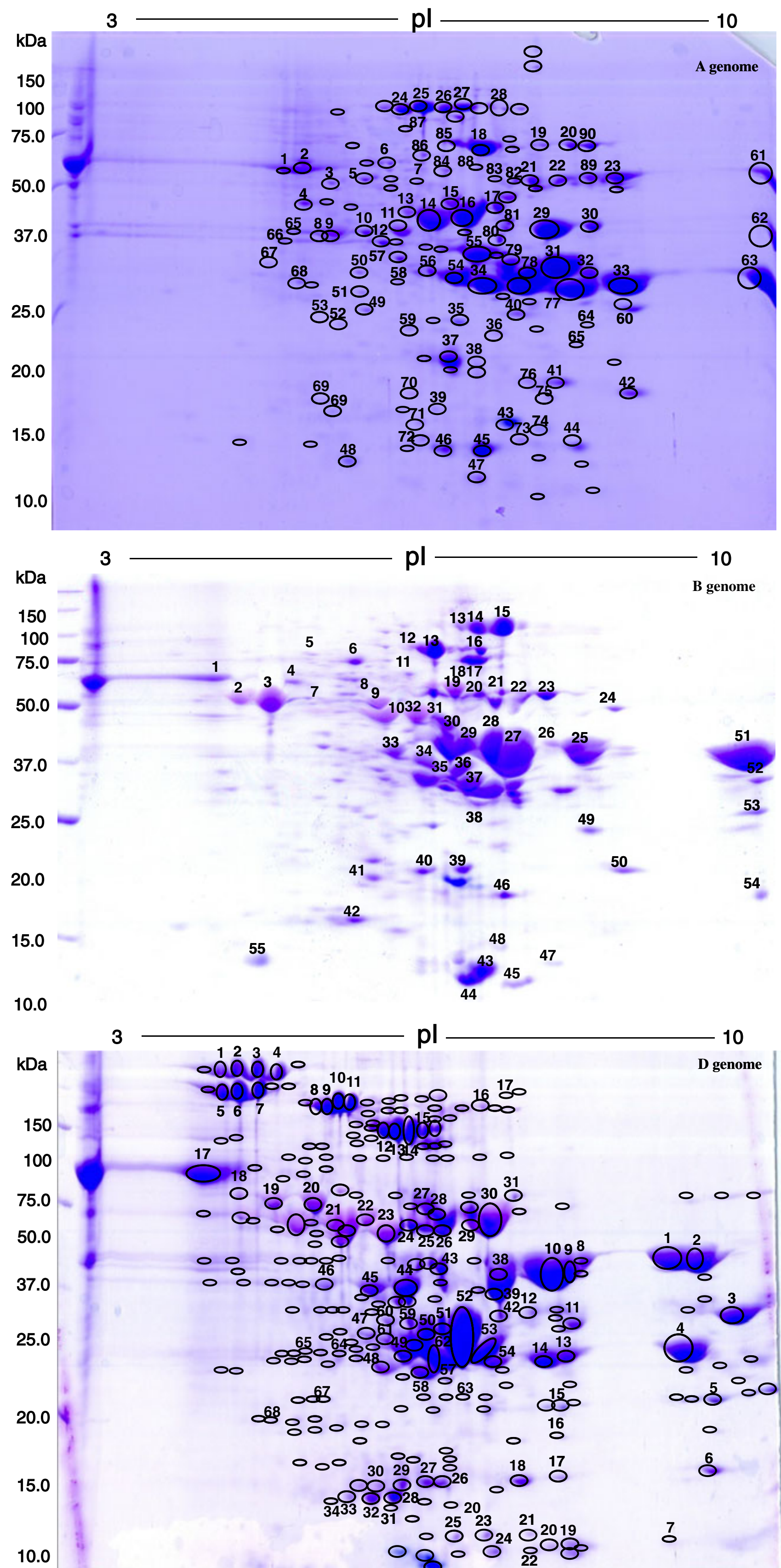

Fig. 1 2D-PAGE resolution of total expressed protein spots from wild relatives of wheat (A genome, *T. urartu*; B genome, *A. speltoides*; and D genome, *A. tauschii*). First-dimension gel electrophoresis (*horizontal*) is represented by IEF ranging from pH 3 (*left*) to pH 10 (*right*). For second-dimension gels (*vertical*) by 12% SDS-PAGE, protein spots were visualized with Coomassie Brilliant Blue R-250. Spots corresponding to glutenin subunits are indicated by *circles*; molecular weight markers are shown on *left* side with sizes

peptide solution was desalted with ZipTip C_{18} (Millipore, Boston, MA, USA), then eluted from the Zip Tip with 70% ACN and 0.1% TFA, and mixed with the matrix on 384-spot MALDI plates. All mass spectra were internally calibrated. After manual acquisition, the spectra were fully processed automatically and further calibrated with Launchpad, using three-point internal calibration with a trypsin autolytic peptide.

Peptide mass fingerprinting (PMF) data searches were conducted with MASCOT search tools (http://www.matrixscience.com) in the NCBInr, MASCOT, and SWISS-PROT public protein databases. "Viridiplantae" (green plants) was selected for the taxonomic category. All peptide masses were assumed to be monoisotopic; [M+H]+, acetyl (K), carbamidomethyl (C), and propionamide (C) were considered to be fixed modifications, while oxidation of methionine was treated as a variable modification. Mass accuracy was set to ±100 ppm, and the maximum number of missed cleavages was set at "1." Identified proteins required more than four matched peptides, and the percentage of sequence coverage had to be >10%. All positive protein identification scores were significant ($P<0.05$). Functional categories were assigned according to biological process, based on SWISS-PROT/TrEMBL and UniProt protein databases (www.uniprot.org).

Results and Discussion

Proteomics Map of A, B, and D Genomes from Wheat Relatives

After we aligned and matched our 2-DE gels, we analyzed the protein spots shown on those gels for the genomes of mature wheat seed. In total, 119, 134, and 193 reproducible spots were detected in gels loaded with protein samples from genomes A, B, and D, respectively. Spot separation was not satisfactory for the p*I* (isoelectric point) span of approximately 4 to 7 and was mixed between 4 and 7 and 3 and 10. Therefore, to avoid overlapping those protein spots and to increase the resolution capacity, we identified proteins covering the p*I* range of 3 to 10, with molecular weights (MW) of 10 to 150 kDa (Fig. 1). A total of 200 protein spots were excised for mass spectrometry (MS) analysis to generate PMFs. These represented 173 unique proteins, based on NCBInr, MASCOT, and SWISS-PROT protein databases (Table 1), and all corresponded to the spot numbers shown in Fig. 1.

We noted that these identified proteins did not always have one-to-one correlations with spots on the gels, according to the proteomics analysis. This may have resulted from polypeptide variants present within different spots, but which were encoded by the same gene (Kerim et al. 2003; Holmes-Davis et al. 2005; Noir et al. 2005). Several possibilities may exist for such differential migration of the same protein. These include posttranslational modification of the protein that differs in vivo, e.g., for phosphorylation, acetylation, or glycosylation (Jensen 2004); alternative splicing of messenger RNAs (mRNAs) during translation (Brett et al. 2002); or chemical modification of the protein during sample preparation.

Comparative Protein Analysis Among the A, B, and D Genomes

We applied an optimized protocol to investigate seed protein from wild relatives of wheat under dormant and storage conditions. Proteins were extracted from three different genomes and were monitored by 2-DE in the p*I* range of 3 to 10. More than 100 protein spots were reproducibly detected in each CBB-stained gel, and proteins that regulated changes in their abundance were found on the appropriate gels among those genomes (Fig. 1). Magnified views of some spots revealed clear differential expressions. A total of 200 spots (90 from A, 55 from B, and 55 from D) were analyzed via MALDI-TOF mass spectrometry. From these, 173 unique proteins were identified, with some spots detecting the same proteins (Table 1). In all, 246 spots (29 from A, 79 from B, and 138 from D) showed low-resolution protein abundance; these were not analyzed by MS (Fig. 1). However, continued advancements in plant proteomics, particularly the development of protein extraction and electrophoresis systems, mean that it is now possible to capture such low-resolution spots, making it possible to analyze them with MALDI-TOF mass spectrometry in the future.

In addition, 196 unique proteins were identified via MALDI-TOF mass spectrometry: 51 from A, 29 from B, and 32 from D. Moreover, 31 proteins were shared between A and B, 22 between A and D, three between B and D, and 28 among all three genomes (Fig. 2).

Functional Categorization Based on Biological Process

All protein sequences detected and identified as part of our wheat proteomics effort were searched against "Viridiplantae" in NCBInr, MASCOT, and SWISS-PROT protein databases coupled to UniProt databases (Kamal et al. 2009a). They were sorted into functional categories according to biological process (Fig. 3; Table 1).

Of the 196 proteins in mature seed, 21.11% were found in genome A, 15.09% in B, and 11.11% in D to be active in energy metabolism. Those roles include electron transport, energy pathways, and metabolic activities. For example, the ATP/ADP carrier protein is involved in moving molecules,

Table 1 Proteins cataloged and identified from wild relatives of the wheat grain proteome

SN	Putative protein name	Acc. no.	Protein source	p*I*	MW	SC	PM	FC	Genome	CCG
20	14-3-3-like protein D	gi|3912949	*Glycine max*	4.76	28,239	32	8	PS	A	A
21	30S ribosomal protein S15,chloroplastic	RR15_WHEAT	*Triticum aestivum*	11.23	10,814	34	7	PS	A	A
72	3-Ketoacyl-CoA thiolase 5, peroxisomal	Q570C8	*Arabidopsis thaliana*	7.07	47,955	27	7	UC	A	A
27	60-kDa chaperonin	gi|49617867	Uncultured bacterium	5.03	44,314	65	6	PS	A	A
5	ABA-inducible protein	gi|33342178	*Triticum aestivum*	5.95	17,518	15	6	DD	A	A
61	Actin-2	Q96292	*Arabidopsis thaliana*	5.37	41,876	37	4	CO	A	A
57	Alpha/beta-gliadin	P02863	*Triticum aestivum*	7.66	32,963	25	5	ST	A	A
14	Alternative oxidase	gi|19912725	*Triticum turgidum*	8.67	36,634	36	6	EM	A	A
24	Beta 3-glucuronyl transferase	gi|32968187	*Triticum aestivum*	9.47	30,115	64	5	PS	A	A
53	Carbon catabolite repressor protein 4 homolog 2	Q9M2F8	*Arabidopsis thaliana*	7.3	67,052	29	3	EM	A	A
69	Cell division protease ftsH homolog	P56369	*Chlorella vulgaris*	9.44	197,171	41	4	CO	A	A
68	Cyclin-P1-1	Q0J9W0	*Oryza sativa*	7.14	27,780	52	9	CO	A	A
87	Cyclophilin	gi|82547216	*Triticum aestivum*	9.15	1,586	15	8	PS	A	A
62	Cysteine synthase, chloroplastic/ chromoplastic	P47999	*Arabidopsis thaliana*	8.13	41,656	52	7	PS	A	A
70	Ent-copalyl diphosphate synthase, chloroplastic	Q38802	*Arabidopsis thaliana*	5.86	93,012	34	9	STD	A	A
19	ER-localized cyclophilin	gi|83700350	*Triticum urartu*	8.93	12,518	23	3	UC	A	A
64	Eukaryotic initiation factor 4A-11	Q40465	*Nicotiana tabacum*	5.38	46,901	47	8	PS	A	A
58	F-box protein At1g10110	O80603	*Arabidopsis thaliana*	6.92	46,109	27	6	UC	A	A
2	H+-ATPase	gi|14334175	*Triticum aestivum*	9.6	15,681	62	5	EM	A	A
37	HistoneH2A.2.1	gi|121974	*Triticum aestivum*	10.63	16,003	52	4	TC	A	A
47	HistoneH2A.2.1	gi|121974	*Triticum aestivum*	10.63	16,003	51	6	TC	A	A
71	Homeobox protein knotted-1-like 2	Q0E3C3	*Oryza sativa*	5.73	33,876	25	3	TC	A	A
73	Homeobox protein knotted-1-like 5	P48002	*Arabidopsis thaliana*	6.03	43,283	36	8	UC	A	A
59	Hordoindoline-B2	Q9LEH8	*Hordeum vulgare*	8.84	16,077	19	4	ST	A	A
63	Malate dehydrogenase, glyoxysomal	P19446	*Citrullus lanatus*	8.67	37,636	42	9	EM	A	A
75	Mitogen-activated protein kinase homolog MMK2	Q40353	*Medicago sativa*	6.15	42,793	26	5	DD	A	A
11	Neutral ceramidase	gi|161702907	*Triticum aestivum*	6.15	86,376	32	4	UC	A	A
76	NPL4-like protein	Q9AS33	*Oryza sativa*	5.32	44,891	37	8	EM	A	A
60	Nucleoside diphosphate kinase 1	A6N0M9	*Oryza sativa* subsp. indica	6.3	16,845	31	2	EM	A	A
84	Nucleoside diphosphate kinase 1	A6N0M9	*Oryza sativa* subs. *indica*	6.3	16,845	18	8	EM	A	A
78	Plastocyanin, chloroplastic	P17340	*Solanum lycopersicum*	5.06	16,992	45	4	EM	A	A
85	Plastocyanin, chloroplastic	P17340	*Solanum lycopersicum*	16,992	5.06	15	7	EM	A	A
66	Probable disease resistance protein At5g66900	Q9FKZ1	*Arabidopsis thaliana*	5.47	92,244	23	9	DD	A	A
80	Probable inactive receptor kinase At1g48480	Q9LP77	*Arabidopsis thaliana*	8.3	71,130	75	6	STD	A	A
88	Protease inhibitor-like protein	gi|162319714	*Triticum aestivum*	8.92	13,443	43	7	TP	A	A

Table 1 (continued)

SN	Putative protein name	Acc. no.	Protein source	p*I*	MW	SC	PM	FC	Genome	CCG
15	Puroindoline a	gi|7672290	*Aegilops kotschyi*	8.55	16,391	65	5	ST, DD	A	A
67	Putative cysteine-rich receptor-like protein kinase 20	O65479	*Arabidopsis thaliana*	6.58	74,057	31	2	DD	A	A
7	Putative F-box protein At4g11580	FB230_ARAT	*Arabidopsis thaliana*	8.27	38,855	47	3	UC	A	A
86	Putative omega-6 fatty acid	gi|1160277	*Triticum aestivum*	8.54	10,597	23	3	EM	A	A
1	Putative resistance protein nb	gi|46358512	*Triticum monococcum*	8.36	62,153	35	4	DD	A	A
16	Putative RIRE2 retrotransposon protein	gi|24899464	*Oryza sativa*	9.23	39,458	32	4	PS	A	A
83	Retinoblastoma-related protein 1	A2YXJ7	*Oryza sativa* subs. *indica*	8.84	111,508	11	3	TC	A	A
50	Serpin-Z1C	Q9ST58	*Triticum aestivum*	5.62	42,881	26	8	CO	A	A
74	*S*-linalool synthase	Q96376	*Clarkia breweri*	6.35	99,789	18	4	EM	A	A
12	ste20-related protein	gi|32400887	*Triticum aestivum*	7.14	25,709	45	5	PS	A	A
38	Storage protein	gi|170696	*Triticum aestivum*	6.8	72,551	41	9	ST	A	A
34	Triticin precursor	gi|7548844	*Triticum aestivum*	9.37	57,318	41	2	ST	A	A
36	Tritin	gi|391929	*Triticum aestivum*	9.69	29,594	37	5	DD	A	A
81	tRNA wybutosine-synthesizing protein 1 homolog	Q8RXN5	*Arabidopsis thaliana*	6.31	72,079	62	5	PS	A	A
8	Ubiquitin	gi|68305063	*Triticum aestivum*	5.69	7,901	56	3	DD	A	A
79	U-box domain-containing protein 3	Q8GWV5	*Arabidopsis thaliana*	5.85	83,778	59	5	PS	A	A
39	Alpha-amylase inhibitor, tetrameric, chain CM3 precursor	gi|21713	*Triticum turgidum*	7.43	18,893	62	3	DD	A	AB
23	Alpha-amylase subfamily Amy2	gi|4261529	*Triticum aestivum*	8.59	3,080	52	3	DD	A	AB
40	Alpha-amylase/trypsin inhibitor	gi|21705	*Hordeum vulgare*	5.31	16,399	72	7	DD	A	AB
43	B3-hordein	gi|82371	*Hordeum chilense*	8.82	6,192	26	2	ST	A	AB
41	B3-hordein(cloneB7)	gi|2371	*Hordeum vulgare*	7.74	30,162	41	4	ST	A	AB
42	B3-hordein(cloneB7)	gi|2371	*Hordeum vulgare*	7.74	30,162	41	3	ST	A	AB
51	Glutenin, low molecular weight subunit	P10385	*Triticum aestivum*	9.04	41,020	27	8	ST	A	AB
52	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic 1	P08735	*Zea mays*	6.46	36,522	26	5	EM	A	AB
18	Hypothetical protein	gi|125605997	*Oryza sativa*	9.02	7,013	43	6	UC	A	AB
30	Hypothetical protein	gi|160701982	*Phaeosphaeria*	6.18	44,314	19	4	UC	A	AB
3	LEA3 protein	gi|25989707	*Triticum aestivum*	8.83	21,838	43	5	DD	A	AB
28	Low molecular weight glutenin protein	gi|50404489	*A. tauschii* × *T. turgidum*	8.92	34,900	62	8	ST	A	AB
35	Low molecular weight glutenin protein	gi|50404489	*A. tauschii* × *T. turgidum*	8.92	34,900	34	2	ST	A	AB
45	Low molecular weight glutenin protein	gi|50404489	*A. tauschii* × *T. turgidum*	8.92	34,900	34	4	ST	A	AB
82	Ribulose bisphosphate carboxylase small chain 1, chloroplastic	P00865	*Glycine max*	8.87	20,073	21	2	EM	A	AB
48	Signal recognition 54 kDa protein 1	P49968	*Triticum aestivum*	9.33	54,511	23	4	STD	A	AB
49	Signal recognition 54 kDa protein 2	P49966	*Triticum turgidum*	9.15	55,078	28	6	STD	A	AB

Table 1 (continued)

SN	Putative protein name	Acc. no.	Protein source	p*I*	MW	SC	PM	FC	Genome	CCG
29	Class II chitinase	gi|62465514	*Triticum aestivum*	8.66	28,599	42	2	DD	A	ABD
4	NBS-LRR type RGA	gi|74129157	*Triticum turgidum*	9.9	4,890	65	4	DD	A	ABD
77	Pentatrico peptide repeat-containing protein, chloroplastic	Q9M9E2	*Arabidopsis thaliana*	8.11	97,696	42	2	DD	A	ABD
65	Pentatrico peptide repeat-containing protein, mitochondrial	Q9LEX5	*Arabidopsis thaliana*	7.89	47,368	51	9	DD	A	ABD
54	Protein synthesis inhibitor II	P04399	*Hordeum vulgare*	9.56	29,863	30	7	PS	A	ABD
32	Trypsin inhibitor CMe precursor (Alpha-amylase/trypsinin)	gi|85682780	*Triticum aestivum*	7.5	16,695	56	7	PS	A	ABD
89	Trypsin/alpha-amylase inhibitor	IACX2_WHEA	*Triticum aestivum*	9.08	13,880	28	6	STD	A	ABD
17	Unknown protein	gi|194704430	*Zea mays*	9.78	19,926	45	4	UC	A	ABD
33	Unnamed protein product	gi|21705	*Triticum aestivum*	5.31	16,399	42	7	UC	A	ABD
6	ATP/ADP carrier protein	gi|944842	*Triticum turgidum*	9.8	35,899	36	8	EM	A	AD
9	ATP/ADP carrier protein	gi|944842	*Triticum turgidum*	9.8	35,899	64	7	EM	A	AD
10	ATP/ADP carrier protein	gi|944842	*Triticum turgidum*	9.8	35,899	65	2	EM	A	AD
13	ATP/ADP carrier protein	gi|944842	*Triticum turgidum*	9.8	35,899	45	7	EM	A	AD
90	ATP/ADP carrier protein	gi|944842	*Triticum turgidum*	9.8	35,899	45	7	EM	A	AD
31	Endogenous alpha-amylase	gi|123975	*Triticum aestivum*	6.77	19,849	36	3	DD	A	AD
26	High molecular weight glutenin subunit	gi|257334	*Triticum aestivum*	5.48	2,877	23	4	ST	A	AD
55	Methionine *S*-methyltransferase	Q9SWR3	*Wollastonia biflora*	5.54	121,564	34	9	TP	A	AD
56	NADPH–cytochrome P450 reductase	Q05001	*Catharanthus roseus*	5.18	78,958	26	7	EM	A	AD
44	Small GTP-binding protein	gi|7548844	*Triticum aestivum*	6.9	22,947	28	3	TP	A	AD
46	STK protein kinase	gi|391929	*Triticum aestivum*	6.47	19,050	62	2	PS	A	AD
25	Transcription factor Myb1	gi|47680445	*Triticum aestivum*	9.05	29,793	21	7	TC	A	AD
32	Alpha-amylase inhibitor 0.53	P01084	*Triticum aestivum*	5.23	13,185	32	8	DD	B	AB
33	Alpha-amylase inhibitor WDAI-3	P10846	*Triticum aestivum*	7.57	4,797	65	6	DD	B	AB
53	alpha-amylase/subtilisin inhibitor	P16347	*Triticum aestivum*	6.77	19,633	42	5	DD	B	AB
2	B1-hordein precursor	gi|23458	*Triticum aestivum*	8.86	6,192	32	2	ST	B	AB
1	Glyceraldehyde-3-phosphate dehydrogenase	gi|148508784	*Triticum aestivum*	7.09	36,854	35	6	EM	B	AB
14	Hypothetical protein	gi|160701982	*Phaeosphaeria nodorum*	6.18	44,314	31	7	UC	B	AB
16	Hypothetical protein At4g30340	Q67ZR3_ARATH	*Arabidopsis thaliana*	6.56	7,296	60	8	UC	B	AB
8	Hypothetical protein SNOG	gi|111063023	*Phaeosphaeria nodorum*	4.96	46,645	14	3	UC	B	AB
11	Hypothetical protein SNOG	gi|111063023	*Phaeosphaeria nodorum*	4.96	46,645	45	4	UC	B	AB
26	Hypothetical protein.	Q5BPP7_ARATH	*Arabidopsis thaliana*	6.55	24,879	10	7	UC	B	AB

Table 1 (continued)

SN	Putative protein name	Acc. no.	Protein source	p*I*	MW	SC	PM	FC	Genome	CCG
12	Low molecular weight glutenin protein	gi|50404489	*A. tauschii × T. turgidum*	8.92	34,900	31	5	ST	B	AB
7	Low molecular weight protein	gi|50404489	*A. tauschii × T. turgidum*	8.92	34,900	32	6	ST	B	AB
23	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit.	Q9FRZ2_WHEAT	*Triticum aestivum*	8.81	19,449	34	3	EM	B	AB
52	Signal recognition particle 54 kDa protein 2	P49966	*Arabidopsis thaliana*	9.15	55,078	32	4	STD	B	AB
44	Chitinase IV precursor	Q2L3V7_WHEAT	*Triticum aestivum*	4.69	29,007	57	4	DD	B	ABD
18	NBS-LRR type RGA	Q3S9N3_WHEAT	*Triticum aestivum*	6.75	9,105	25	4	DD	B	ABD
43	Pentatrico peptide repeat-containing protein At5g15280	Q9LXF4	*Arabidopsis thaliana*	7.64	139,608	27	9	DD	B	ABD
38	Probable protein phosphatase 2C 68	Q0J2L7	*Oryza sativa*	6.16	37,704	32	6	PS	B	ABD
55	Probable protein phosphatase 2C 75	Q9FLI3	*Arabidopsis thaliana*	5.45	46,042	35	6	CO	B	ABD
40	Trypsin/alpha-amylase inhibitor CMX2	Q43691	*Triticum aestivum*	9.08	13,889	54	2	STD	B	ABD
9	Unnamed protein product	gi|18910	*Hordeum vulgare*	8.86	33,857	61	2	UC	B	ABD
13	Unnamed protein product	gi|21795	*Triticum aestivum*	11.48	11,402	25	6	UC	B	ABD
37	4-coumarate-CoA ligase-like 7	Q9M0X9	*Arabidopsis thaliana*	8.59	59,849	42	5	DD	B	B
50	50S ribosomal protein L23, chloroplastic	Q5SD14	*Huperzia lucidula*	10.04	10,734	62	7	PS	B	B
41	Acidic endochitinase P	P17513	*Nicotiana tabacum*	4.89	27,469	43	6	DD	B	B
51	Adenosyl homo-cysteinase	P32112	*Triticum aestivum*	5.65	53,436	18	7	EM	B	B
42	Aldehyde oxidase 1	Q7G193	*Arabidopsis thaliana*	6.02	149,553	28	4	DD	B	B
21	Alpha-2-thionin	VSWTA2	*Triticum aestivum*	8.17	49,204	32	9	DD	B	B
31	Armadillo repeat protein 1	gi|186695201	*Triticum aestivum*	6.09	33,634	16	4	UC	B	B
10	Beta amylase	gi|32400764	*Triticum aestivum*	8.6	31,100	32	3	EM	B	B
47	Chalcone synthase	Q9SEP4	*Arabis alpina*	6.43	42,658	42	7	PS	B	B
36	DEAD-box ATP-dependent RNA helicase 42	Q8H0U8	*Arabidopsis thaliana*	5.69	133,032	18	4	UC	B	B
15	Disrupted meiotic cDNA 1	Q281P7_AEGBI	*Aegilops bicornis*	8.05	1,530	26	6	CO	B	B
44	Ent-isokaur-15-ene synthase	A4KAG8	*Oryza sativa*	5.52	92,407	35	3	DD	B	B
24	Epstein–Barr virus EBNA-1-like protein	Q6K227_ORYSA	*Oryza sativa*	5.16	9,932	32	2	DD	B	B
17	Flavonoid *O*-methyltransferase	Q38J50_WHEAT	*Triticum aestivum*	5.7	38,545	12	3	PS	B	B
22	G protein, alpha subunit	Q18LC3_TRITU	*Triticum turgidum*	6.88	9,929	23	3	STD	B	B
4	Gamma-hordein-3	gi|708280	*Hordeum vulgare*	6.7	33,168	18	4	ST	B	B
6	Glceraldehyde-3-phosphate dehydrogenase	gi|148508784	*Triticum aestivum*	7.09	36,854	64	6	EM	B	B
30	GTP binding	gi|15219943	*Arabidopsis thaliana*	5.28	22,930	60	5	ST	B	B
49	Peroxidase 1	P37834	*Oryza sativa*	7.03	35,308	72	5	DD	B	B
48	Poly (ADP-ribose) polymerase 3	Q9FK91	*Arabidopsis thaliana*	5.14	91,533	34	2	PS	B	B

Table 1 (continued)

SN	Putative protein name	Acc. no.	Protein source	p*I*	MW	SC	PM	FC	Genome	CCG
54	Protein argonaute 3	Q7XTS3	*Oryza sativa*	9.2	122,751	41	4	PS	B	B
34	Puroindoline-B	Q10464	*Triticum aestivum*	9.06	16,792	42	4	ST	B	B
29	Putative acyl transferase 4	gi|151175357	*Triticum aestivum*	5.72	46,392	29	7	PS	B	B
28	Putative ADP-ribosylation factor	Q70AI8_WHEAT	*Triticum aestivum*	6.07	8,173	35	5	EM	B	B
27	Putative low temperature and salt responsive protein	Q8H1Z1_WHEAT	*Triticum aestivum*	5.58	7,804	28	2	DD	B	B
39	Pyruvate decarboxylase isozyme 3	Q0D3D2	*Oryza sativa*	5.62	62,642	16	7	PS	B	B
45	Reticulon-like protein B18	Q8LDS3	*Arabidopsis thaliana*	9.8	51,422	62	4	UC	B	B
20	Thionin precursor, leaf	S07648	*Hordeum vulgare*	7.36	14,701	56	3	DD	B	B
35	Uncharacterized GPI-anchored protein	Q84VZ5	*Arabidopsis thaliana*	6.57	21,301	32	6	UC	B	B
46	Cytochrome c biogenesis protein ccsA	P48269	*C. reinhardtii*	6.95	39,983	15	5	EM	B	BD
19	Cytochrome P450 monooxygenase	Q2MIZ1_MEDTR	*Medicago truncatula*	8.17	49,204	44	9	EM	B	BD
32	Alpha-amylase/trypsin inhibitor	IAAC2_WHEAT	*Triticum aestivum*	6.86	15,449	71	9	DD	D	ABD
34	Alpha-amylase/trypsin inhibitor	IAAC2_WHEAT	*Triticum aestivum*	6.86	15,449	26	4	DD	D	ABD
44	Chitinase IV precursor	gi|4741848	*Triticum aestivum*	4.69	29,007	37	2	DD	D	ABD
41	NBS-LRR type RGA (fragment)	Q3S9P1_WHEAT	*Triticum aestivum*	8.97	20,128	16	4	DD	D	ABD
55	Pentatrico peptide protein At3g53360	Q9LFI1	*A. thaliana*	6.3	85,674	62	5	DD	D	ABD
52	Pentatricopeptide protein At1g66345	Q3ECH5	*A. thaliana*	7.82	62,332	25	2	DD	D	ABD
11	Protein phosphatase 2C	gi|151384864	*Triticum aestivum*	5.32	30,911	18	4	PS	D	ABD
30	Trypsin/alpha-amylase inhibitor	IACX2_WHEAT	*Triticum aestivum*	9.08	13,880	60	8	STD	D	ABD
5	Unknown	gi|195655553	*Zea mays*	8.23	9,355	25	4	UC	D	ABD
8	Unnamed protein product	gi|157349358	*Zea mays*	6.15	57,336	23	3	UC	D	ABD
13	Vernalization insensitive 3	gi|207175055	*Triticum aestivum*	6.45	78,020	14	6	DD	D	ABD
27	ATP synthase subunit alpha	ATPA_WHEAT	*Triticum aestivum*	6.11	55,261	26	4	EM	D	AD
35	Endogenous alpha-amylase/subtilisin inhibitor	IAAS_WHEAT	*Triticum aestivum*	6.77	19,621	25	3	DD	D	AD
48	Glutenin, high molecular weight subunit	P10388	*Triticum aestivum*	6.21	89,315	35	4	ST	D	AD
2	High molecular weight glutenin subunit	gi|32328665	*Triticum aestivum*	8.34	14,944	31	8	ST	D	AD
33	Metallothionein-like protein 1	MT1_WHEAT	*Triticum aestivum*	4.44	7,371	30	2	DD	D	AD
16	NADH-ubiquinone oxidoreductase	NU1M_WHEAT	*Triticum aestivum*	8.69	35,908	23	5	EM	D	AD

Table 1 (continued)

SN	Putative protein name	Acc. no.	Protein source	p*I*	MW	SC	PM	FC	Genome	CCG
14	Small GTP-binding protein	gi|57547575	*Triticum aestivum*	6.9	22,947	20	4	STD	D	AD
40	STK protein kinase	gi|71912119	*Triticum aestivum*	6.47	19,050	35	7	PS	D	AD
22	Transcription factor HBP-1b(c1)	HBP1C_WHEAT	*Triticum aestivum*	6.87	51,755	14	7	TC	D	AD
1	y-Type high molecular weight glutenin subunit	gi|24474926	*Aegilops ventricosa*	8.85	19,908	10	3	ST	D	AD
9	Cytochrome P450	gi|26655531	*Triticum aestivum*	6.82	59,212	18	8	EM	D	BD
15	1-Cys peroxiredoxin PER1	REHY_WHEAT	*Triticum aestivum*	6.08	23,950	37	2	DD	D	D
43	23.1 kDa heat shock protein	gi|147225064	*T. monococcum*	5.04	23,070	55	6	DD	D	D
42	23.9 kDa heat shock protein	gi|147225060	*Triticum turgidum*	5.06	23,852	55	4	DD	D	D
31	60S ribosomal protein L35	RL35_WHEAT	*Triticum aestivum*	11.26	14,356	56	9	PS	D	D
51	ABC transporter B family member 2	Q8LPK2	*A. thaliana*	8.97	135,209	32	9	TP	D	D
26	Adenylosuccinate synthase	T06792	*Triticum aestivum*	5.93	50,886	37	5	PS	D	D
25	Adenylosuccinate synthetase	PURA_WHEAT	*Triticum aestivum*	5.93	50,886	12	4	PS	D	D
29	ADP-ribosylation factor	Q5XUV1_WHEAT	*Triticum aestivum*	6.43	20,597	63	9	EM	D	D
12	Auxin-responsive Aux/IAA family	gi|40253392	*Triticum urartu*	5.4	15,916	13	4	CO	D	D
28	Beta-expansin 1	gi|42601003	*Triticum aestivum*	8.98	11,254	73	7	CO	D	D
50	Deoxyhypusine synthase	Q9AXQ9	*Musa acuminata*	5.49	41,585	41	3	PS	D	D
10	Dimeric alpha-amylase inhibitor	gi|65993756	*Triticum aestivum*	6.54	15,250	10	7	DD	D	D
53	Dynamin-related protein 3B	Q8LFT2	*A. thaliana*	5.95	86,643	28	3	CO	D	D
23	Expansin EXPB2	gi|44894798	*Triticum aestivum*	6.93	30,672	25	5	CO	D	D
21	Gamma gliadin	gi|133741924	*Triticum aestivum*	8.88	16,195	57	9	ST	D	D
54	Glutathione synthetase, chloroplastic	O23732	*Brassica juncea*	6.59	59,740	42	4	EM	D	D
39	Glutathione transferase	gi|20067419	*Triticum aestivum*	6.35	24,996	22	5	PS	D	D
4	Grain softness protein	gi|215231212	*Triticum timopheevii*	8.54	19,652	10	3	ST	D	D
47	Grain softness protein	gi|1821214	*Triticum aestivum*	8.42	19,335	34	6	ST	D	D
7	Lt1.1 protein	gi|12054814	*Triticum aestivum*	6.34	5,575	21	5	DD	D	D
37	Putative 1,4-beta-xylanase	gi|40644790	*Triticum aestivum*	12.18	19,735	15	5	EM	D	D
49	Putative F-box/kelch-repeat protein	Q9CAE9	*A. thaliana*	5.16	42,323	62	9	UC	D	D
45	rbcL gene product (30 AA)	gi|14017612	*Triticum aestivum*	4.66	3,424	25	7	PS	D	D
3	Ribosomal protein L3-B2	gi|118152402	*Triticum aestivum*	10.07	44,562	14	5	PS	D	D
46	Small heat shock protein	HS21C_WHEAT	*Triticum aestivum*	9.64	26,579	50	3	DD	D	D
6	Small heat shock protein, chloroplastic	P11890	*C. rubrum*	5.89	23,285	34	3	DD	D	D

Table 1 (continued)

SN	Putative protein name	Acc. no.	Protein source	p*I*	MW	SC	PM	FC	Genome	CCG
24	Small heat shock protein, chloroplastic	HS21C_WHE	*Triticum aestivum*	9.64	26,579	21	6	DD	D	D
19	WRKY18 transcription factor	gi|189172039	*Triticum aestivum*	8.97	30,360	12	3	TC	D	D
38	WRKY45 transcription factor	gi|126508732	*Triticum aestivum*	7.14	30,856	23	7	TC	D	D
17	ZCCT1	gi|45390711	*T. monococcum*	7.74	1,656	17	2	DD	D	D
18	ZCCT1-TD	gi|45390745	*Triticum turgidum*	10.02	1,656	14	5	DD	D	D
20	ZCCT2	gi|45390727	*T. monococcum*	5.92	1,635	24	7	DD	D	D

2D-PAGE and MALDI-TOF mass spectrometry were utilized, followed by coupling to MASCOT databases

Criteria: *SN* spot number, *Acc. no.* accession number, *pI* isoelectric point, *MW* molecular weight (Da), *SC* sequence coverage (%), *PM* peptide match, *FC* functional category, *CO* Cellular organization, *DD* disease/defense, *EM* energy metabolism, *PS* protein synthesis, *ST* storage protein, *STD*, signal transduction, *TC* transcription, *TP* transport facilitation, *UC* unclear classification, *CCG* cross-correlation among genome

ions, or electrons across cell membranes, within the cell, or in a tissue fluid, whereas adenine nucleotides are transferred across the inner mitochondrial membrane (Iacobazzi and Palmieri 1995). Ribulose bisphosphate carboxylase small chain 1, which acts chloroplastically as RUBISCO, catalyzes two reactions: the carboxylation of D-ribulose 1,5-bisphosphate (the primary event in carbon dioxide fixation) and the oxidative fragmentation of the pentose substrate. Both reactions occur simultaneously and in competition at the same active site during photorespiration and photosynthesis (Berry-Lowe et al. 1982).

As disease- or defense-related proteins, 18.89% were identified in genome A, 28.30% in B, and 37.0% in D. One example is an NBS-LRR type RGA protein, which is triggered in response to injury or in the presence of a foreign body and which minimizes damage to the organism or aids in the prevention or recovery from infection that results from such attack (Bozkurt et al. 2007). In addition, an ABA-inducible protein is involved in water or osmotic stress signaling that results from a stimulus and leads to an increase or decrease in the concentration of solutes outside the plant cell (Anderberg and Walker-Simmons 1992). Another 15.56% of proteins were identified in genome A, 15.09% in B, and 16.67% in D as functioning in protein folding/stability and flavonoid synthesis. They include the 30S ribosomal protein S15 in chloroplasts, as the passive partner for protein synthesis as well as occasional translation within structures. Ribosomes coordinate that synthesis by placing mRNA, aminoacyl-tRNA, and some associated protein factors (Ogihara et al. 2000).

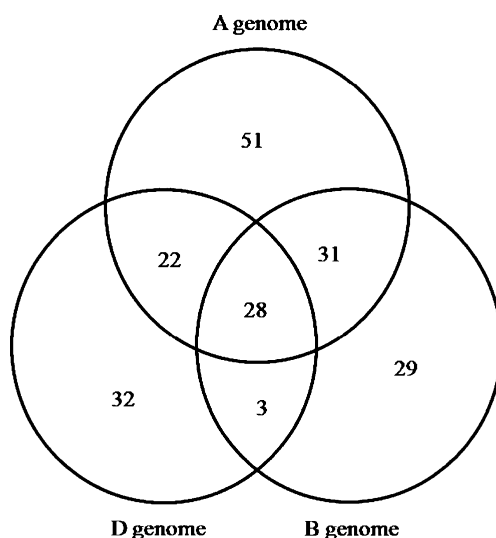

Fig. 2 Cross-correlation of different proteome analyses for wild relatives of wheat (A, B, and D genomes)

As storage proteins, 14.44% were located in genome A, 11.32% in B, and 11.11% in D. For example, gluten (glutenin and gliadin) is a major determinant of baking performance and viscoelasticity, both of which are responsible for high-quality bread that has good leavening and a light, porous crumb. Glutenin contains two kinds of isoforms—high molecular weight and low molecular weight subunits (Kamal et al. 2009b). Puroindoline is another storage protein that may act as a membranotoxin, probably through its antibacterial and antifungal activities. It contributes to the defense mechanism of plants against predators, gram-positive bacteria *Staphylococcus aureus* and *Clavibacter michiganensis*, and gram-negative *Escherichia coli*, *Pseudomonas syringae* pv phaseoli, *Agrobacterium tumefaciens*, and *Erwinia carotovora*. It also has synergistic activity with PINB against bacteria and helps determine grain texture and hardness (Day et al. 2006; Kamal et al. 2009c).

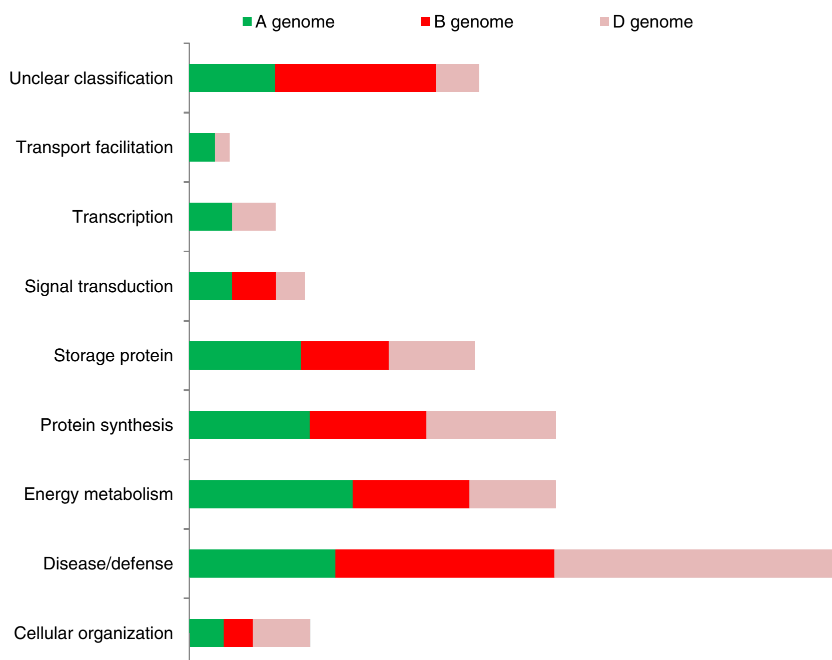

Fig. 3 Functional categories of all identified proteins according to biological process. Each combination of distinct proteins (Table 1) was manually classified via UniProt database. After assessment, percentage of proteins within each class was determined for A, B, and D genomes

Signal transducer proteins accounted for 5.56% of the total in genome A, 5.66% in B, and 3.70% in D. These include the signal recognition 54-kDa protein 1, an α-G protein, and the trypsin/α-amylase inhibitor CMX2. Signal recognition proteins are targeted to a membrane during translation and depend upon two key components—the signal recognition particle (SRP) and the SRP receptor. SRP is a cytosolic particle that transiently binds to the endoplasmic reticulum (ER) signal sequence (the large ribosomal unit) in a nascent protein as well as to the SRP receptor in the ER membrane (www.uniprot.org). G protein is a Cu(2+) ion signal transducer protein that increases in phosphatidic acid and phosphatidyl butanol similar to the behavior detected with that metal (Navari-Izzo et al. 2006).

Proteins are also involved in the transfer of genetic information from DNA to mRNA by DNA-directed RNA polymerase and from RNA to mRNA by RNA-directed RNA polymerase. (www.uniprot.org) Here, transcription-related proteins accounted for 5.56% of the total in both genomes A and D. These include transcription factors WRK 18, WRK 45, HBP-1b, and Myb 1 (www.uniprot.org).

For cellular organization, protein distributions were 4.44% in genome A, 3.77% in B, and 7.41% in D. These function in cell cycles and division and in general plant growth. For example, actins are highly conserved proteins that are involved in various types of cell motility. They are ubiquitously expressed in all eukaryotic cells and are essential components of the cell cytoskeleton, where they are important for cytoplasmic streaming, cell shape determination, cell division, organelle movement, and extension growth. Actins are strongly expressed in nearly all vegetative tissues and remain at high levels even in older tissues. However, little or no expression is detected in mature pollen sacs, ovules, embryos, or seeds (Yamada et al. 2003).

The facilitation of transport involved 3.33% of the proteins in genome A and 1.84% in D. Examples are methionine *S*-methyltransferase, which catalyzes biosynthesis of *S*-methylmethionine (SMM) from adenosyl-L-homocysteine (AdoMet) and methionine. SMM production (by MMT1) and degradation (by HMT-1, HMT-2, and HMT-3) constitute the SMM cycle, which is probably required for achieving short-term control of the AdoMet level in plants. MMT is also able to catalyze selenium-methylmethionine (SeMM) from AdoMet and selenium-methionine (SeMet), possibly playing a role in phloem sulfur transport (Kocsis et al. 2003).

No proteins for transcription or transporter facilitation were identified in the B genome, and the remainder could not be classified by any UniProt protein databases (i.e., 11.11% in genome A, 20.75% in B, and 5.56% in D).

In conclusion, we have now developed a proteome reference map, the data from which will be a useful tool in future studies among the A, B, and D genomes for wild relatives of wheat. These protein profiles will be constructive for comparisons with those generated during other studies of those relatives, such that researchers will be able to determine quickly how proteins of interest are affected by genetics and environment. This information can then lead to the identification of biological markers for resistance to disease and insects; tolerance to cold, heat, and drought; and improvements in crop quality traits.

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References

Anderberg RJ, Walker-Simmons MK (1992) Isolation of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinases. Proc Natl Acad Sci U S A 89:10183–10187. doi:dx.doi.org

Andon NL, Hollingworth S, Koller A, Greenland AJ, Yates JR III, Haynes PA (2002) Proteomic characterization of wheat amyloplasts using identification of proteins by tandem mass spectrometry. Proteomics 2:1156–1168

Appels R, Lagudah ES (1990) Manipulation of chromosomal segments from wild wheat for the improvement of bread wheat. Aust J Plant Physiol 17:253–266

Berry-Lowe SL, McKnight TD, Shah DM, Meagher RB (1982) The nucleotide sequence, expression, and evolution of one member of a multigene family encoding the small subunit of ribulose-1,5-bisphosphate carboxylase in soybean. J Mol Appl Genet 1:483–498

Bliffeld M, Mundy J, Potrykus I, Futterer J (1999) Genetic engineering of wheat for increased resistance to powdery mildew disease. Theor Appl Genet 98:1079–1086

Bozkurt O, Hakki EE, Akkaya MS (2007) Isolation and sequence analysis of wheat NBS-LRR type disease resistance gene analogs using degenerate PCR primers. Biochem Genet 45:469–486. doi:dx.doi.org

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Brett D, Pospisil H, Valcárcel J, Reich J, Bork P (2002) Alternative splicing and genome complexity. Nat Genet 30:29–30

Crouch JH, Payne TS, Dreisigacker S, Wu H, Braun HJ (2009) Improved discovery and utilization of new traits for breeding. In: Dixon J, Braun H-J, Kosina P, Crouch J (eds) Wheat facts and futures 2009. CIMMYT, Mexico, pp 42–51

Damerval C, de Vienne D, Zivy M, Thiellement H (1986) The technical improvements in two-dimensional electrophoresis increase the level of genetic variation detected in wheat-seedling proteins. Electrophoresis 7:52–54

Day L, Bhandari DG, Greenwell P, Leonard SA, Schofield JD (2006) Characterization of wheat puroindoline proteins. FEBS J 273:5358–5373. doi:dx.doi.org

Donnelly BE, Madden RD, Ayoubi P, Porter DR, Dillwith JW (2005) The wheat (*Triticum aestivum* L.) leaf proteome. Proteomics 5:1624–1633

Dupont FM (2008) Metabolic pathways of the wheat (*Triticum aestivum*) endosperm amyloplast revealed by proteomics. BMC Plant Biol. doi:10.1186/1471-2229-8-39

Farooq S, Shah TM, Asghar M (1996) Interspecific hybridization for wheat improvement-VIII. Production and cytogenetic analysis of F1 hybrids of wheat with *Aegilops ovata*. Cer Res Commun 24:60–67

Holmes-Davis R, Tanaka CK, Vensel WH, Hurkman WJ, McCormick S (2005) Proteome mapping of mature pollen of *Arabidopsis thaliana*. Proteomics 5:4864–4884

Huxley A (1992) The new RHS dictionary of gardening. MacMillan, New York. ISBN 0-333-47494-5

Iacobazzi V, Palmieri F (1995) Nucleotide sequence of a cDNA encoding the ADP/ATP carrier from wheat (*Triticum turgidum*). Plant Physiol 107:1473–1473. doi:dx.doi.org

Islam N, Tsujimoto H, Hirano H (2003a) Wheat proteomics: relationship between fine chromosome deletion and protein expression. Proteomics 3:307–316

Islam N, Tsujimoto H, Hirano H (2003b) Proteome analysis of diploid, tetraploid and hexaploid wheat: towards understanding genome interaction in protein expression. Proteomics 3:549–557

Jensen ON (2004) Modification-specific proteomics: characterization of post-translational modifications by mass spectrometry. Curr Opin Chem Biol 8:33–41

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

Kamal AHM, Kim KH, Shin KH, Seo HS, Tsujimoto H, Heo HY, Choi JS, Park CS, Woo SH (2009b) Diversity of novel glutenin subunits in bread wheat (*Triticum aestivum* L.). J Plant Biol 52:533–542

Kamal AHM, Park CS, Heo HY, Chung KY, Cho YG, Kim HS, Song BH, Lee CW, Woo SH (2009c) Proteomics approach on puroindoline gene of pre-harvest sprouting wheat. Kor J Breed Sci 41:205–212

Kerim T, Imin N, Weinman JJ, Rolfe BG (2003) Proteome analysis of male gametophyte development in rice anthers. Proteomics 3:738–751

Kocsis MG, Ranocha P, Gage DA, Simon ES, Rhodes D, Peel GJ, Mellema S, Saito K, Awazuhara M, Li C, Meeley RB, Tarczynski MC, Wagner C, Hanson AD (2003) Insertional inactivation of the methionine *s*-methyltransferase gene eliminates the *s*-methylmethionine cycle and increases the methylation ratio. Plant Physiol 131:1808–1815. doi:dx.doi.org

Ling HQ, Qiu J, Singh RP, Keller B (2004) Identification and genetic characterization of an *Aegilops tauschii* ortholog of the wheat leaf rust disease resistance gene Lr1. Theor Appl Genet 109:1133–1138

Majoul T, Bancel E, Triboi E, Hamida J, Branlard G (2003) Proteomic analysis of the effect of heat stress on hexaploid wheat grain: characterization of heat-responsive proteins from total endosperm. Proteomics 3:175–183

Navari-Izzo F, Cestone B, Cavallini A, Natali L, Giordani T, Quartacci MF (2006) Copper excess triggers phospholipase D activity in wheat roots. Phytochemistry 67:1232–1242. doi:dx.doi.org

Noir S, Bräutigam A, Colby T, Schmidt J, Panstruga R (2005) A reference map of the *Arabidopsis thaliana* mature pollen proteome. Biochem Biophys Res Commun 337:1257–1266

Ogihara Y, Isono K, Kojima T, Endo A, Hanaoka M, Shiina T, Terachi T, Utsugi S, Murata M, Mori N, Takumi S, Ikeo K, Gojobor T, Murai R, Murai K, Matsuoka Y, Ohnishi Y, Tajiri H, Tsunewaki K (2000) Chinese spring wheat (*Triticum aestivum* L.) chloroplast genome: complete sequence and contig clones. Plant Mol Biol Report 18:243–253

Sofalian O, Valizadeh M (2009) Investigation of seed storage proteins in some wild wheat progenitors using SDS-PAGE and acid-PAGE. Not Bot Hort Agrobot Cluj-Napoca 37:179–182

USDA (2010) USDA agricultural projections to 2019. Office of the Chief Economist, World Agricultural Outlook Board, US, Department of Agriculture, prepared by the Interagency Agricultural Projections Committee, Long-term Projections Report OCE-2010-1, 100 pp.

van Wijk KJ (2001) Challenges and prospects of plant proteomics. Plant Physiol 126:501–508

Yamada K, Lim J, Dale JM, Chen H, Shinn P, Palm CJ et al (2003) Empirical analysis of transcriptional activity in the *Arabidopsis* genome. Science 302:842–846. doi:dx.doi.org

Yan Y, Hsam SLK, Yu J, Jiang Y, Zeller FJ (2003) Allelic variation of the HMW glutenin subunits in *Aegilops tauschii* accessions detected by sodium dodecyl sulphate (SDS-PAGE), acid polyacrylamide gel (A-PAGE) and capillary electrophoresis. Euphytica 130:377–385