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RESEARCH ARTICLE

Comparative proteomic analysis of cold responsive proteins in two wheat cultivars with different tolerance to spring radiation frost

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Abstract Spring radiation frost (SRF) is a severe environmental stress which impairs wheat yield and productivity worldwide. To better understand the mechanism of wheat (*Triticum aestivum*) responding to SRF, a comparative proteomic analysis was performed to analyze the changes of the key proteins in two wheat cultivars Jimai22 and Luyuan301 with high and low tolerance to SRF respectively. A total of 43 differentially expressed proteins (DEPs) which mainly involved in carbohydrate metabolism, amino acid metabolism, resistance proteins and antioxidant enzymes, photosynthesis and cellular respiration proteins, cell-wall related proteins, protein translation/processing/degradation and signal transduction were isolated and identified by two-dimensional electrophoresis and MALDI-TOF-TOF MS. The results revealed that of the 21 DEPs in Jimai22 responding to the SRF, 13 DEPs were upregulated and 8 DEPs were downregulated, and that of the 22 DEPs in Luyuan301, 9 DEPs were upregulated and 13 DEPs were downregulated. These DEPs might be responsible for the stronger cold resistance of Jimai22 compared to Luyuan301. The expression pattern and function analysis of these DEPs were very significant to understanding the mechanism of the SRF responses in wheat.

Keywords common wheat, spring radiation frost, proteomic analysis, 2-DE, MALDI-TOF-TOF MS

1 Introduction

Wheat is one of the most important food crops in the world. Low-temperature injury during winter and spring can be particularly destructive on wheat production. The winter hardiness of modern cultivars and good management practices have reduced winterkilling of wheat, so injury during winter is not a big problem nowadays. While, with the frequent occurrence of climatic anomalies, SRF has become a significant limiting factor on wheat production. SRF injury frequently occurs when low temperatures coincide with sensitive plant growth stages [1–3], from pistil and stamen differentiation stage to anther differentiation stage [4–7]. The research into cold tolerance at the vegetative stages of wheat development has revealed that genetic variation does exist which was controlled by few genetic loci with major effects, such as *VRN, Fr* and *CBF* transcription factors [8–12]. To date, there have been few studies on the underlying mechanisms involved in the SRF response at the reproductive stages of common wheat [13–15].

Transcriptome profiling has contributed markedly to our understanding of cold stress in species including *Arabidopsis* and rice. However, it has some limitations because mRNA levels are not always correlated to those of corresponding proteins, due in part to post-transcriptional regulation. Recently, proteomic analysis has become a preferred strategy to reveal the dynamics of expression under cold stress.

Protein synthesis and accumulation in developing wheat grains has been extensively investigated [16–18], with most studies focusing on plant proteomic analysis under abiotic stress conditions [19,20]. Also comparative proteomic analysis of wheat seedling roots, leaves, and grains, as well as embryos, between cultivars differing in tolerance to drought and salt has been reported [21–23]. Some work has been conducted to uncover proteomic changes in wheat under cold stress. Danyluk et al. [24]

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found that 18 proteins were transiently induced in the three wheat cultivars after cold treatment. There are 53 kinds of proteins sustaining high levels of expression in the induction of freezing tolerance required for four weeks, in which 34 kinds of proteins of the best freezing tolerance cultivars also had a higher level of expression. Sara et al. [25] analyzed the wheat plants grown at 20 or 4°C until entering the reproductive stage and a cross-comparison on the leaf proteomes experiments, which found a series of proteins related to cold tolerance. However, most of the above mentioned studies were focused on the cold responding at the vegetative stage.

Our previous studies revealed that Jimai22 has almost the same development process and frost resistance with another wheat cultivar Luyuan301 at the vegetative stage, but in the event of SRF, Jimai22, at pistil and stamen differentiation stage, was less affected, while Luyuan301 was injured to bald seriously. In the present study, we performed comparative proteome analyses using two-dimensional electrophoresis (2-DE) and MALDI-TOF MS. The objectives were to determine the changes of protein expression in metabolic pathways of *wheat (T. aestivum)* after cold treatment at the reproductive stage and improve our understanding about the effects of SRF on plant growth and metabolism, which will provide valuable information for breeding commercial cultivars with SRF tolerance.

2 Materials and methods

2.1 Plant materials and stress treatment

Experiments were carried out on two wheat cultivars, Jimai22 and Luyuan301. Seeds were germinated and grown at 2°C for vernalization. After 40 days, the seedlings were transplanted to greenhouse and grown under 22/16°C day/night cycle. Forty days after transplanting, most of the young spikes had developed to pistil and stamen differentiation stage, and then the seedlings were divided into two groups. One was used as the control sample at 22/16°C in green house, while the other was moved to a chilling chamber for frost treatment at 0°C for 48 h. The young spikes and leaves were harvested and mixed together, stored in liquid nitrogen for protein extraction.

2.2 Physiological analysis

The fresh weights (FW) of second leaves were measured immediately after harvesting. The leaves were floated on deionized water for 24 h at 4°C under low irradiance. The turgid leaves were then quickly weighed (TW) and dry weights were determined after oven-drying at 70°C for 48 h. The relative water content (RWC) was calculated as: RWC/% = (FW-DW)/(TW-DW) × 100 [26].

Total soluble sugars of wheat leaves (about 100 mg) were extracted in boiling water for 30 min and determined by anthrone reagent using glucose as standard. The absorbance was read at 630 nm and the sugar concentration was determined from a glucose standard curve [27].

2.3 Protein extraction and quantification

Soluble proteins from leaf tissues were homogenized separately to a fine powder in liquid nitrogen, and were transferred into 50 mL tubes. The pellets were added to 25 mL tricholoroacetic acid/acetone (1:9), 65 mmol·L^{-1} DTT and incubated at −20°C for 1 h and centrifuged for 45 min at 12000 r·min^{-1}. The supernatant was removed, 25 mL pure acetone was added and incubated at −20°C overnight. This step was repeated three times and the pellets were lyophilized. Powdered pellet (200 mg) was dissolved in 0.5 mL 2D protein extraction buffer and ultrasonic for 3 min in an ice bath. After centrifugation for 45 min at 12000 r·min^{-1}, the supernatant was filtered (0.22 μm). Finally, the protein concentration was determined with a 2-D Quant Kit (Sangon, Shanghai, China) using BSA (2 mg ·mL^{-1}) as the standard following the manufacturer's instructions. The protein samples were kept at −80°C until used.

2.4 2-DE and image analysis

Protein samples of 80 μg were loaded onto an Immobiline DryStrip (GE, pH 4–7) and were then placed on an IPG strip to perform IEF. Isoelectric focusing was conducted in five steps: 30 V for 12 h; 500 V for 1 h; 1000 V for 1 h; 8000 V for 8 h; and 500 V for 4 h. The focused strips were then treated twice in equilibration solution for 15 min. For the second dimension, the proteins were separated on 12.5% SDS-polyacrylamide gels. After SDS-PAGE, gels were stained with silver staining. The images of the stained gels were acquired using a GS-800 calibrated densitometer (Bio-Rad) and analyzed using PDQuest software, version 7.2.0 (Bio-Rad). After background subtraction and spot detection, spots were matched and normalized using the method of total density in the gel image. Student's *t*-tests ($n = 3$, $P < 0.05$) were performed to determine if the proteins were differentially accumulated.

2.5 In-gel digestion and MS analysis

Protein spots showing at least a 2-fold difference ($P < 0.05$) in abundance between control and treatment were excised manually into 1.5 mL microtubes. Gel pieces were first washed with 200 μL of bleaching solution (100 mmol·L^{-1} NH_4HCO_3/30% acetonitrile), repeated 2–3 times until colorless. The dry particles were digested with 5 μL of 10 ng·μL^{-1} trypsin enzyme (Promega, USA), and incubated at 37°C for 20 h.

Tryptic peptides were analyzed with a 4800PlusMALDI TOF/TOFTM Analyzer (Applied Biosystems, USA). The spots were analyzed with parameters of laser intensity 5200, acceleration voltage 8 kV, grid voltage 91.25% and delay time 250 ns, UV nitrogen laser 355 nm, and 40–50 times of single scan of MS signal accumulation. The MS/MS ion search was performed using MASCOT (http://www.matrixscience.com) searching the NCBI (National Center for Biotechnology Information) non-redunant database. The parameters for searching were: monoisotoic peptide masses, ±0.2 Da peptide mass tolerance; one missed cleavage site, enzyme of trypsin, modifications allowed for oxidation of methionine and carboxy-amidomethylation of cysteine. Only significant hits as defined by the MASCOT probability analysis ($P < 0.05$) were accepted. BLAST homology and similarity searches were conducted with the protein *Viridiplantae* index of the non-redundant NCBI database.

3 Results

3.1 Physiological responses induced by low temperature stress

The relative water content assay (RWC) was performed and results showed that the RWC of the two cultivars were both decreased after the cold treatment (Fig. 1a). This indicated that the leaves of wheat were subjected to chilling injury. RWC of Jimai22 was higher than Luyuan301, which did not the significant differences.

Soluble sugars content was detected. Both of the two wheat cultivars were relatively low and at similar levels as shown in Fig. 1b. After the stress treatment, both the sugar increased, and the contents showed significant differences between Jimai22 and Luyuan301 plants.

3.2 2-DE analysis of cold-responsive total proteins in Jimai 22 and Luyuan 301

2-DE analysis was carried out and a representative gel is shown in Fig. 2. Approximately 1100–1400 protein spots were detected on silver-stained gels in both Jimai 22 and Luyuan 301, and proteins were better separated in both directions of the isoelectric and molecular weight. We detected approximately 1200 proteins in total and 24 (2%) of them were considered to have changed significantly between cold-treated and untreated plants. The results showed that the occurrence of cold-treated resulted in both up- and downregulation of a series of genes, which may relate to the morphological and physiological changes of cold-treated plants.

3.3 Identification of the differentially expressed proteins (DEPs) by MALDI-TOF MS

A total of 49 protein spots showed at least 2-fold differences in abundance between the two cultivars in response to cold stress. Forty-three DEPs from the 49 protein spots between cold-treated and untreated plants were identified by MALDI-TOF MS. The results revealed that 21 proteins in Jimai22 differed in the protein map, including 13 upregulated and 8 downregulated (Table 1); 22 proteins in Luyuan301 differed including 9 upregulated and 13 downregulated (Table 2). These DEPs were mainly involved in seven biologic processes, carbohydrate metabolism, amino acid metabolism, resistance proteins and antioxidant enzymes, photosynthesis and cellular respiration proteins, cell-wall related proteins, protein translation/processing/degradation and signal transduction. The protein levels indicated that wheat cold tolerance was involved in the changes in cell metabolism and signal or biochemical pathways. The peptide sequence data for the

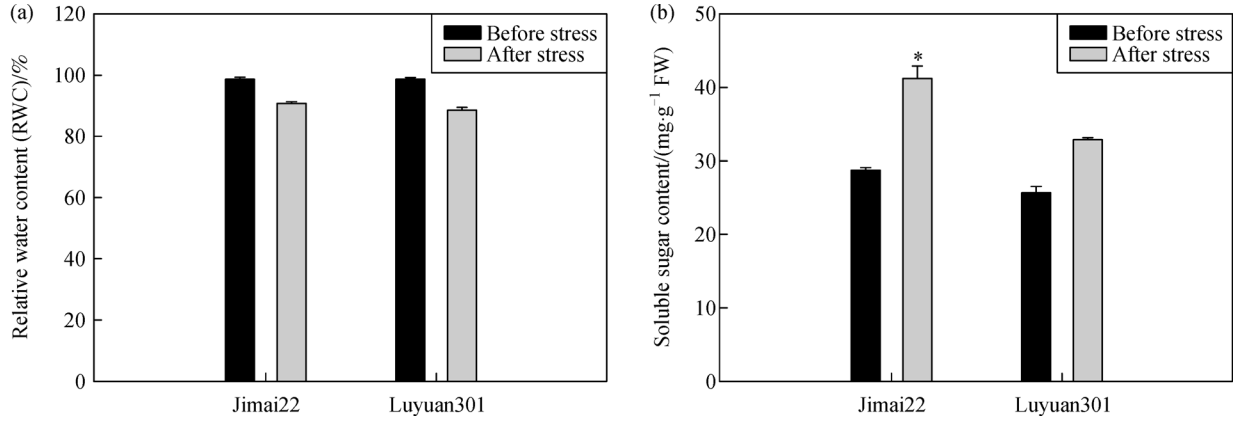

Fig. 1 Physiologic responses induced by cold treatment. (a) Effect of cold treatment on relative water content (RWC); (b) soluble sugar content of cold-treated and untreated plants. Values are the means±SD of three differences from each cultivar. * significant differences from the corresponding untreated plants at $P < 0.05$ under the cold treatment.

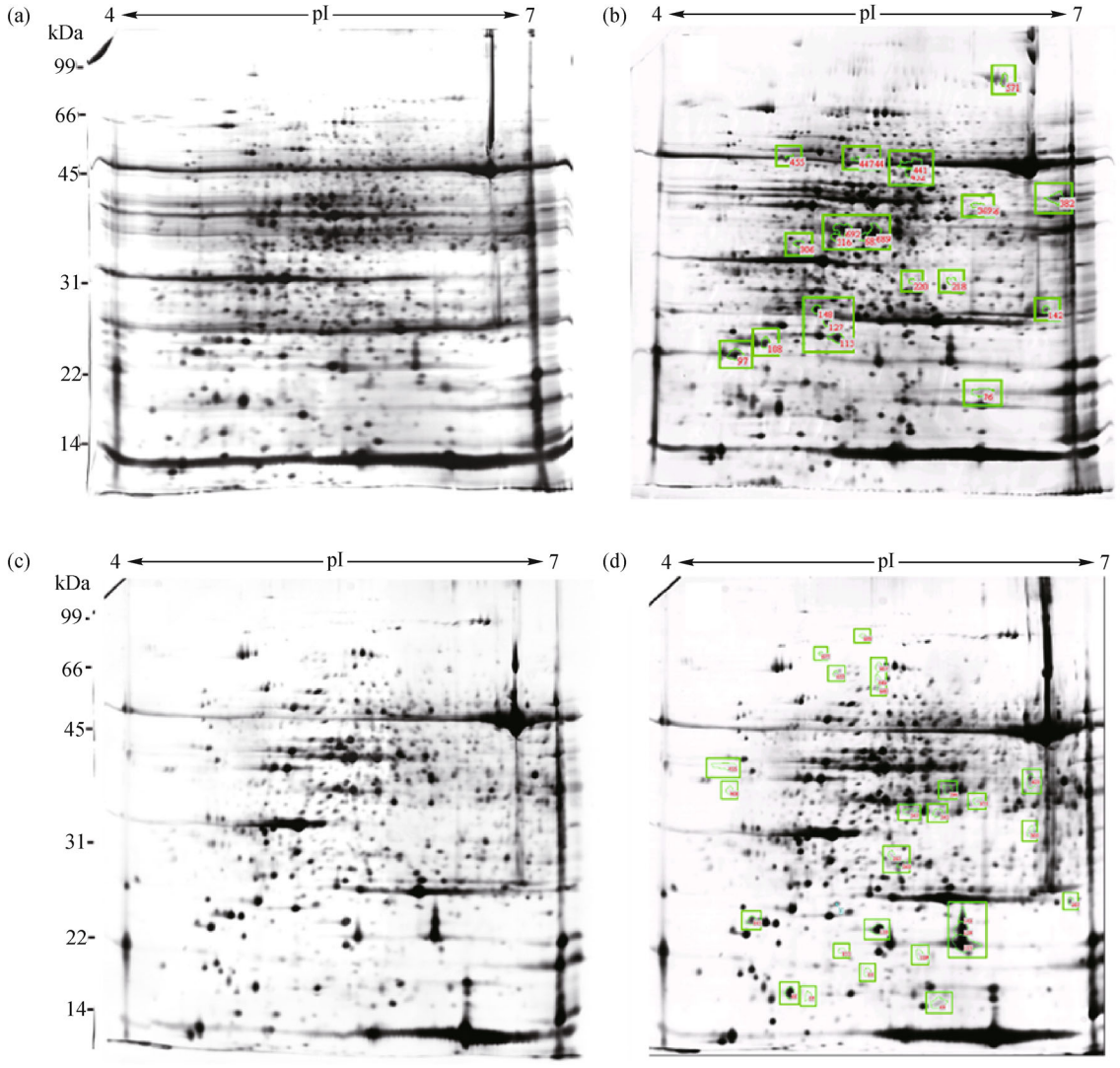

Fig. 2 Comparison of 2-DE gel maps proteins in cold-treated plants with untreated plants. Forty-three of the spots at least a 2-fold change under cold treatment with $P < 0.05$ were analyzed. (a) 2D-images of proteins from untreated Jimai22 plants; (b) 2D-images from Jimai22 plants after cold-treatment for 48 h; (c) 2D-images of proteins from untreated Luyuan301 plants; (d) 2D-images from Luyuan301 plants after cold-treatment for 48 h. Proteins that increased under cold stress are numbered on the 2-D map.

43 different expression proteins identified by MS was listed in Table S1.

4 Discussion

4.1 Carbon metabolism proteins

A number of proteins important in sugar metabolic pathways had the same expression pattern in cold-treated plants compared to the untreated plants. Fructose-bisphosphate aldolase (spot 682 and 689), an important enzyme associated both in glycolysis and pentose phosphate pathway, which could provide ATP and substrate that is essential for the organism. The upregulated of this enzyme implied the sugar metabolism alteration in Jimai22, while in Luyuan301 was downregulated (spot 428) compared to their untreated plants [28].

RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) is a bifunctional enzyme that catalyzes both the carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP), thus fixing carbon dioxide as the first step of the Calvin cycle. Compared to untreated plants, the relative contents of Rubisco proteins (spots 76, 142, and 366) and Rubisco activase1 (spot 444) in the total proteins of Jimai22 in cold-treated plants were lower than untreated plants, which suggested that the capability of carbon fixation and assimilation might be relatively decreased in

Table 1 Proteins of Jimai22 responsive to cold stress identified by MALDI-TOF-MS

Spot	Protein name	Theoretical pI	Theoretical Mw /kDa	Score	Sequence coverage/%	Peptides matched	Fold change	Accession No.	Species
682	Chloroplast fructose-bisphosphate aldolase	5.94	42217	600	26	6	2.08	ACM78035	*Triticum aestivum*
220	Predicted protein	6.45	32854	87	5	1	−2.73	BAK01718	*Hordeum vulgare*
571	Victorin binding protein	6.48	112298	141	3	5	2.00	AAA63798	*Avena sativa*
692	PREDICTED:quinone oxidoreductase-like protein At1g23740	8.29	39840	196	8	2	2.55	XP_003574429	*Brachypodium distachyon*
127	Light-harvesting complex I, partial (chloroplast)	8.11	24439	277	10	4	2.61	AAB29485	*H. vulgare*
76	RecName: ribulose bisphosphate carboxylase small chain PW9, chloroplastic;	8.52	19784	566	41	5	−3.27	P26667	*T. aestivum*
369	Predicted protein	6.36	47882	145	11	3	−4.54	BAJ95933	*H. vulgare*
148	Predicted protein	7.67	29.70	502	33	7	−2.42	BAJ97959	*H. vulgare*
441	Unnamed protein product	6.32	47517	248	12	5	4.52	CAA25265	*H. vulgare*
218	Hypothetical protein SORBI DRAFT_09 g001130	6.99	31865	343	11	3	−4.34	XP_002439133	*Sorghum bicolor*
455	ATP synthase beta subunit	5.29	52213	339	16	6	−2.78	ABR67209	*Pseudosasa japonica*
432	Predicted protein	6.00	52038	50	7	2	2.12	BAJ89632	*H. vulgare*
113	Predicted protein	5.63	25926	683	35	5	3.32	BAK01399	*H. vulgare*
142	RbcL, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	6.60	47600	215	9	3	−3.94	CAA44027	*T. aestivum*
689	Fructose-bisphosphate aldolase	7.63	41924	379	15	4	4.27	ACG36798	*Zea mays*
306	Predicted protein	5.08	35439	168	13	5	−2.13	BAJ86516	*H. vulgare*
316	Putative mitochondrial cysteine synthase precursor	5.36	22585	285	24	3	3.09	ACJ54643	*Aegilops speltoides*
366	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit	9.42	7895	99	18	2	−2.54	ACI22694	*Secale cereale*
444	Ribulose-1,5-bisphosphate carboxylase activase isoform 1	8.62	47341	293	13	4	−2.89	AAA63163	*H. vulgare*
97	Predicted protein	4.75	16185	89	7	1	5.51	BAK07537	*H. vulgare*
447	ATP synthase beta subunit	5.13	50859	234	11	4	−2.89	ABH02606	*Festuca rubra*

cold-treated plants. And in Luyuan301 plants, Rubisco proteins (spot 667) and Rubisco activase1 (spot 640) were upregulated, while Rubisco activase (spot 394) was downregulated compared to untreated plants.

Malate dehydrogenase (MDH) (spot 375) was commonly found in animals, plants, bacteria and other organisms, is a key enzyme in the glucose metabolism, which catalyzes the reversible conversion between malic acid and oxaloacetate.

Differential accumulation of these proteins implied a shift of sugar metabolic direction after cold stress and then resulted in abundance changes of components in sugar pool, which is likely to be an important reason for drastic morphological and physiological modification caused by cold.

4.2 Amino acid metabolism proteins

The abundance of some enzymes involved in amino acid metabolism showed significant differences between cold-treated and untreated plants. Cysteine synthase (spot 316) was significantly accumulated in cold-treated plants. The result indicated that the effect of cold stress was also reflected in a dynamic modification of both amino acid catabolism and anabolism, and also suggested the specificity for synthesis and/or degradation of amino acids after cold treatment.

4.3 Resistance proteins and antioxidant enzymes

Plant resistance to cold stress involves a series of inducible

Table 2 Proteins of Luyuan301 responsive to low-temperature stress identified by MALDI-TOF-MS

Spot	Protein name	Theoretical pI	Theoretical Mw /kDa	Score	Sequence coverage/%	Peptides matched	Fold change	Accession No.	Species
149	Cold shock protein-1	5.74	21884	601	54	5	−2.70	BAB78536	*Triticum aestivum*
123	Germin-like protein 1	6.01	22017	173	15	2	−2.27	BAA74702	*Oryza sativa*
138	Pentatricopeptide repeat-containing protein	8.19	71386	61	1	1	−6.98	NP_190408	*Arabidopsis thaliana*
66	Predicted:low quality protein:50S ribosomal protein L12-2	6.77	22351	370	19	3	−3.67	XP_003566962	*Brachypodium distachyon*
667	Ribulosebisphosphate carboxylase, partial (chloroplast)	6.34	52111	44	4	2	2.88	AAA68039	*Pandanus tectorius*
345	Predicted: probable pyridoxal biosynthesis protein PDX1.1-like	6.11	33676	179	11	4	−2.02	XP_003558759	*B.distachyon*
347	Predicted protein	6.73	40202	102	7	2	−4.93	BAK02219	*Hordeum vulgare*
145	Predicted protein	4.75	16185	69	7	1	−2.33	BAK07537	*H. vulgare*
261	Predicted protein	6.45	32854	143	9	3	−3.24	BAK01718	*H. vulgare*
455	ATPase subunit I (chloroplast)	5.47	47261	52	6	2	2.65	CAB88710	*Spinacia oleracea*
394	Ribulose-bisphosphate carboxylase activase	5.01	26084	186	18	3	−5.30	S25484	*Nicotiana tabacum*
640	Ribulose 1,5-bisphosphate carboxylase activase isoform 1	8.62	47341	130	11	3	2.24	AAA63163	*H. vulgare*
84	Eukaryotic translation initiation factor 5A; Short = eIF-5A	5.60	17483	95	17	2	2.75	Q9SC12	*Senecio vernalis*
428	Predicted: fructose-bisphosphate aldolase	6.86	37977	154	10	2	−2.13	XP_003563638	*B.distachyon*
109	Putative glycine-rich protein	5.63	19556	473	41	3	2.97	BAD06324	*T. aestivum*
167	Predicted protein	9.25	27800	232	20	3	−2.26	BAJ85545	*H. vulgare*
112	EM_EST:AJ602339 AJ602339.1	5.78	13731	94	17	1	2.97	AJ602339	*T. aestivum*
57	Predicted: low quality protein: 50S ribosomal protein L12-2	6.77	22351	257	19	3	3.85	XP_003566962	*B.distachyon*
653	Predicted: ras GTPase-activating protein binding protein 1-like	5.30	51777	51	8	3	2.26	XP_003581124	*B.distachyon*
49	Heat shock protein 17.3	5.58	17399	230	36	5	−3.17	CAA41218	*T. aestivum*
375	Malate dehydrogenase	8.23	36347	70	11	2	2.17	AAD56659	*Glycine max*
242	Predicted protein	6.45	32854	130	5	1	−2.21	BAK01718	*H. vulgare*

defensive responses, which include the synthesis of various cold-related proteins and multiple enzymes with antioxidant activity.

Quinone oxidoreductase-like protein (spot 692) belongs to zinc-containing alcohol dehydrogenase family and quinone oxidoreductase subfamily. It has recently been demonstrated that purified NAD(P)H:quinone oxidoreductase 1 (NQO1) is able to scavenge superoxide (O2(.-)) though the rate of reaction of O2(.-) with NQO1 is much lower than the rate of enzymatic dismutation catalyzed by superoxide dismutase (SOD). This gene was confirmed to play an important role as the force generators for fish in a low water environment. In addition, NADH-quinone oxidoreductase 3 subunit gene was associated with cold adaptation of common carp to some extent in oxidation of respiratory chain electron transport system [29].

Vitamin B6 is an essential coenzyme for numerous metabolic enzymes and is a potent antioxidant. The *de*

novo pathway of vitamin B6 biosynthesis has only been described recently and involves the protein Pyridoxal phosphate synthase protein (PDX1, spot 345).

Cold-shock proteins (CSPs) (spot 149) are nucleic acid binding proteins containing a cold shock domain (CSD), which are highly conserved in bacteria, plants and animals. They play a critical role for transcription, translation, development and stress responses by the RNA chaperones function.

4.4 Photosynthesis and cellular respiration proteins

A light-harvesting complex (spot 127) is a complex of subunit proteins that may be part of a larger supercomplex of a photosystem, the functional unit in photosynthesis. The increase of LCHI aggregates in the study may suggest that LCHI aggregates may represent a protective mechanism to cold-stress plants.

ATPases (spot 455 and 447) are membrane-bound enzyme complexes/ion transporters that combine ATP synthesis and/or hydrolysis with the transport of protons across a membrane. ATPases can harness the energy from a proton gradient, using the flux of ions across the membrane via the ATPase proton channel to drive the synthesis of ATP. The plasma membrane proton pump ATPase [30] is important in transport across the plasma membrane. As a primary transporter, it mediates ATP-dependent H(+) extrusion to the extracelllar space, thus creating pH and potential differences across the plasma membrane that activate a large set of secondary transporters. ATPases (spot 455 and 447) of Jimai22 were downregulated while spot 455 of Luyuan301 was upregulated when they compared to non-stressed plants.

4.5 Cell-wall related proteins

Germin and Germin-like protein have various proposed roles in plant developmental stages and stress-related processes. It may function through the controlling of the cell wall extensibility in the early plants development, but their expression is also regulated by pathogens and abiotic stress factors such as photoperiod [31].

Glycine-rich RNA binding proteins (GRPs; spot 109) have been found in the cell walls of many higher plants and form a group of structural protein components of the wall in addition to extensins and proline-rich proteins. GRPs are involved in post-transcriptional regulation of gene expression in plants under various conditions and, in most cases, they are accumulated in the vascular tissues and their synthesis is part of defense mechanisms in plants [32].

4.6 Protein translation, processing and degradation

Regulation of gene expression is achieved at several levels; transcriptional, post-transcriptional, translational, and post-translational. Pentatricopeptide repeat proteins (PPR; spot 138) are encoded by a large gene family, which are involved in plant development, organelle biogenesis, restoring of cytoplasmic male sterilities, RNA processing and editing in mitochondrial and chloroplasts, plastid-to-nucleus retrograde signaling, and responses to environmental stresses. PPR proteins have been implicated in RNA editing, RNA processing, RNA splicing and translational activation. Many miRNAs were processed and edited from PPR proteins, which may be directly or indirectly involved in plant resistance to environmental stresses.

Changes of translation activity were considered to be primary stress responses [33]. Spots 57 and 66 were 50S ribosomal protein which involved in the protein translation and synthesis.

Eukaryotic translation initiation factor (eIF-5A) (spot 84) is important in the regulation of biologic growth, aging and environmental responses. It's upregulation can enhance the plants stress tolerance.

Heat shock proteins (spot 49), also known as stress proteins, are a group of highly structurally conserved peptides, which are widely found in prokaryotic, eukaryotic cells and are involved in cell damage and repair.

4.7 Signal transduction

Ras GTP binding protein (spot 653) is an evolutionary conserved eukaryotic GTPase, which is likely to be involved in nuclear translocation of proteins and cell cycle progression [34]. However, little is known about the function of Ras in plant stress response.

5 Conclusions

Cold tolerance is a complex phenomenon in most plants, and involves numerous mechanisms at the cellular, tissue, organ and whole plant levels. In the present study, a comparative proteomic analysis of wheat subjected to cold treatment for 48 h at the pistil and stamen differentiation stage was performed. The physiologic results showed the relative higher RWC and soluble sugars may have increased the cold stress tolerance of Jimai22 compared to Luyuan301. Forty-three different cold-responsive proteins were identified (Table 1 and Table 2). The proteins identified were implicated in seven physiological process; including carbohydrate metabolism, amino acid metabolism, resistance proteins and antioxidant enzymes, photosynthesis and cellular respiration proteins, cell-wall related proteins, protein translation/processing/degradation and signal transduction.

The same proteins involved in the two wheat cultivars may had different expression mode, such as Fructose-bisphosphate aldolase, Rubisco proteins and ATPase. Other proteins were cultivar specific and involved in many different biochemistry pathways, for example,

quinone oxidoreductase-like protein, cold-shock protein, germ-like protein and 50S ribosome protein. The different expression of these proteins in various pathways may cause the different cold tolerance for the two wheat cultivars. The unknown proteins represent novel mechanisms for cold tolerance in wheat. Further study is needed to reveal their structures and functions.

The proteins identified in this study represent only a small part of the wheat proteome responsive to cold treatment, and other cold-responsive proteins still need to be identified. The identification of cold-responsive proteins provides not only new insights to cold stress responses but also a good starting point for further dissection of their functions using genetic and approaches.

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Compliance with ethics guidelines Shujuan Zhang, Guoqi Song, Yulian Li, Jie Gao, Jiao Wang, Guiju Chen, Haosheng Li, Genying Li and Zhendong Zhao declare that they have no conflict of interest or financial conflicts to disclose.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

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