A proteomic analysis to identify cold acclimation associated proteins in wild wheat (*Triticum urartu* L.)

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Abstract To gain a better understanding of cold acclimation process in wheat, we applied a 2-DE based proteomic approach to discover changes in proteome profile of a diploid wild wheat (*Triticum urartu* L.) during prolonged cold stress treatment. To this end, plants were grown in pots and the growing seedlings (4-leaf stage) were exposed to cold stress. After 4 weeks of cold acclimation (4–6 °C) and subsequent treatment for 12 h at −2 °C, samples were collected from control and stressed plants and were subjected to proteome patter
n analysis. Among approximately 450 reproducible protein spots displayed in each given 2-DE gels, 34 proteins changed significantly in abundance in response to cold stress. Among them, 25 and 9 proteins were up and down-regulated under stress condition, respectively. Analysis by matrix-assisted laser desorption ionization time of flight/time of flight mass spectrometry coupled with non-redundant protein database search allowed the identification of 20 cold-induced proteins. Integrated proteomic and database survey resulted in identification of several cold stress related proteins such as pathogenesis related protein, cold regulated protein, cold-responsive LEA/RAB-related COR protein, oxygen-evolving enhancer protein and oxalate oxidase. The presumed functions of the identified proteins were mostly related to cold acclimation, oxidative stress and photosynthesis. The possible implications of differentially accumulated proteins in acquiring systemic tolerance to freezing stress following exposure to prolonged low temperature will be discussed.

Keywords Proteomics · Two-dimensional gel electrophoresis (2-DE) · Mass spectrometry · Cold acclimation · *Triticum urartu* · Low temperature

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Introduction

Low temperature is one of the most important environmental factors that limits productivity, geographical distribution and growing season of many plant species. Cold stress imposes major limitation on agriculture. For example, it has been estimated that a 1 °C decrease in the mean temperature of the world would result in a 40 % yield loss in rice [1]. The vulnerability of plants to temperature fluctuations varies during different growth phases. For example, the majority of crop species could not tolerate even a short period of low temperature during reproductive stage of growth which almost results in sterility and significant reduction in grain yield [2]. During development, plants have evolved sophisticated mechanisms to circumvent low temperature or freezing damage by using different strategies. Some plants have gained the ability to avoid freezing stress by overwintering as dormant seeds which contain little freezable water [3, 4]. Some may completely avoid freezing by preventing the formation of ice

Table 1 Differentially accumulated proteins identified using combined mass spectrometry (MS) and tandem mass spectrometry (MS/MS) analysis

Spot ID	Exp pI/MW[a]	The pI/MW[b]	MS score[c]	% Cov[d]	Protein identity/accession number	Fold change[e]	
						LT/C	FT/C
11	5.11/28	5.00/18.32	482	44.76	Group3 Late embryogenesis abundant protein [*Triticum aestivum*]/A7VL25	1.51**	1.18
2	6.76/14	6.28/13.1	112	56.66	Pathogenesis related protein 4 (PR-4) [*Triticum aestivum*]/Q9SQG4	+	+
4	6.19/29	6.41/23.5	334	22.92	Oxalate oxidase [*Lolium perenne*]/Q8L695	−1.62*	−5.6**
5	5.61/38	8.81/39.1	211	18.93	Enoyl-[acyl-carrier-protein] reductase [NADH] 1, chloroplastic [*Oryza sativa*]/Q6Z0I4	1.4	1.54*
7	5.29/41	5.44/29.7	278	16.99	Cysteine synthase, chloroplastic [*Triticum urartu*]/M8A9L0	1.48	1.72**
8	5.17/39	5.45/37.2	79	20.01	Thiamine thiazole synthase, chloroplastic [*Oryza sativa*]/Q7XXS4	1.69*	1.43
10	5.07/37	7.01/38.38	82	5.89	Fructose-bisphosphate aldolase [*Arabidopsis thaliana*]/Q9SJQ9	−1.39	−1.59*
13	5.14/24	5.37/16.32	178	28.99	Ribosomal protein L12, chloroplastic [*Oryza sativa*]/O22386	2.9**	2.2**
14	5.05/24	5.37/16.32	235	35.17	Ribosomal protein L12, chloroplastic [*Oryza sativa*]/O22386	+	+
23	5.34/18	5.33/14.79	301	34.32	Ribosomal protein S12 [*Oryza sativa*]/Q0D8L0	−1.51*	−1.32
15	4.88/25	4.85/17.16	299	35.01	Cold-responsive LEA/RAB-related COR protein [*Triticum aestivum*]/Q9M4T9	2.02**	1.59**
16	4.85/24	4.85/17.16	302	26.99	Cold-responsive LEA/RAB-related COR protein [*Triticum aestivum*]/Q9M4T9	1.37	1.51**
20	4.41/18	4.75/13.6	521	40.08	Cold-responsive protein WCOR14 [*Triticum aestivum*]/B9VUA8	+	+
19	4.48/31	4.45/22.18	98	7.78	Chloroplast 29 kDa ribonucleoprotein [*Oryza sativa*]/A6N1F5	+	+
24	5.59/15	8.80/19.46	609	89.33	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [*Triticum aestivum*]/Q9FRZ4	−1.54**	−1.48**
1	6.12/16	8.59/19	530	97.06	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [*Triticum durum*]/Q575T3	−1.52*	−1.2
25	5.68/15	8.80/19.46	601	87.87	Ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit [*Triticum aestivum*]/Q9FRZ4	−4.05**	−3.18**
29	6.32/45	6.09/34.51	543	41.29	Oxygen-evolving enhancer protein 1 [*Leymus chinensis*]/A5JV93	1.74*	1.49
30	6.60/26	7.89/25.29	592	45.21	Superoxide dismutase [*Triticum aestivum*]/P93606	1.64*	3.48**
27	5.96/31	9.13/31.27	152	23.16	Putative 3-beta hydroxysteroid dehydrogenase/isomerase protein [*Oryza sativa*]/Q65XW4	+	+

C control, *LT* low temperature, *FT* freezing temperature

* significance at 0.05

** significance at 0.01

[a] Experimental isoelectric point (pI) and molecular weight (MW)

[b] Theoretical isoelectric point (pI) and molecular weight (MW)

[c] Mass spectrometry score from combined peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/MS)

[d] Percent of sequence coverage

[e] Fold change in expression (mean% vol stress/mean% vol control)

nucleators (supercoiling) and/or lowering the freezing point by synthesis and accumulation of cryoprotectant substances such as antifreeze proteins, amino acids, and sugars [4–6]. Plants may also avoid freezing by delaying the transition from vegetative to reproductive phase.

The process that is induced in plants in response to exposure to low or moderately sub-optimal temperatures and allows them to increase their freezing tolerance is referred to as cold acclimation [7]. Cold acclimation is achieved through changing the metabolic processes which lead to the accumulation of anti-freezing and antioxidative substances, and modulation of lipid and protein composition of plasma membrane [8, 9]. Cold acclimation involves precise reprogramming of gene expression which is accompanied with the synthesis of new transcription factors and effector genes collectively referred as cold-regulated (COR) genes [10]. These genes include transcription factors, chaperones, metabolic enzymes, late embryogenesis-abundant (LEA) proteins, dehydrins and antioxidative enzymes [11, 12].

Environmental stresses, such as low temperature, salinity and drought stress, that result in cellular dehydration

often induce similar changes to plant gene expression and metabolism and there almost exist a cross-talk in their signaling pathways [10, 13]. Therefore, identification of the gene expression signatures associated with each of these stresses will improve our understanding about the tolerance mechanisms. The hypothesis that stress-responsive genes are likely to be involved in establishment of systemic tolerance has led to great efforts to study the gene expression profile during different stresses.

At transcriptome level, microarray analysis of gene expression has contributed greatly to our understanding of the cold stress response and provided a detailed list of cold-responsive genes in many plant species [14–18]. However, the existence of an mRNA in a cell does not necessary mean that the corresponding encoded protein is expressed and exists in its functional conformation. Since the mRNA level does not always correlate well with the level of the corresponding protein, as a key player in the cell, it is insufficient to predict protein expression level from quantitative mRNA data [19]. This is mainly due to post-transcriptional regulation mechanisms such as nuclear export and mRNA localization, transcript stability, translational regulation and protein degradation, and a significant error and noise in quantitative measurement of both mRNA and protein [19, 20].

Analysis of large number of proteins in response to specific treatments is feasible through techniques such as two-dimensional gel electrophoresis (2-DE) and mass spectrometry. Through analysis of the proteome profile of cold stressed and control plants, different families of proteins have been identified to be associated with plant response to cold stress by being newly synthesized, increased or decreased [21–24]. Cold stress associated proteins are mainly involved in signal transduction, protein synthesis and processing, host-defense mechanisms, carbohydrate and energy metabolism and amino acid metabolism. In the current study, we applied a 2-DE based proteomics approach to dissect the proteome changes associated with cold acclimation in a diploid wild wheat which was expected to display less complex proteome than hexaploid bread wheat (*Triticum aestivum*).

Materials and methods

Plant growth conditions and cold stress treatments

Wheat seeds (*Triticum urartu* L.) were collected from Kurdistan Mountains, Kurdistan province, Iran and were cultured in pots containing mixture of soil: sand: mulch (2:1:1) in green-house under controlled conditions. Plant seedlings (control and treatment groups) were well watered twice a week for 5 weeks until they reached the 4–5 leaf

stage. Before to subject seedlings to low temperature, samples from control plants were harvested by cutting at the base of leaf number one. The harvested leaf samples were quickly wrapped in aluminum foil pouch and immediately frozen in liquid nitrogen and stored at −80 °C. Plant seedlings in treatment group were transferred to a cold room and were subjected to cold stress by setting temperature at 4–6 °C. The seedlings were grown at this temperature regime under controlled condition (14 h light and 10 h dark, 60 % relative humidity) for 4 weeks. At the end of this step, leaf samples from stressed seedlings were harvested same as above, the remaining cold harnessed seedlings were subjected to −2 °C for 12 h and samples from freeze stressed seedlings were also collected.

For determination of lethal temperature 50 (LT_{50}) the procedure described by [25] was used with some modifications. Same experimental condition was set up but after subjecting plant seedlings to −2 °C for 12 h they were cooled at a rate of 2 °C/h down to −17 °C. Thirteen seedlings were removed at 2 °C intervals and cooled at 8 °C/h. The plant seedlings were transferred to normal conditions and the LT_{50} was determined on the basis of regrowth after 2–3 weeks.

Protein extraction

Harvested leaves were finely ground in liquid nitrogen using mortar and pestle, then the total proteins were extracted following the procedure described by [26] with some modifications. Briefly, one gram of the resulted powder resuspended in an ice-cold solution of 10 % w/v trichloroacetic acid (TCA) in acetone with 0.07 % w/v Dithiothreitol (DTT) for at least 1 h at −20 °C, and centrifuged for 20 min at $35,000 \times g$. The pellets were rinsed twice with acetone containing 0.07 % w/v DTT for 1 h at −20 °C and then lyophilized. The resulting pellet was solubilized in lysis buffer (7 M urea, 2 M thiourea, 4 % 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 35 mM TRIS, 1 % w/v DTT, and 1 % v/v Ampholyte pH 3.5–10) for 1 h at room temperature and then centrifuged at $12,000 \times g$ for 15 min. The supernatant was carefully collected and aliquted and stored at −80 °C until 2-DE analysis. The protein concentrations were quantified according to the Bradford method using bovine serum albumin (BSA) as standard [27].

Two-dimensional gel electrophoresis (2-DE)

Two-dimensional gel electrophoresis (2-DE) was performed as described previously [28].The immobilized pH gradient (IPG) strips (pH 4–7, 17 cm in length, Bio-Rad) were rehydrated at room temperature for 12–16 h in 320 μL rehydration solution (8 M w/v urea, 2 % w/v CHAPS,

20 mM DTT, 2 % v/v IPG buffers (pH 3–10) and 0.002 % Bromophenol blue) in a reswelling tray (GE Healthcare). For analytical and preparative gels, 120 μg and 1.5 mg proteins were loaded, respectively. IEF was performed at 20 °C with Multiphor II and a DryStrip Kit (Amersham Pharmacia Biotech). The running conditions were as follows: 150 Vh at 0–300 V, 300 Vh at 300–500 V, followed by 2,000 Vh at 500–3,500 V and finally 39,500 Vh at 3,500 V. Focused strips were equilibrated for 15 min in 8 ml equilibration solution (50 mM Tris–HCl buffer, pH 8.8, 6 M w/v urea, 30 % w/v glycerol, 2 % w/v SDS, 1 % w/v DTT and 0.002 % bromophenol blue).

The second dimension was performed on a 12.5 % SDS–polyacrylamide gel using a Protean II Xi Cell (Bio-Rad). The protein spots in analytical gels were visualized by silver nitrate according to [29]. Preparative gels were stained with colloidal coomassie brilliant blue (CBB) G-250. Triple replicate gels were run for each treatment.

Image acquisition and 2-DE gel analysis

The silver-stained 2-DE gels were scanned using a GS800 calibrated densitometer (Bio-Rad) in transmissive mode. Spot detection, quantification, and matching were performed using Melanie 6.02 software (Genebio, Geneva, Switzerland) as described previously [30]. The molecular masses of proteins on gels were determined by co-electrophoresis of a standard protein marker (Amersham Pharmacia Biotech) and pI of the proteins were determined by migration of the protein spots on 17 cm IPG (pH 4–7, linear) strips. The volume of each spot from three replicate gels was normalized against total spot volume, quantified, and subjected to one-way analysis of variance ($p < 0.01$). Only those spots that were present on all three replicate gels were quantified and enrolled to statistical analysis.

Mass spectrometry based protein identification and database search

Differentially accumulated protein spots were manually recovered from preparative CBB stained gels, destained for 1 h at room temperature using a freshly prepared washing solution consisting of 100 % acetonitrile/50 mM ammonium biocarbonate ($NH4CHO3$) (50:50 v/v). Trypsin digestion and MALDI-TOF/TOF analysis were performed as described before [28]. Briefly, a 1 μL aliquot of trypsin digested peptides was applied directly to the ground steel MALDI target plate. Positive-ion MALDI mass spectra were obtained using a Bruker ultraflex III in reflectron mode. MS spectra were acquired over a mass range of m/z 800–4,000. For each spot the ten strongest peaks of interest, with a S/N greater than 10, were selected for MS/MS fragmentation. Brukerflex Analysis software was used to process the spectral data and peak list

generation for both the MS and MS/MS spectra. Combined mass spectral and tandem mass spectral data were submitted to database searching using a locally-running copy of the Mascot program (Matrix Science Ltd., version 2.1), through the Bruker Biotools interface (version 3.1). Search criteria included: enzyme, trypsin; variable modifications, oxidation (M); peptide tolerance, 200 ppm; MS/MS tolerance, 0.8 Da; instrument, MALDI-TOF-TOF. The search criteria also included carbamidomethyl (C) as a fixed modification for all alkylated samples. The database search was run against NCBI non-redundant protein database Viridiplantae (20080423; 5552184 sequences; 1912145351 residues).

Results and discussion

Cold acclimation is a cumulative process and requires an optimal temperature which its threshold for winter cereals is thought to be 10 °C [31, 32]. There is an inverse relationship between the rate of cold acclimation and temperature. Plants with cold acclimation ability usually acclimate to cold and freezing stresses within a short period of time (1–2 days) [33]. Extending the duration of cold acclimation may result in full adaptation to subsequent freezing temperatures [34]. In order to mimic farm conditions and to reach full cold acclimation capacity, we exposed plant seedlings to low temperature (4–6 °C) condition for a period of four weeks at 14/10 h day/night photoperiod (Fig. 1). Lethal temperature 50 (LT_{50}) is the most commonly employed method for assessing differences in cold hardiness of winter cereals [35]. To measure the cold hardiness capacity of *T. urartu,* we set up a freezing test on cold acclimated seedlings. Figure 2 shows the percentage of seedling death by function of lowering temperature for *T. urartu*. As shown, the LT_{50} for this wheat accession is estimated to be −11 °C. This result clearly showed that cold acclimation process had been well established in *T. urartu,* even though that our controlled growth conditions may significantly differ from that in its native growing location.

To investigate cold acclimation and freezing tolerance associated proteins in *T .urartu*, we employed a 2-DE based proteomics approach coupled with tandem mass spectrometry protein identification. Using Melanie software we could successfully identify and quantify more than 450 reproducible protein spots. To identify differentially accumulated proteins, the percentage volume (as normalized expression value of each spot) of the reproducibly detected spots were determined and subjected to one way analysis of variance (one-way ANOVA). Of them, 34 protein spots changed significantly ($p \leq 0.05$) in abundance in response to low and freezing temperature. Among them, 25 and 9 proteins were up and down-regulated under stress condition, respectively.
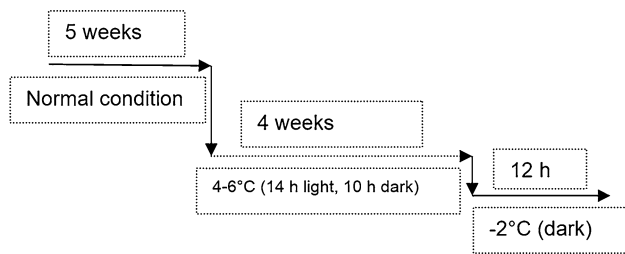
Fig. 1 Schematic diagram of the temperature regimes and growth conditions
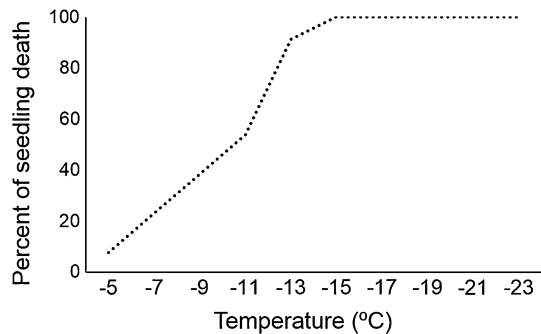

Fig. 2 The percentage of seedling death has been plotted as function of decrease in temperature to determine lethal temperature 50 (LT_{50}). As it can be seen the LT_{50} is estimated to be −11 °C for *T. urartu*

Identity of the differentially accumulated proteins

To identify the candidate up or down regulated proteins, differentially accumulated spots were recovered from CBB-stained preparative gels and were subjected to trypsin digestion, MALDI-TOF/TOF mass spectrometry analysis, and non-redundant protein database search. To increase the chance of protein identification, a combined peptide mass fingerprinting (PMF) and tandem mass spectrometry (MS/ MS) was applied. The MS analysis resulted in identification of 20 proteins representing 17 unique protein species. Supplementary figures (A, B, and C) show the overall leaf proteome pattern of *T. urartu* under control, cold, and freezing conditions. Figure 3 shows the position of the identified proteins on a 2-DE gel of total proteins extracted from leaves of cold stressed seedlings. Two candidate proteins were identified in multiple spots including cold-responsive LEA/RAB-related COR protein (spots 15 and 16) and Rubisco small subunit (spots 1, 24, and 25). The change in abundance of the identified proteins are shown in cropped images for spots 13, 30, 15 and 16 (Fig. 4; Table 1). Some of the candidate spots were found as newly synthesized proteins in response to cold acclimation such as those identified in spots 14, 2, 27, 19 and 20 (Fig. 5). Some proteins such as those identified in spots 16, 20, 19, 30, and 27 steadily increased with decrease in temperature.
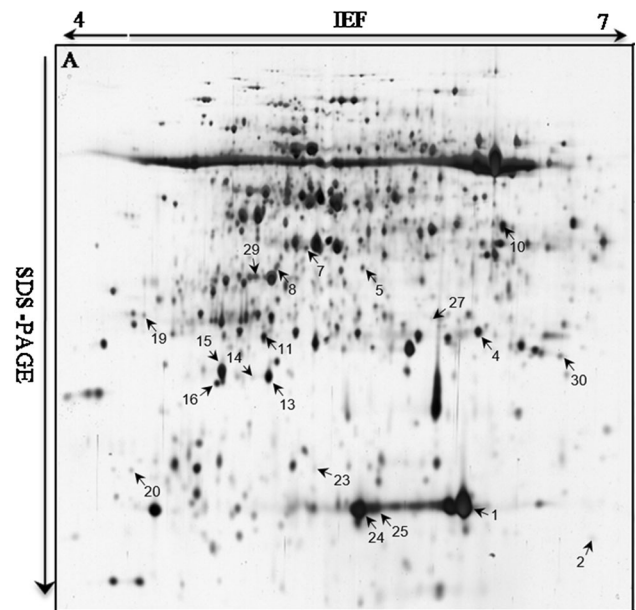

Fig. 3 A representative 2-DE gel image of leaf proteins of wild wheat (*Triticum urartu* L.) under cold stress. Total soluble leaf proteins were subjected to 2-DE to establish a proteome reference map. The gel positions of the identified proteins are shown using an *arrow* and a *number* which refers to match ID as given to each spot during software analysis
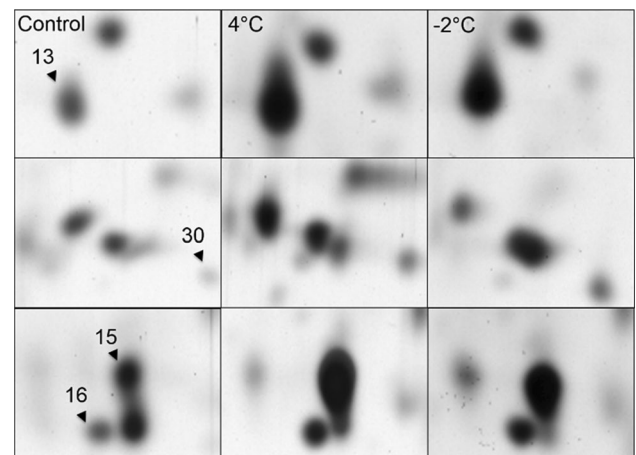

Fig. 4 Cropped images show the details of up/down regulation of some of the candidate cold responsive proteins. For details of protein identities refers to data presented in Table 1

The biological function of the candidate cold responsive proteins

The candidate proteins were found to be involved in several physiological processes including cold acclimation, energy metabolism, antioxidative response and photosynthesis. Several of the candidate proteins were found as being directly involved in cold acclimation. Spots 15 and 16 were matched to cold-responsive LEA/RAB-related COR protein and spot 20 was identified as cold-responsive
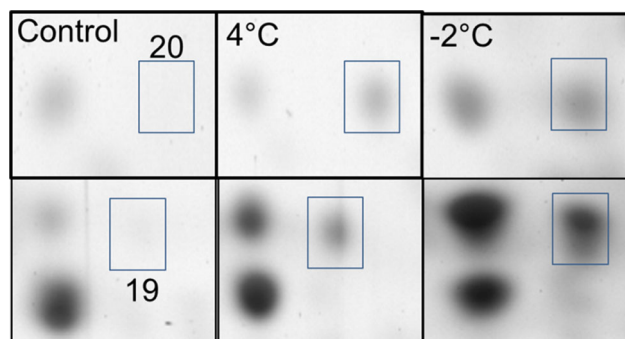
Fig. 5 Cropped images show expression patterns of two newly expressed proteins (*spots 19* and *20*) in response to cold acclimation

protein WCOR14. In addition, spot 11 was identified as group3 LEA protein. All of these proteins were accumulated in response to cold acclimation. Cold responsive proteins (COR) belong to different protein families which are induced or activated during cold acclimation. The cold responsive proteins are independently categorized as LEA proteins, DHA (dehydrin), RAB (responsive to abscisic acid (ABA)), and COR. Since the majority of these proteins belong to LEA proteins, they are collectively referred to as LEA/CORs [12, 36]. Excess accumulation of some dehydrin proteins such as WCOR410 in a freezing tolerant cultivar of wheat has been well correlated with cold acclimation capacity [37]. It has been well documented that the accumulation of these proteins protect plant cells against freezing damage specifically by stabilizing the plasma membrane and preventing protein aggregation [38]. Studies have also shown that ectopic overexpression of members of these protein families increases freezing tolerance in model plants [39]. Group3 LEA proteins possess tandemly repeated 11-mer amino acid motifs and are expressed during late embryogenesis, low temperature and salinity [40]. Ectopic expression of group3 LEA proteins in rice and yeast increased tolerance to salt, dehydration and freezing [41–43]. Change in abundance of some LEA proteins have also been reported in previous proteomic studies of cold acclimation [21, 24, 44, 45].

Superoxide dismutases (SODs) constitute a large family of metalloenzymes that catalyze the conversion of highly toxic superoxide radicals into less toxic H_2O_2 and O_2 [46]. In our study, SOD was significantly accumulated in response to cold acclimation and subsequent freezing stress. SOD constitutes the first line of defense against reactive oxygen species (ROS) which thought to increase when plants are encountered to dehydration stresses such as cold, salinity, heat, and drought [47, 48]. Despite their damaging effects on biological membranes and other biomolecules such as DNA and proteins, ROS are extensively utilized as signaling molecules to transduce the extracellular signals to cellular machinery to activate defense

mechanisms [49]. Studies have shown that ectopic introduction of SOD in tobacco and alfalfa improved resistance to low temperature [50, 51]. In addition, increase in abundance of SOD after cold acclimation may reflect the increased demand for protective role of this antioxidative enzyme and may also contribute to enhanced cold tolerance. Oxalate oxidase (OXO, spot 4) steadily decreased in abundance with decrease in temperature. OXO catalyzes the conversion of oxalic acid and O_2 to CO_2 and hydrogen peroxide (H_2O_2) and thereby acts as a source of hydrogen peroxide in the cells. This reaction is known as one of the major routes of H_2O_2 production in the cells during pathogen attack [52]. Decrease in abundance of this protein during cold acclimation may slow down the rate of H_2O_2 production (as a potential ROS) and further enhance the plant response to cold stress.

Spot 2 which showed significant up regulation in response to cold stress was identified as pathogenesis related protein (PR-4). PR-4 is a member of pathogenesis related proteins and is well known as responsive to cold stress [13]. In line with our results, a significant change in abundance of some PR proteins has been reported in bread wheat under cold stress [22, 24].

One of the cold stress induced proteins (spot 7) was identified as cysteine synthase (CS). CS catalyzes the final reaction in cysteine biosynthetic pathway which is also known as a key rate limiting step in the biosynthesis of tripeptide glutathione [53]. Glutathione level in the cells has been shown to correlate with plant adaptation to extreme temperature and tolerance to xenobiotic compounds and abiotic stresses [53]. A significant accumulation of CS has also been reported in rice [54], *Lolium perenne* [55], and wheat [44] challenged with cold or freezing stresses. CS over production may contribute in enhanced glutathione content which is thought to correlate with tolerance to abiotic stresses.

Several of the candidate cold responsive proteins were found as being involved in photosynthesis and energy metabolism including rubisco small subunit (spots 1, 24 and 25), oxygen-evolving enhancer protein 1 (spot 29), and fructose-bisphosphate aldolase (spot 10). Plants usually respond to cold stress by lowering the energy metabolism related processes in order to manage the oxidative burst imposed by low temperature. Photosynthesis is thought to be one of the first processes influenced by cold stress [45]. Rubisco small subunits were significantly down regulated upon exposure to cold stress. The small subunit of rubisco is synthesized in the cytoplasm as a precursor and then is transported into the chloroplast where it is incorporated into rubisco enzyme complex. In line with our results, a significant reduction in levels of rubisco subunits has been reported in rice [56] and wheat [44, 45] challenged with cold stress.

Thiamine thiazole synthase (spot 8) showed an increase in abundance in cold acclimated seedlings. It is involved in biosynthesis of the thiamine precursor thiazole [57]. Thiamine is an essential cofactor required by an array of enzymes involved in the production of acetyl-CoA, the tricarboxylic acid cycle (TCA), the pentose phosphate pathway/Calvin cycle, branched chain amino acid biosynthesis, and isoprenoid biosynthesis [58]. Arabidopsis plants subjected to environmental stresses such as cold, salinity, drought, and oxidative stresses accumulated thiamine and transcripts encoding thiamine biosynthesis enzymes [59]. Studies have also linked the exogenous application of thiamine to enhanced tolerance to oxidative and abiotic stresses [59–61]. Accumulation of thiamine thiazole synthase enzyme in cold acclimated seedlings in this study suggested a link between thiamine accumulation and cold acclimation process.

We next sought to identify common proteins responsive to low temperature by comparing up/down regulated proteins in this study with that reported for bread wheat elsewhere. When our protein dataset was compared with that reported by [24, 62] for winter wheat a significant overlap was observed in identified proteins. Interestingly, members of COR/LEA proteins were significantly enriched in cold acclimated plants and emerged as markers for cold stress tolerance. In addition, a significant change in abundance of general stress responsive proteins such as those related to photosynthesis, oxidative stress and energy metabolisms including rubisco, oxygen evolving enhancer protein, SOD, and ribosomal proteins were detected in cold acclimated plants. Comparing our protein dataset with that reported for a spring and a winter cultivar during different cold acclimation periods (0, 3, and 21 days) showed a little overlap in differentially expressed proteins [22]. Only pathogenesis related protein and fructose-bisphosphate aldolase were shared between our protein dataset with that reported by [22]. In another study that analyzed the proteome response of a spring wheat cultivar to prolonged cold stress a significant overlap was detected in differential expression of general stress responsive proteins such as rubisco, fructose-bisphosphate aldolase, and ribosomal proteins [44]. These observations clearly show the significant differences in the response of different wheat cultivars to cold acclimation and highlight the importance of the selection of suitable cultivar for assessing the cold acclimation response at least at protein level.

Conclusion

A 2-DE based proteomics approach was applied to monitor the proteome changes associated with cold acclimation and subsequent freezing stress. Several different classes of proteins were identified as being involved in establishment of cold acclimation in wild wheat *T. uratu*. Cold acclimation was accompanied with increased abundances of some cold regulated proteins such as LEA/RAB proteins, dehydrins, ROS scavenging proteins. In addition, low temperature modulated the abundance of proteins related to photosynthesis and metabolism. Taken together, this study highlighted that cold acclimation is achieved through reprogramming gene expression and activation and accumulation of cold regulated proteins and it is mostly related to better capability in circumventing oxidative stresses.

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