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Proteome response of winter-hardy wheat to cold acclimation

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ABSTRACT:
The proteome analysis of winter crops during cold acclimation and overwintering can provide important information for designing breeding processes. The current experiment was carried out to investigate the proteome changes in frost-tolerant winter wheat (cv. Norstar) during different cold acclimation (CA) periods under field conditions in a cold and high-altitude region by two-dimensional gel-based proteomic techniques. The results showed that frost tolerance significantly increased by CA and the lethal freezing temperatures (LT_{50}) 10, 14, and 18 weeks after seed sowing were -28°C, -22°C, and -10°C, respectively. By the beginning of the reproductive stage (double ridge stage), the LT_{50} values had decreased significantly. Around 1000 protein spots were distinguished by Coomassie staining on the gels. The changes in the proteins during the CA often occurred in those with a functional role in photosynthesis, energy production (glycolysis), transcription, chaperone-like activities, membrane and cytoskeleton reorganisation, transport, redox adjustments, and signalling. The results revealed that changes in chloroplast proteins, certain transcription factors such as MADS-box transcription factor 26, and antioxidant enzymes (ascorbate peroxidase) show a similar trend to freezing tolerance, and their expression decreases with the onset of reproductive growth and the loss of freezing tolerance. During the acclimation period, most of the changes were focused on defence systems and cytoskeleton rearrangement, while, photosynthesis, and energy production became the main priority at the beginning of reproductive growth.

Keywords:
antioxidant, freezing tolerance, glycolysis, photosynthetic, proteomics, cold-responsive proteins

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INTRODUCTION

The climate changes which have occurred in recent decades have caused extensive modifications in the onset of cold periods in winter crops. Climate change will increase the occurrence and intensity of heatwaves and cause significant changes in the timing of the beginning of cold periods, as well as the intensity and incidence of extreme freezing spells. Therefore, the process of cold acclimation during the autumn and winter has been disturbed and this will have a significant impact on the level of final freezing tolerance. Early and late cold spells are one of the most important factors in reducing production in cold and high-altitude regions (Yadav *et al.* 2020). Given the increase in the world's population, it seems necessary to increase the production of agricultural products and reduce the effects of environmental stress on plants. Forecasts indicate that the world's population will reach more than 10 billion people by 2050, and to establish food security the amount of food production must increase by 60–100% (FAOSTAT 2021). Meanwhile, providing food security will become more complicated under cold stress conditions. However, cold-tolerant crops can improve their freezing tolerance by exposure to lethal temperatures (LT) and non-freezing temperatures, which is referred to as cold acclimation (Ritonga & Chen 2020). Exposure to autumn thermal conditions under field conditions is an obvious

example of cold acclimation (CA), especially in cold areas where the air temperature gradually decreases during the autumn season. Wheat (*Triticum aestivum* L.) is the most common winter cereal crop and as an important strategic product has a significant cultivation area in cold semi-arid regions. This is partially due to the dominant rainfall during winter and the relative adaptation of wheat to cold conditions.

In the semi-arid Mediterranean cold region, the soil temperature during the winter may fluctuate between 0 to -4°C, while the plant shoots may experience temperatures as low as -15°C. The freezing conditions which occur in the soil and the severe cold in the upper parts of the plants means that they can be simultaneously subjected to drought (dehydration) and cold stress (Yadav *et al.* 2020). During the CA process in plants, extensive metabolic and protein changes occur at the cellular level and the cells reprogramme their internal conditions (Ritonga & Chen 2020). The formation and increase of freezing tolerance in winter plants require certain changes in physiological processes including the initiation of the CA process. It can be concluded that even hardy winter cereals are not freeze tolerant at the beginning of the development period and their tolerance to freezing is strongly influenced by the development stage and the CA gained during their vegetative growth (Mahfoozi *et al.* 2001; Laudencia-Chingcuanco *et al.* 2011) Winter crops are able to adapt numerous intercellular processes, plasma membrane structures, energy production and consumption pathways, and the accumulation of specific structural, protective, and antifreeze proteins during CA (Juurakko *et al.* 2021). In winter cereals, the initiation and progress of CA seems to be carried out only during the vegetative stages, and on entering the reproductive stage (the emergence of reproductive primordia and the double ridge stage), the ability to acclimatise to cold is lost or stopped (Mahfoozi *et al.* 2001). The control effect of developmental stages on improving freezing tolerance is characterised as phenological regulation (Li *et al.* 2021). Consequently, all environmental or genetic factors regulating the speed of growth stages are important to determine the maximum cold tolerance and achieve successful overwintering in cold regions.

The physiological aspect of CA under controlled conditions and the improvement of freezing tolerance in plants has been widely investigated in Arabidopsis, tobacco, and winter cereals such as wheat and barley (Hassan *et al.* 2021; Li *et al.* 2021; Wójcik-Jagła *et al.* 2021; Wei *et al.* 2022). Although it is well established that under controlled conditions CA facilitates freezing tolerance in seedlings, temperature fluctuations under field conditions significantly diverge from controlled conditions and the rate of acclimatisation and the mechanisms involved in it have not been sufficiently elucidated. Under natural field conditions, winter cereals can experience both non-freezing and sub-zero temperatures during vegetative growth. Although there is some scattered information about wheat proteome response to cold acclimation, the protein changes following the onset of the reproductive stage have not yet been well identified. Understanding the nature of proteome changes on entering the reproductive stage provides valuable information for breeders.

It has been determined that the experience of mild, non-detrimental freezing during the vegetative period has a greater effect on improving freezing tolerance compared to acclimation with non-freezing temperatures, and the effect of sub-zero acclimation on gene expression and protein accumulation differs significantly from CA (Le *et al.* 2015). Acclimatization to sub-zero temperatures seems to be somewhat different from CA. Protein changes at sub-zero temperatures are mostly related to the processes of removing ice nuclei and some rearrangements in the cell walls, such as adding pectin and sugar side chains (Takahashi *et al.* 2021). Some alternations in cell wall synthesis and the accumulation of antifreeze protein (inhibitor of ice recrystallization) in extracellular space are more emphasised under sub-zero acclimation (Herman *et al.* 2007). Assessing the proteome response to both CA and sub-zero acclimation may provide valuable information about the cellular and molecular mechanisms involved in the improvement of freezing tolerance and such information may in turn offer a valuable basis for refining processes aimed at the improvement of freezing tolerance. The presence of very different acclimatisation conditions in the field with controlled conditions such as the presence of cold and dry winds, variable solar radiation during the day or on different slopes of land, snow cover, the occurrence of other environmental stresses such as drought and waterlogging, and pathogenic factors all clearly emphasise the need to investigate the cellular and molecular mechanisms involved in CA under field conditions. Although some studies have investigated CA in controlled conditions, it appears that the proteome response to CA under field conditions is different from that in controlled conditions. Therefore, this study aimed to gain a better understanding of the proteome response of winter-hardy wheat to different periods of CA under field conditions in a cold region of Iran.

MATERIAL AND METHODS

Experimental site. The current experiment was carried out under field conditions at Firoozkooh (N 55°35′, E 50°52′; 1979 m a.s.l. altitude), in the eastern region of Tehran, Iran. The region is mountainous and has very cold winters, and given the trend of decreasing air and soil temperatures during autumn, it is considered a very suitable acclimation environment for very cold-tolerant cultivars. The temperature conditions of the region are shown in Table 1.

Table 1. The meteorological characteristics of the Firoozkooh region.

Months	JAN	FEB	MAR	APR	MAY	JUNE	JULY	AUG	SEP	OCT	NOV	DEC
Average minimum temperature	-10.4	-9.1	-4	1.5	5	8.9	12.6	11.8	6.7	1.7	-4.1	-8.9
The number of days with a maximum temperature equal to or lower than 0°C	11.9	6.1	1.4	0.1	0	0	0	0	0	0	3.5	6.9
The number of days with a minimum temperature equal to or lower than 0°C	30.4	27.3	27	10.6	1.4	0	0	0	0.5	9.9	24.1	29.5
The number of days with a minimum temperature equal to or lower than 4°C	28.5	24.4	17.2	2.3	0	0	0	0	0	0.8	11.6	22
GDD (based on 18°C)	679.9	576	476	270.3	151.6	39.3	7.3	2.9	70.6	241.4	425.3	577.6
Minimum recorded temperature	-26.6	-24.5	-16	-13	-2.5	2	5	3	-1	-8.8	-15.6	-24
Relative humidity	67	63	56	49	44	43	44	40	43	49	57	64
Monthly rainfall (mm)	36.9	30.9	41.3	39.6	25.6	10.8	16.7	7.9	5.2	16.8	22.4	28.7
Snowy days	3.9	5.1	3.2	0.8	0	0	0	0	0	0.1	1.4	4

The seeds of a winter bread wheat (*Triticum aestivum*) cv. Norstra were sown on September 23rd and were harvested in different acclimation periods to evaluate cold tolerance and proteome changes. The Norstar cultivar is a forest-tolerant red winter wheat developed by Agriculture and Agri-Food Canada (AAFC), and released to the public in 1977. Simultaneously, some seeds were also sown in pots under greenhouse conditions where the air temperature was set at an 18/12°C (day/night) regime and used as unacclimated plants (control) 5 weeks after sowing (T1). To evaluate freezing tolerance, 10 (T2), 14 (T3), and 18 (T4) weeks after planting, 160 seedlings were harvested from the field and greenhouse along with the roots and transferred to the laboratory. After removing the leaves from near the crown and cutting off the roots, 10 crowns were placed in aluminium cylinders with a diameter and height of 15 cm and the cylinders were then filled with wet sand. The cylinders were transferred to the programmed freezer with a temperature decrease rate of 2°C per hour. Between -2 to -32°C, one cylinder was taken out of the freezer every hour and after defrosting at room temperature, the crowns were planted in pots under greenhouse conditions (25°C). The LT50 (the temperature which causes 50% of plants to die) was evaluated based on the survival and regrowth rate of the crowns which had experienced freezing temperatures.

Proteome analysis. The green and developed leaves of the plants were cut in the field (10, 14, and 18 weeks after planting) and immediately frozen in liquid nitrogen after being placed in aluminium foil. Protein extraction was carried out according to the method proposed by Damerval *et al*. (1986) and based on trichloroacetic acid (TCA)/acetone precipitation. The protein concentration of the leaf samples was determined using the 2-D Quant Kit procedure (GE Healthcare/Amersham Biosciences, Freiburg, Germany). The absorbance was read using a spectrophotometer (Spectrophotometer UVIKON-930, Italy) at a wavelength of 480 nm, and the protein concentration in the samples was estimated using a standard curve. The first step of electrophoresis (Isoelectric focusing) was carried out by adding 150 mg of protein from the extracted solution into the IPG-strip holder (for 13 cm strips). The IPG strips measuring 13 cm in length and with a pH range of 4–7 were then placed inside the IPG-strip holder. The maximum current intensity of the device was 50 microamperes and the separation process was carried out at a temperature of 20°C. The device was set for 14 hours without passing any current for rehydration operation and then the first stage of separation was carried out for 1 hour, during which time the electric potential difference in both the negative and positive poles reached 250 volts. In the second stage, the electric potential difference increased up to 500 V in 1 hour, in the third stage, this difference increased to 4000 V in 1 hour, and in the last stage, this difference was maintained for 5:35 hours. After completing the IEF steps, the strips were placed in separate jars and stored at -20°C for further use.

Following equilibration of the IPG strips, the second step of electrophoresis (SDS-PAGE) was performed. In this step, the proteins which were previously separated according to electric charge are separated according to weight. For this purpose, an SE 600 (Hoefer) electrophoresis tank for 13 cm IPG strips was used. 11.25% acrylamide gel was used in this step. To prepare the separation buffer, first 240 ml of HCl with a concentration of 1 normal was added to 183 g of Tris, and then 40 ml of 10% SDS was also added and its volume was brought to 500 ml with distilled water. The gels were stained according to the method proposed by Kang *et al.* (2002) using Coomassie Brilliant Blue (CBB G250). The stained gels were scanned using a UMAX Power Look III scanner and Power Scan 3.0 (Nonlinear Dynamics) software. Progenesis Samespot software (Nonlinear Dynamics) was used to analyse the spots and identify the changes between the treatments. Comparisons of the spots were made between sampling dates. After comparing the data related to the volume and density of the extracted spots and after calculating the standard deviation, T-tests were

performed for each of the comparisons and the number of spots which had changed at the statistical level of 5% was determined. The identification of the proteins was performed by Mass Spectrometry (MS) and Peptide Mass Fingerprint. Matrix-Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) MS (Bruker Daltonics) was used. The Liquid Chromatography-Electrospray Ionization (LC-ESI) MS/MS (Waters) method was used for those spots that were not identified by the aforementioned method and according to the instructions suggested by Amme *et al.* (2005).

RESULTS AND DISCUSSION

The investigation of the temperature conditions of the region during the experiment indicated that in the initial ten days of October, the temperature was above 10°C. After planting the seeds, the temperature gradually decreased until November reaching approximately -1.5°C, and for 20 days, the temperature remained at around 4°C. Despite the increase in temperature during November to nearly 10°C, this increase was not persistent and the temperature continued to decrease, reaching -7°C in December. This trend shows that the plants experienced the most serious sub-zero acclimation during December, which can significantly affect the expression process of cold-induced proteins and stimulate their accumulation. Although an increase in temperature up to 5°C occurred in December, this increase did not last and in the middle of December a sharp drop in temperature was observed and the temperature dropped to -15°C. A trend of increasing temperature was observed from January, and during March, the temperature increased by about 10°C. Crown dissection and shoot apex evaluation at 10, 14, and 18 weeks after sowing showed that the plants initiate the reproductive phase 18 weeks after sowing. Although it was expected that a period of 10 weeks of cold experience would suffice for vernalisation and the formation of the double ridge on the shoot apex to be achieved (as a sign of the beginning of the reproductive stage), the plants were still in the vegetative stage. This seems to be due to the prolonged sub-zero temperature and its inhibiting effect on the development of the apical shoot apex. At 14 weeks after planting, the double ridges were evident on the shoot apex. At 18 weeks after planting, the advanced reproductive stages were recorded by enlarging spikelets on the shoot apex.

During the investigation of the extracted proteome from different acclimation periods or control conditions, approximately 1000 protein spots were recorded on two-dimensional electrophoresis gels. A comparison of the protein spots between the plants 10 weeks after planting under field conditions (cold hardened) and the plants grown under control conditions showed that the expression or accumulation of 140 protein spots exhibited a significant increase, while 48 proteins showed down-regulation (Fig. 1). Some of the identified proteins in the comparison were: Cytochrome P450-like (with a 2.31-fold increase), ribulose-1 5-bisphosphate carboxylase activase (with a 2.25-fold increase), glyceraldehyde-3-phosphate dehydrogenase (with a 2.09-fold increase), Fructose- bisphosphate aldolase (with a 2.03-fold increase) and the AP2 domain transcription factor (with a 2-fold increase), which showed the highest amount of up-regulation, respectively. The mentioned proteins are mainly involved in the photosynthetic redox reaction, the biosynthesis of some secondary metabolites, photosynthesis, and response to cold and light stimuli, glycolysis, and transcription. The enzyme RuBisCO activase is necessary for the formation of carbamate (the connection of CO_2 to the amino acid lysine in the active site of the Rubisco enzyme). This enzyme also plays a crucial role in the inhibition of certain competitive inhibitors such as 2-Carboxy-D-arabitinol 1-phosphate at the active site of the Rubisco enzyme. These findings are consistent with research showing that RuBisCO activase plays an important role as a chaperone in the biochemical limitations of photosynthesis in cereal crops under abiotic stresses (Perdomo *et al.* 2021). The AP2/ERFs transcription factor is a member of the C-repeat/ Dehydration-Responsive Element-Binding-Factor family and their role in cold stress conditions is well defined, initiating a complicated regulatory network to control reactive oxygen species, protect key enzymes, and maintain internal structures (Ritonga *et al.* 2021). On the other hand, the greatest decrease was related to spots No. 690 (unidentified with 77% reduction), 542 (unidentified, with 70% reduction), 837 (Adenosine kinase-like protein, with 50% reduction), 951 (the metal ion transmembrane transporter, with 47% reduction) and 489 (UDP-glucuronate decarboxylase/catalytic, with 45% reduction). These proteins play a role in the processes of purine ribonucleoside reuse (489) and ion transfer (951). UDP-glucuronate decarboxylase plays an important role in intracellular metabolic processes and it seems to be involved with pectin biosynthesis. However, these findings do not support previous research which showed the up-regulation of UDP-glucuronate decarboxylase under long-term cold acclimation (Borg *et al.* 2021).

The changes in the expression or accumulation of some proteins during cold acclimation were similar to freezing tolerance (Fig. 2). The names and functions of the proteins which exhibited similar changes in freezing tolerance are shown in Table 2. The results showed that the chaperone-like activities, changes, and reorganization of membrane lipids, maintaining the structure of proteins, photosynthesis, energy production, and scavenging of reactive oxygen species were the most consistent with the LT process and such activities seem to make a significant contribution in plant adaptation to cold and play key roles in the development of freezing tolerance. The results are mainly in line with our previous findings under field conditions (Janmohammadi *et al.* 2014).

Table 2. The names and functions of the identified proteins whose expression trend was similar to the freezing tolerance trend during the sampling dates.

Spot No.	Protein	Function
772	70 kDa heat shock protein	Protein folding, response to abiotic stress
993	Extracellular lipase	degradation of lipid
263	Pyruvate dehydrogenase kinase isoform 2	Phosphorylation, signal transduction
359	Fructose-bisphosphate aldolase	Glycolysis
398	ribulose-1,5-bisphosphate carboxylase activase	Photosynthesis, response to cold and light
407	Photosystem II stability/assembly factor HCF136	Photosynthesis
419	cytosolic malate dehydrogenase	Glycolysis, malate metabolism, oxidation, and reduction
423	Ps16 protein	Transcription, response to cold stress
427	MRNA-binding protein	Metabolic processes of the cell
443	chloroplast fructose-bisphosphate aldolase	Glycolysis
446	Cytochrome c	Electron transfer in the respiratory chain
448	glycerol-3-phosphate dehydrogenase	oxidation and reduction
454	fructose-bisphosphate aldolase	Glycolysis
563	mitochondrial cysteine synthase	Amino acid biosynthesis
567	Cytochrome P450-like	oxidation and reduction
707	MLA27-2	Apoptosis, defense processes
932	protein At2g37660, chloroplast precursor	Defense and metabolic processes
153	ascorbate peroxidase	Scavenging, oxidation and reduction
144	Regulator of chromosome condensation/beta-lactamase-inhibitor protein II	Transcription
190	putative 20 kDa chaperonin, chloroplast	Protein folding
412	MADS-box transcription factor 26	Regulation of transcription
650	Os03g0189600	oxidation and reduction

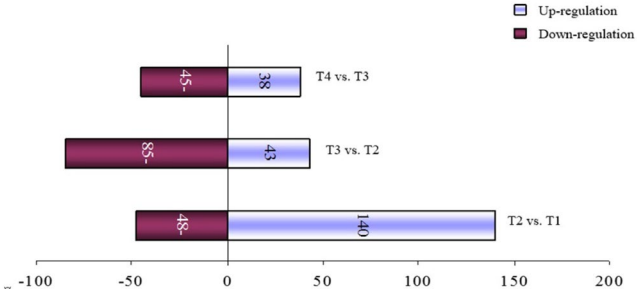

Fig. 1. A comparison of the protein spots between different sampling dates and the number of down-regulated and up-regulated proteins. T1: Plant grown under greenhouse conditions as unacclimated or control, T2, T3, T4: plants acclimated 10, 14, and 18 weeks under field conditions, respectively

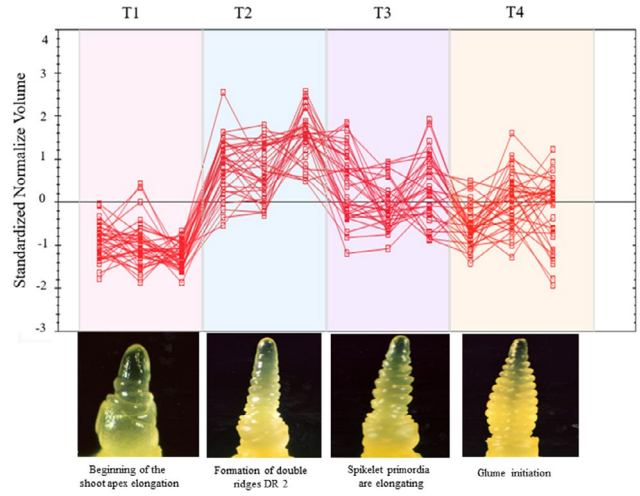

Fig. 2. The expression or accumulation trend of some proteins was quite similar to the pattern of the improvement of freezing tolerance during cold acclimation periods.

The comparison of the proteome between 14 and 10 weeks after planting under field conditions showed that by the beginning of the reproductive stage the expression or accumulation of some proteins such as spots No. 742 (unidentified with a 1.64-fold increase), 752 (Putative purple acid phosphatases, with a 1.61-fold increase), 542 (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, with a 1.56-fold increase, Fig. 3), 569 (ATP synthase CF1 beta subunit, with 1.42-fold), 692 (Oxygen-evolving enhancer protein 2, with 1.37-fold), 838 (Aconitate hydratase cytoplasmic, 1.35-fold) and 783 (Histone H2B.2,

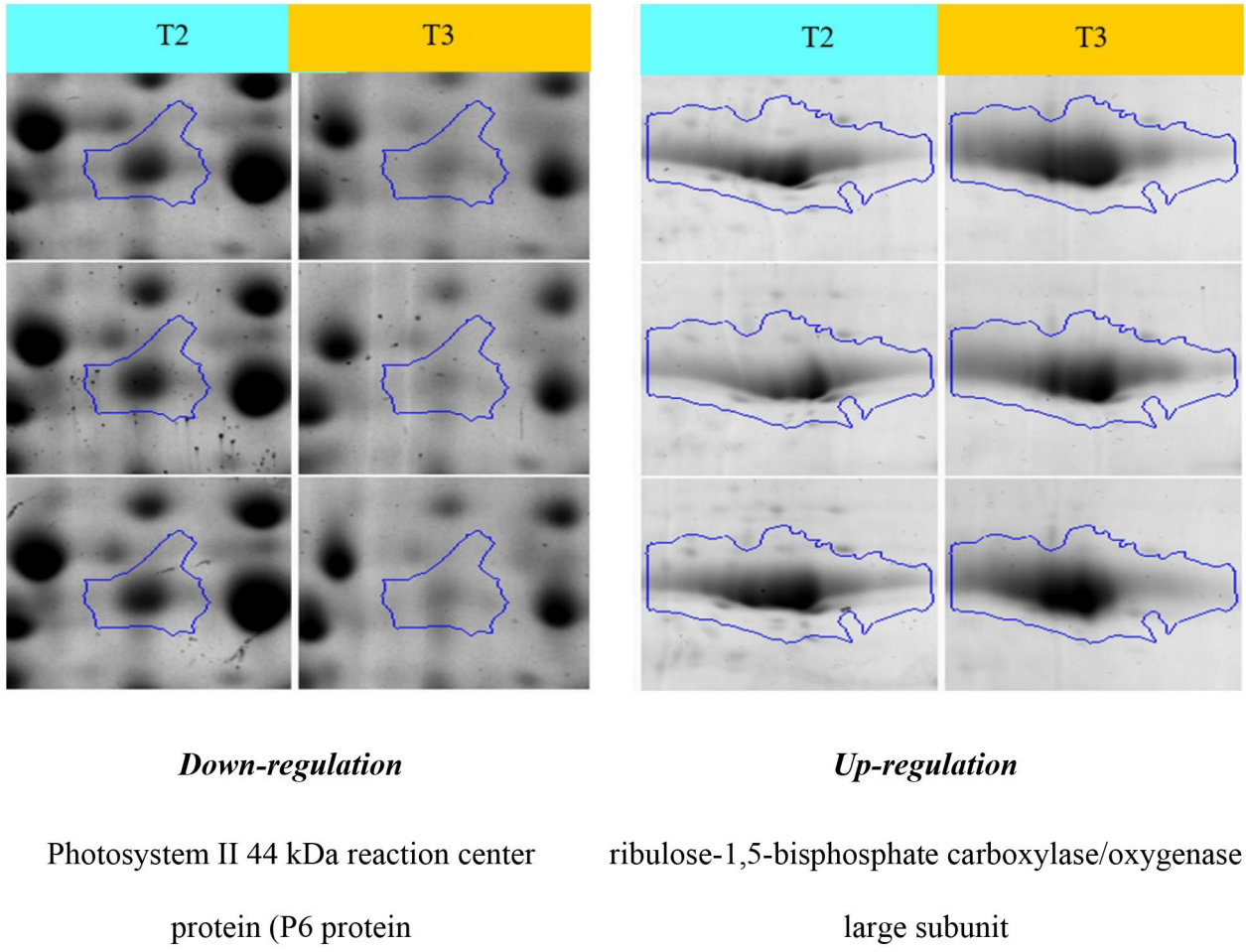

Fig. 3. An example of up and down-regulation in wheat seedlings (cv. Norstar in the Firoozkooh region during the transition from the stage of maximum freezing tolerance (T2) to the beginning of reproductive growth (T3).

with 1.34-fold) had increased. An overview of the categories and function of the mentioned proteins indicates the strengthening of the photosynthetic apparatus, the energy production pathways, and intracellular metabolic activities during this sampling period. Furthermore, the highest down-regulation was related to spots No. 692 (actin, with a 57% decrease), 951 (dehydration response element binding protein, by 51%), 945 (aldehyde-lyase/threonine aldolase, by 48%), 468 (ribulose 1) 5-bisphosphate carboxylase, large subunit, by 43%) and 335 (Photosystem II 44 kDa reaction centre protein, by 41%, Fig. 3).

During the transition from the T3 stage (early reproductive development) to T4, the expression of proteins such as Oxygen-evolving enhancer protein 2 and ribosomal protein S1, UDP-glucose pyrophosphorylase, Fructose-bisphosphate aldolase, both alpha and beta enzyme subunits Rubisco as well as Rubisco activase all increased. The increase in the expression of the enzymes involved in photosynthesis (490, 648, 707, 802, and 939) can be attributed to the improvement of thermal conditions and the start of the rapid growth of the plants after passing the rosette period. The investigation of the quantum performance of photosystem II also demonstrated a significant increase during this period. The enzyme UDP-glucose pyrophosphorylase plays a role in converting glucose 1-phosphate and UTP to UDP-glucose, and considering the numerous uses of UDP-glucose inside the cell (such as starch synthesis), it seems that the increase in the expression of this enzyme indicates an increase in photosynthetic production. The rapid growth of the plant coincided with the initiation of the reproductive stage. Furthermore, the increase in the expression of fructose-bisphosphate aldolase enzyme, which is involved in the glycolysis pathway, also played a significant role. This enzyme breaks down the fructose 1,6-bisphosphate molecule into two molecules of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, which contribute to the reactions of this pathway in the production of energy and some intermediates which are necessary for the biosynthesis of other metabolites.

The cell membrane is the primary site for receiving signals related to temperature, and the membrane fluidity may be decreased under cold stress (Dhaliwal & Angeles-Shim 2022). Cold stress changes the physical and chemical composition of cell membranes and is also associated with disruption to the order and rearrangement of microtubules and actin microfilaments (Byun *et al.* 2021). The cytoskeletal components can act as LT sensors under cold stress, so actin changes act as a mediator for transmitting the external signal into the cell and connecting it with the calcium-dependent pathway (Pareek *et al.* 2017). It seems that following vernalisation and the rise in temperatures the amount of free actin in the cell decreased and this can be attributed to the lower activity of actin depolymerizing factors (Inada 2017). Therefore, alterations to the cytoskeletal components were suggested to be imperative for the development of LT tolerance in plants. In terms of the down-regulation of spot No. 951, it can be stated that dehydration-responsive element-binding proteins include a large family of proteins such as CBF transcription factors which are involved in the regulation of gene expression related to freezing tolerance. The decrease in the expression of the mentioned protein may refer to the occurrence of the suppression of LT-induced genes by the initiation of the reproductive phase (Laudencia-Chingcuanco *et al.* 2011). With the progress of the reproductive stage (18th week), the expression of most of the proteins involved in the process of photosynthesis and energy production increased, and the plant reprogrammed for the new stage based on energy production and increased growth.

CONCLUSION

The findings reveal that winter-hardy wheat seedlings can follow the acclimation process even in sub-zero temperatures under field conditions. Sub-zero acclimation significantly improves frost tolerance and LT50 reached -28°C during the vegetative growth. Our results show that the changes in some proteins were largely similar to the freezing tolerance trend. Most cold-induced spots were classified as photosynthetic proteins, transcription factors, and molecular chaperones. The role of proteins involved in cell membrane changes, cytoskeleton rearrangement, and the scavenging of reactive oxygen species was also evident during cold acclimation. We found that the expression or accumulation of cold-induced proteins can be affected by developmental stages, and the highest accumulation of LT-induced proteins was observed during the vegetative period. On entering the reproductive stage and the formation of the double ridge stage on the shoot apex, the expression of some LT-induced proteins decreased. However, some proteins are up-regulated by the initiation of the reproductive phase and the loss of frost tolerance, which often play a role in respiratory pathways, protein protection (chaperone), energy supply, glycolysis, and transcription, and their expression or accumulation increases with the progress of the developmental stages. After passing through the double ridge stage and the initiation of the reproductive stage, the expression of some proteins related to CBF genes such as the APETALA2/ethylene response factor (AP2/ERF) decreased.

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REZIME

Botanica SERBICA

Proteomski odgovor otporne ozime pšenice na privikavanje na hladnoću

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Proteomska analiza ozimih useva tokom hladne aklimatizacije i prezimljavanja može pružiti važne informacije za osmišljavanje procesa oplemenjivanja. Aktuelni eksperiment je sproveden kako bi se istražile promene proteoma u ozimoj pšenici otpornoj na mraz (cv. Norstar) tokom različitih perioda aklimatizacije na hladnoću (CA) u poljskim uslovima u region koji se odlikuje velikom nadmorskom visinom i niskim temperaturama pomoću dvodimenzionalne proteomike zasnovane na gelu. Rezultati su pokazali da je otpornost na mraz značajno povećana zahvaljujući CA i letalne temperatura smrzavanja (LT50) 10, 14 i 18 nedelja nakon setve semena su iznosile -28°C, -22°C i -10°C, respektivno. Do početka reproduktivne faze (faza dvostrukog grebena), LT50 se značajno smanjio. Oko 1000 proteinskih tačaka se može razlikovati nakon Coomassie bojenjem na gelovima. Proteini promenjeni tokom CA su često imali funkcionalnu ulogu u fotosintezi, proizvodnji energije (glikoliza), transkripciji, aktivnostima sličnim šaperonu, reorganizaciji membrane i citoskeleta, transportu, redoks prilagođavanju i signalizaciji. Rezultati su otkrili da promene u proteinima hloroplasta, nekim transkripcionim faktorima kao što je transkripcioni faktor 26 sa MADS domenom i antioksidativnim enzimima (askorbat peroksidaza) imaju sličan trend sa tolerancijom na smrzavanje, a njihova ekspresija opada sa početkom reproduktivnog rasta i gubitkom tolerancije smrzavanja. Tokom perioda aklimatizacije, većina promena je bila usmerena na odbrambene sisteme i preuređenje citoskeleta, dok su sa početkom reproduktivnog rasta glavni prioritet postali fotosinteza i proizvodnja energije.

Ključne reči: antioksidant, tolerancija smrzavanja, glikoliza, fotosintetski, proteomika, proteini koji reaguju na hladnoću