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SOLUBLE PROTEINS INDUCED BY LOW TEMPERATURE TREATMENT IN THE LEAVES OF SPRING AND WINTER WHEAT CULTIVARS

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Abstract

Quantitative and qualitative changes in total leaf soluble proteins were determined in a spring cv. Zagross and in a winter cv. Azar 2 cultivar of allohexaploid wheat (*Triticum aestivum* L.) exposed to 4°C for two weeks. The data obtained were used to test whether these different genotypes of wheat responded differentially to cold treatment. Seedlings were grown in a controlled growth chamber for 14 d at 20°C and then transferred to 4°C for 14 d before returning them to 20°C (cold treatment), or else they were maintained continuously at 20°C (control treatment). The plants were sampled every 48 h for total leaf fresh weight measurements. Total leaf soluble proteins were extracted. Proteins concentration was either determined by a colorimetric method, or size-fractionated on SDS-PAGE. Clear cold-induced increases in proteins quantity occurred during the low temperature treatment irrespective of cultivar. However, the electrophoretic protein patterns showed differences between-cultivar and between-temperature treatment. Ten new cold-induced polypeptides (17, 19, 30, 77, 83, 90, 100, 166, 180 and 200 kDa) were produced from 2 d at 4°C reaching their maximum amounts on 6-10 d at 4°C regardless of cultivar. With increasing exposure to 4°C, fewer new cold-induced higher molecular weight (HMW) polypeptides (166, 180 and 200 kDa) was observed compared with those produced over the first week regardless of cultivar. During the second week, many new cold-induced lower molecular weight (LMW) polypeptides were detected at 4°C. This alteration in polypeptide composition from HMW to LMW occurred about 4 d earlier in the cold-treated seedlings of winter wheat compared with spring wheat. Perhaps cold-shock proteins are a component of this cold-induced response.

Introduction

Among abiotic stresses, low temperature (cold and frost) constitutes one of the major hazards to agriculture and is an important factor that limits the survival, productivity and geographical distribution of plants in large areas of the world (Boyer, 1982). Exposure to a low non-lethal temperature usually induces a variety of biochemical, physiological and molecular changes in plants which can results in an acclimation response that is characterized by a greater ability to resist injury or survive an otherwise lethal low temperature stress (Levitt, 1980). This process is known as cold acclimation (Howarth & Ougham, 1993; Hughes & Dunn, 1996). Low temperature can result in the synthesis of cold shock proteins (Hughes & Dunn, 1996). In *Lolium temulentum*, progressive changes in gene expression resulted from few days or longer exposure to low temperatures of 5°C and these alterations occurred in parallel with an increase in chilling or freezing tolerance

(Ougham & Howarth, 1988). On the other hand, one feature of cold hardening in some plant species is an accumulation in soluble protein content *e.g.*, *Medicago sativa* (Mohapatra *et al.,* 1987), *Triticum aestivum* and *Secale cereale* (Howarth & Ougham, 1993; Hughes & Dunn, 1996; Sarhan *et al*., 1997; Kolesnichenko *et al*., 1997, 2000), *Hordeum vulgare* (Crosatti *et al*., 1996, 1999; Cattivelli *et al*., 1997; Bravo *et al*., 1999) and *Brassica napus* (Sangwan *et al*., 2001; Lee *et al*., 2002; Karimzadeh *et al*., 2003). The accumulation of a high molecular weight *e.g.* 200 kDa (Howarth & Ougham, 1993; Sarhan *et al*., 1997) and 310 kDa (Kolesnichenko *et al*., 1997) polypeptide is a major change that occurred during hardening in wheat. The latter cold stress protein (310 kDa) was also reported in rye by Kolesnichenko *et al*., (2000).

Increases in levels of certain RNA species as a result of cold exposure (5°C) in alfalfa and spinach (*Spinacia oleraceae*) were detected by Mohapatra *et al*., (1987) and Guy & Haskell (1987). Changes in gene expression and the synthesis of cold shock or cold acclimation proteins is correlated with enhanced cold tolerance (Hughes & Dunn, 1996; Thomashow, 1998). Synthesis of specific proteins is an important mechanism involved in increasing freezing tolerance during cold acclimation (Antikainen *et al.,* 1996; Sarhan *et al.,* 1997; Guy, 1999). Bravo *et al*., (1999) reported the accumulation of an 80-kDa DHN-like protein (P-80) in barley under cold acclimation 6/4°C (day/night) in cold-acclimated leaves. The low temperature-induced accumulation of proteins (12, 26, 40, 50, 66 180 and 200 kDa) encoded by the *Wheat Cold Shock* (*WCS*) 120 gene family was reported for more that 20 wheat genotypes (Sarhan *et al*., 1997). In this gene family, 6 different genes viz., *WCS40*, *WCS*66, *WCS80*, *WCS120*, *WCS180* and *WCS200* are involved (Hude *et al.,* 1992). Another wheat cold-induced protein, *WCS19* that consists of 190 amino acids, is produced only in the leaves at low temperature and stimulated by light (Chauvin *et al.,* 1993).

In the present study, we examined whether spring and winter cultivars of wheat responded differently to cold treatment through differential protein accumulation. The aim of the work reported here was to test this hypothesis by comparing quantitative amounts and electrophoretic patterns of the total soluble proteins that accumulated in leaves of spring and winter cultivars of allohexaploid wheat in a treatment comprising shifts from 20 to 4 back to 20°C.

Materials and Methods

Plant material and growth conditions: Seeds of a spring (cv. Zagross) and a winter (cv. Azar 2) wheat (*Triticum aestivum*, 2n = 6x = 42) cultivars supplied from the Seed and Plant Improvement Institute (SPII), Karaj, Iran, were initially grown in plastic pots (150 mm diameter × 150 mm deep) filled with a mixture of five parts soft mold leaves and two parts loamy sand. The Zagross cultivar with spring habit is semi-susceptible to cold and tolerant to drought, and is grown in temperate and semi-aried regions of Iran. Azar 2, a recently-released winter habit cultivar, has a partial resistance to yellow rust and is tolerant to cold and drought. Azar 2 is cultivated in elevated regions of the country in the cold winter season.

Two replicate pots per cultivar per temperature treatment per sample time, each containing 5 seedlings were established. The pots were kept in a controlled growth chamber at a constant air temperature of 20 ± 1°C with illumination provided by white fluorescent tubes at a fluence rate of 140 W m^{-2} PAR at soil level for 12 h d^{-1} (0800-2000

h). Seedlings were maintained in these conditions until experimental day 14. The day of sowing was designated as experimental day 0. At 1000 h on day 14, after taking a sample, the remaining seedlings were either transferred to a constant air temperature of 4 ± 1°C at the same fluence rate and photoperiod as above for 14 d and then returned to 20°C on day 28 until the end of the experiment (day 40; cold treatment), or they remained at a constant 20°C throughout the experimental period (control treatment).

Sampling times: Over the, 14-40 days experimental period 3 × 0.5 g of total leaf fresh weight samples (Dunn *et al*., 1990, 1998; Crosatti *et al*., 1996) were sampled randomly every 48 h from two pots of five seedlings. In other words, at 1000 h, the sampling times for each cultivar-low temperature treatment combination were days 14 (before transfer to 4°C), 16, 18, 20, 22, 24, 26, 28 (during exposure to 4°C), 30, 32, 34, 36, 38 and 40 (after the return of cold-treated seedlings to 20°C (Fig. 1). At the same sampling times and daytime, samples were also taken from the controls.

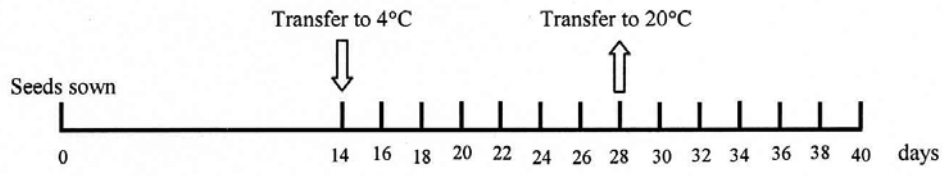

Fig. 1. Experimental protocol. The arrows indicate the days of the downshift and upshift of temperatures and sampling days.

Protein extraction: Total soluble proteins were extracted from the leaves with a modification of the method described by Guy *et al*., (1992). This consisted of homogenization with a chilled mortar and pestle using a buffer containing ice-cold 50 mM Tris-HCl, pH 7.5; 2 mM EDTA and 0.04% (v/v) 2-mercaptoethanol. The homogenate was centrifuged at 4000 rpm for 30 min., at room temperature (Masoodi-Nejad & Yazdi-Samadi, 1992). Supernatant was re-centrifuged for 20 min., and stored at –20°C for analysis (Hames & Rickwood, 1990).

Quantification of protein using the bradford assay: Protein extracts were thawed and their concentration determined by a colorimetric method as described by Bradford (1976) using a commercially available reagent (Bio-Rad protein assay dye reagent). In the Bradford assay, protein concentration is determined by quantifying the binding of the dye, Coomassie Brilliant Blue G-250, to the unknown protein solution, as compared to known standards. Tubes containing 100 µl aliquots of known concentrations of Bovine Serum Albumin (BSA; 0.156 mg l^{-1} to 10 mg l^{-1} in 0.15 M NaCl), were prepared. Blank tubes containing 100 µl of 0.15 M NaCl were also prepared. One ml Coomassie Brilliant Blue solution was added to each tube and the mixtures vortexed. The reactions were left at room temperature for 2 min. The absorbance at wavelength of 595 nm was determined against the blank and the standard curve of absorbance versus protein concentration plotted (Copeland, 1994). Reactions containing dilutions of the soluble protein extracts (unknown concentrations) were set up as above and the absorbance at 595 nm determined. The proteins concentration of the extracts was determined from the standard curve, using an Unicam 8620 UV/VIS (USA) Spectrophotometer.

Table 1. Mean squares (MS) of the ANOVA for leaf soluble proteins of wheat (*Triticum aestivum* L.) cultivars.

Source of variation	df	MS
Cultivar (cv.)	1	0.000039$^{n.s.}$
Temperature treatment (T)	1	0.201094***
Sampling time (S)	13	0.006149*
cv. × T	1	0.000001$^{n.s.}$
cv. × S	13	0.000629$^{n.s.}$
T × S	13	0.011591***
cv. × T × S	13	0.001610$^{n.s.}$
Error	112	0.003493
Total	167	

$^{*}P<0.05$; $^{***}P<0.001$; $^{n.s}$., non significant.

Protein electrophoresis: The leaf proteins homogenate was mixed with buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 5% (v/v) 2-mercaptoethanol, 10% glycerol and 0.01% bromophenol blue and then heated at 100°C for 2 min., and centrifuged at 4000 rpm for 30 min. Supernatant was stored at –20°C for later analysis. Protein extracts were thawed and separated, as polypeptides, by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in 15% gels; 10 µg of soluble total protein was loaded in each well. Gels were fixed in trichloroacetic acid and stained in 0.1% (w/v) coomassie brilliant blue G-250, 10% (v/v) glacial acetic acid and 40% (v/v) methanol. The gels were placed in freshly prepared destaining solution (40% methanol, 10% glacial acetic acid) with gentle shaking for 30 min. This process was repeated until the gels were sufficiently destained. The gels were then photographed and/or stored in 10% glacial acetic acid.

Statistical analysis: The quantitative amounts of total protein were statistically analysed using three- factorial balanced analysis of variance (ANOVA) on the basis of randomized complete design (RCD) with three replications. Cultivars, temperature treatments and sampling times were considered as factors with 2, 2 and 14 levels, respectively. The protein data were analyzed after log x+1 transformation. Analysis of variance was conducted using Multi-Factorial Balanced Model in Minitab Statistical Software (Minitab Inc., State College, PA, USA; Fry, 1993; Ryan & Joiner, 2001); identification of differences in response of the cultivars to the temperature treatments at the sampling times are based on the outcome of these tests. An additional analysis was carried out in order to test between-temperature treatments difference within each cultivar at each sampling time (Montgomery, 2001).

Results

Leaf total soluble proteins increase in both cultivars at 4°C: ANOVA analysis showed (Table 1) that there were significant differences between-temperature treatments (P<0.001), between-sampling times (P<0.05) and their interaction (P<0.001). Considering all samplings times, both cultivars showed a similar response to a 20 to 4°C temperature change (P>0.05). Transfer of seedlings from 20 to 4°C on day 14 resulted in significant increases in leaf total protein amounts in both cultivars during the low temperature period compared to seedlings maintained throughout at a constant temperature of 20°C (Fig. 2a & b). The return of cold-treated seedlings to 20°C resulted in the production of proteins in leaves to levels similar to those in the controls in both the spring and the winter wheat cultivars.

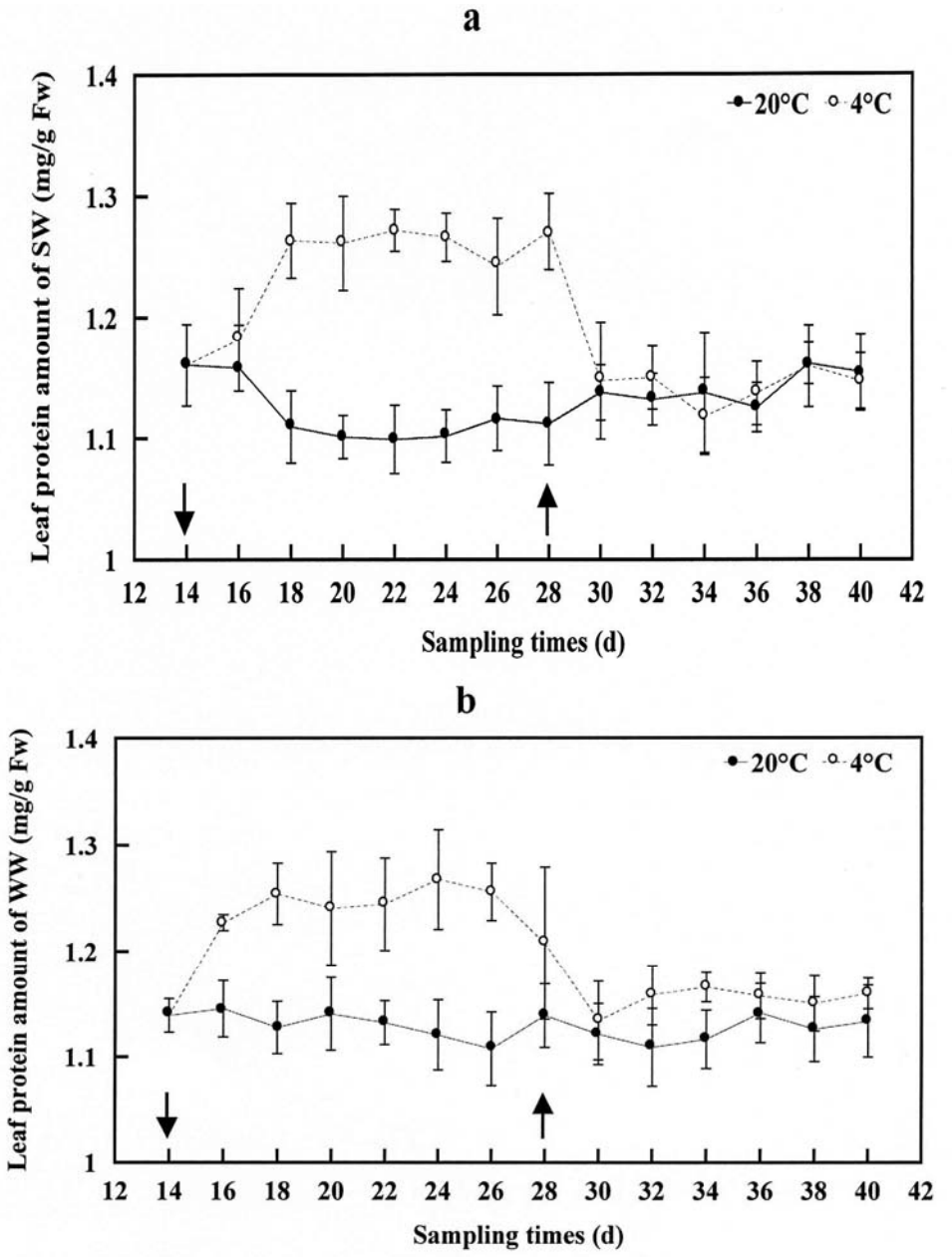

Fig. 2. The curve showing the changes of mean (n = 3) quantitative amount of total leaf soluble proteins of a) spring wheat cv. Zagross and b) winter wheat cv. Azar 2 grown either at a constant 20°C (solid line) or at 20°C followed by transfer to 4°C on day 14 for 14 d and then transferred back to 20°C on day 28 (dotted line). The arrows show the timing of the dowshift and upshift of temperatures.

SW = Spring wheat, WW = Winter wheat, d = day

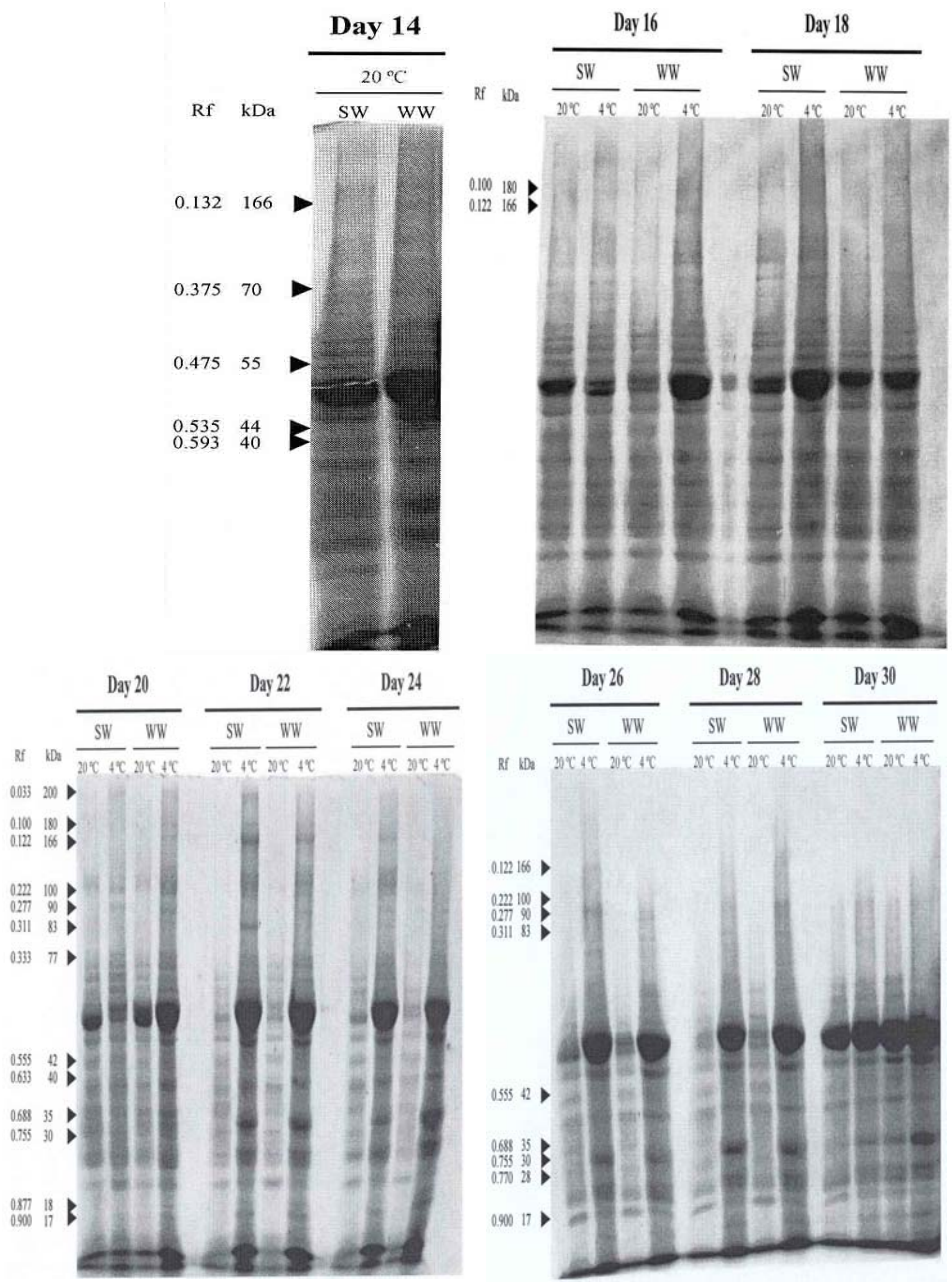

Fig. 3. SDS-PAGE patterns of total leaf soluble protein fractions from a spring wheat (SW; *Triticum aestivum* L.) cv. Zagross and a winter wheat (WW) cv. Azar 2 grown either at a constant 20^{o}C (shown as 20^{o}C), or at 20^{o}C followed by transfer to 4^{o}C on day 14 for 14 d and then transferred back to 20^{o}C on day 28 (shown as 4^{o}C). The arrows indicate bunds which were induced on experimental days 14-18 and experimental days 20-30.

Table 2. Differences observed in the electrophoretic patterns of total soluble leaf proteins of spring (cv. Zagross) and winter (cv. Azar 2) wheats (*Triticum aestivum* L.) in two temperature treatments.

Sampling times (days)	Observed polypeptides in spring wheat (kDa)		Observed polypeptides in winter wheat (kDa)	
	only at 4°C	only at 20°C	only at 4°C	only at 20°C
16	180*	---------	166, 180	---------
18	166, 180	---------	166, 180	---------
20	90, 200	---------	17, 90, 166, 180, 200	---------
22	17, 19, 30, 77, 83, 90, 100, 180, 200	35, 42	17, 19, 30, 77, 83, 90, 100, 180	35, 42
24	17, 30, 77, 83, 100, 180, 200	35, 42	17, 19, 30, 77, 83	35, 42
26	17, 30, 77, 83, 90, 166	35, 42	17, 30, 77, 83, 90, 100	35, 42
28	17, 30, 77, 83, 90, 100	35, 42	17, 30, 77, 83, 90, 100	35, 42

* Observed at low density

New polypeptides detected in both cultivars at 4°C: Quantitative changes into leaf soluble protein were similar in response to cold treatment in both the spring and winter wheat cultivars. However, to check if there were any qualitative changes in polypeptide composition between cultivars, the total soluble proteins were size-fractionated on SDS-PAGE. On day 14 (before transfer to 4°C), two polypeptides with molecular weights of 40 and 55 kDa were detected at low density in the spring cultivar but not in the winter cultivar (Fig. 3a). Conversely, at this sampling time, 44 and 166 kDa-polypeptides were observed in the winter but not in the spring wheat. Over the low temperature period (days 14-28), other differences in the electrophoretic patterns of proteins in the spring and winter wheat cultivars in two temperature treatments were detected and are summarized in Table 2 (Fig. 3a & b). Two days following transfer to low temperature (day 16), cold-induced polypeptides were induced in both cultivars (e.g. 180 kDa in spring and 166 and 180 kDa in winter wheat; Fig. 3a; Table 2). In general, the major changes occurred during the second week at low temperature in both cultivars: the maximum number of changes occurred on day 22 (8 d at 4°C; Fig. 3b; Table 2). However, at this sample time (day 22), two polypeptides of 35 and 42 kDa were also detected at low density in both cultivars at 20°C but not at 4°C. In both cultivars, during the low temperature period, 10 different new cold-induced polypeptides (17, 19, 30, 77, 83, 90, 100 at low density and 166, 180 and 200 kDa at higher density) were detected for a period of time at 4°C but not at 20°C. The protein concentration of some of the above-mentioned polypeptides differed between cultivars and between temperature treatments within a cultivar.

Discussion

Cold-induced quantitative changes in total leaf proteins: The 14-d exposure to 4°C, resulted in significant increases in total proteins in the leaves of both cultivars. However, following the return of cold-treated seedlings to 20°C, the total leaf protein amount was similar to that in the controls maintained at 20°C. In fact, following the transfer of cold-treated seedlings to 20°C (*i.e.* days 30-40), protein accumulation tailed off. Thus, we observed a clear difference between-temperature treatment within a cultivar during the cold treatment period. Though both cultivars responded similarly to temperature alteration over the experimental period (*i.e.* days 14-40), the most substantial changes in proteins amount occurred during the first week of exposure to 4°C: the winter wheat cultivar responded more obviously and rapidly (particularly over the first 48 h of low temperature) than the spring wheat cultivar to temperature changes. This more rapid

response to a low temperature by the winter wheat cultivar indicates a more sensitive response compared with the spring wheat. In agreement with these data, Karimzadeh *et al*., (2000) also reported a significant cellular protein accumulation during the first week (in particular 7 d at 4°C) of low temperature treatment in winter wheat compared with spring wheat. In another experiment on spring (cv. Reyhan) and winter (cv. Makouei) and cultivars of barley (*Hordeum vulgare* L.) exposed to 4°C for 14 d, low temperature induced increases in proteins quantity over the second week of the exposure to cold treatment irrespective of cultivar however, these changes were also detected in the winter cultivar 2 d before comparable changes in the spring cultivar (Unpublished data). The cold-induced accumulation of leaf soluble proteins was also detectable in non cereal plants *e.g*., canola. Using spring and winter cultivars (Hyola 401 and Symbol, respectively) of canola (*Brassica napus* L.) seedlings exposed to 4°C for 3 weeks, Karimzadeh *et al*., (2003) also reported clear cold-induced increases in soluble protein amounts at low temperature treatment in winter canola but not in spring canola. These increases appeared 8 d following the cold exposure to 4°C and were maintained until 2 d following the return to 15/10°C (day/night) in the leaves of the winter canola cultivar.

Cold-induced electrophoretic changes in total leaf proteins: Changes in the electrophoretic patterns of the leaf soluble proteins showed between temperature treatments differences within a cultivar and between-cultivar differences. When winter wheat seedlings were transferred from 20 to 4°C, the appearance of two new polypeptides of 166 and 180 kDa and the increases in the density of some of pre-existed protein patterns in the leaves were consistent with the increase in total soluble protein found on experimental day 16 (2 d at 4°C). Such response was not detected in the spring wheat cultivar at this sampling time.

In general, both wheat cultivars showed a cold-induced quantitative protein amount in their leaves but they differed regarding the timing of appearance of new polypeptides. Overall, during the two week exposure to low temperature treatment, a range of new cold-induced polypeptides (10 polypeptides of 17, 19, 30, 77, 83, 90, 100, 166, 180 and 200 kDa) were produced in the cold-treated leaves regardless of cultivar. Surprisingly, with increasing time, only a few new cold-induced higher molecular weight (HMW) polypeptides (166, 180 and 200 kDa) which were mostly produced over the first week at 4°C in the leaves of both wheat cultivars, were then replaced by numerous new cold-induced lower molecular weight (LMW) polypeptides during the second week of exposure to 4°C. This alteration in polypeptide profiles occurred about 4 d earlier in the cold-treated seedlings of winter wheat compared with spring wheat. Sarhan & Perras (1987) studied the changes of epicotyl protein patterns of three allohexaploid wheat (cv. Clenlea, spring wheat; cvs. Fredrik and Norstar, winter wheat) cultivars following 10, 20 and 30 d at 2°C in darkness in comparison with seedlings maintained at 22°C for 3, 4 and 5 d. They reported that low temperature resulted in the production of a new low temperature-induced protein with a molecular weight of 200 kDa. This protein was observed strongly in both wheat cultivars and in a lower density in the spring wheat. They also reported increases in three polypeptides (42, 47 and 48 kDa) and decreases in five polypeptides (63, 67, 80, 89 and 93 kDa) in cold-acclimated epicotyls compared with non-acclimated ones (Sarhan & Perras, 1987). One of the reported cold-induced polypeptides (200 kDa) was also detected in wheat cultivars studied in the present work.

In addition to this, we detected a range of other protein patterns at low temperature exposure in the spring and winter wheat cultivars.

Perras & Sarhan (1989) studying freezing tolerance proteins in leaves, crown and roots of winter wheat cvs., Norstar and Fredrik reported that the major changes in protein patterns were observed in seedlings following 10 d at 6/2°C (day/night) compared with those maintained at 24/20°C (day/night) for 9 d. During cold hardening, the 200 kDa new-low temperature protein accumulated in the leaves, roots and crown. Moreover, an increase in the concentration of 8 polypeptides (31, 38, 43, 52, 68, 74, 77 and 180 kDa) was also reported in those three tissues but the concentration of three others (34, 142 and 157 kDa) decreased in the tissues in hardened seedlings. The 200 kDa-protein accumulated in leaves and crown tissues rather than root tissues (Perras & Sarhan, 1989). Most of our findings are in agreement with the resultant data reported (Sarhan & Perras, 1987; Perras & Sarhan, 1989; Sarhan *et al.*, 1997). Hence, the work reported here, and earlier published observations indicate that the pattern of polypeptide synthesis at low temperatures (2, 4 or 6°C) is remarkably similar regardless of cultivar. Indeed, the response is more to do with the wheat genome's reaction to the low temperature and less to genotypic influences. Presumably, it is the way in which these cold-induced polypeptides are utilized that distinguishes winter from spring varieties.

To what extent can the observation of new polypeptides as a result of low temperature treatment be attributed to the induction of cold shock proteins? Ougham (1987), Ougham & Howarth (1988), and Howarth & Ougham (1993) reported that prolonged exposure of *Lolium temulentum* to 5°C resulted in alterations in synthesis of polypeptides which were qualitatively different from those at 20°C. This, together with low temperature-induced mRNAs, were components of the cold hardening response (Howarth & Ougham, 1993). Mohapatra *et al*., (1987) also detected increases in levels of certain RNA species as a result of cold exposure (5°C) in alfalfa. Moreover, Tabaei-Aghdaei *et al*., (2000) reported that dehydrin and non-specific lipid transfer protein (nsLTP) mRNA sequences and polypeptides increased more during acclimation to cold (6/2°C day/night for 14 d) and drought (watering withheld for 6 d), in *Agropyron desertorum* than in *Lophopyrum elongatum* crowns. As mentioned earlier, wheat, cold acclimation rapidly induces the expression of a specific set of *Wheat Cold Shock* (*Wcs)* genes, which subsequently fail to express upon de-acclimation. *Wcs* are regulated by low temperature at the transcriptional level and winter wheat cultivars exhibit higher levels of expression than spring wheat cultivars (Limin *et al.*, 1995). In this species and other cereals, the expression of several genes during cold acclimation was found to be positively correlated with the capacity of each genotype to develop freezing tolerance. Among these, the *Wcs 120* gene family in wheat encodes a group of highly abundant proteins ranging in size from 12 to 200 kDa (Sarhan *et al.*, 1997). Limin *et al*., (1997) using the ditelocentric series in the hexaploid Chinese Spring wheat, mapped the *wcs 120*, *wcs 200* and *wcs 66* genes to 6DL, 6AL and 6BL, respectively. In the present work, we detected cold-induced proteins ranging in size from 17 to 200 kDa in wheat cultivars which are in the same molecular weight range proteins reported by Sarhan *et al*., (1997). Hence, it may be that the new cold-induced proteins induced by low temperature in the wheat cultivars examined in the present work are cold shock-like proteins and could well be encoded by the *Wcs 120* gene family. Cloutier (1983) reported that two protein patterns of 30 and 40 kDa had an important role in freezing tolerance in six wheat

cultivars following 4 weeks at 2°C compared with seedlings remained at 24°C for 2 d. In contrast, in the present report, the 40 kDa-polypeptide appeared to be unaffected by temperature changes during most sampling times in the spring and the winter wheat cultivars. Moreover, in our research, three other polypeptides of 28, 35 and 42 kDa were produced in the leaves of the 20°C-grown seedlings of both cultivars on days 22-28 but, none of these were detected in the leaves of the corresponding 4°C grown seedlings.

Quantitative and qualitative differences in soluble protein content between non-acclimated and cold-acclimated plants which were clearly observed in the present work, have been found in some plants species (Guy, 1990). A feature of hardening of wheat was the accumulation of a HMW (200 kDa) polypeptide which may protect important enzymes (Cloutier, 1983; Perras & Sarhan 1989; Howarth & Ougham, 1993). Bravo *et al*., (1999) reported the accumulation of an 80-kDa DHN-like protein (P-80) in barley under cold acclimation 6/4°C (day/night) in cold-acclimated leaves, pointed out that three other polypeptides (64, 67 and 73 kDa) became apparent later during cold-acclimation of barley. They attributed these polypeptides to be either the products of other DHN-like genes of this species or proteolytic products of P-80 (Bravo *et al.*, 1999). Moreover, a cold-induced 310 kDa protein was reported in cold-hardened winter wheat seedlings (Kolesnichenko *et al.*, 1997) and in winter rye plants (Kolesnichenko *et al.*, 2000).

Our results showed that greater accumulation of leaf soluble protein occurred over the first week of exposure to 4°C regardless of wheat cultivar: the winter cultivar responded more rapidly over the first 48 h of cold treatment than the spring cultivar. The SDS-PAGE studies illustrated 10 new cold-induced LMW and HMW polypeptides ranging from 17 to 200 kDa which were produced mostly during the first week at 4°C irrespective of cultivar. The LMW ones were more prominent over the second week of exposure to 4°C in both cultivars. Also, in this respect, the winter wheat cultivar was notable in responding 4 d earlier that the spring wheat cultivar. In the Tehran laboratory, work is in progress studying the effect of low temperature stress on chlorophyll fluorescence and its relationship with cold-induced proteins. In future we also aim to study the influence of Ca^{++} on cold-induced proteins of wheat to further understand the tolerance mechanisms that result in the greater adaptation of the winter wheat compared with the spring wheat genotypes to cold stress.

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