UDC (UDK) 633.11:631.55(55)

Mohsen JANMOHAMMADI,
Siroos MAHFOOZI and Reza TVAKKOL-AFSHARI [1]

INFLUENCE OF VEGETATIVE/REPRODUCTIVE TRANSITION ON PROLINE AND PROTEIN ACCUMULATION AND EXPRESSION OF FREEZING TOLERANCE IN WHEAT CULTIVARS GROWN IN TEMPERATE AND COLD CLIMATES

ABSTRACT

This project was initiated to determine the effect of different hardening conditions on developmental traits, accumulation of compatible solutes and freezing tolerance (FT) in winter and spring habit cultivars grown under field conditions in Iran. For this purpose, three cultivars with different vernalization requirements were planted in the field during autumn at Zanjan and Karaj, which represent cold and temperate regions, respectively. FT, as measured by means of LT_{50} values and developmental progress estimated from final leaf number (FLN) and shoots apex morphology were monitored throughout the cold seasons. Result showed that 'Pishtaz' spring wheat quickly initiated reproductive growth in comparison with other cultivars as measured by minimum final leaf number (FLN) and timing of double ridge formation. The phenological development of the winter cultivars (CDC Osprey and Pishagam) in cold region was greatly delayed relative to temperate region, and this delay in development was associated with higher and longer FT expression. Cold and sub-zero temperatures, especially in cold region, resulted in slow advancement to the early reproductive phase after vernalization fulfilment and continuation of freezing tolerance. Soluble protein and proline concentrations in leaves of winter cultivars increased during cold acclimation and subsequently decreased by vegetative/reproductive transition. These observations confirm the hypothesis that the phenological development regulate accumulation of compatible solutes, expression of FT, and acclimation conditions of regions determine the level and duration of expression of FT in wheat.

Keywords: Cold acclimation, Compatible solutes, Developmental traits, Vernalization

INTRODUCTION

Low temperature (LT) and freezing stress are major factors limiting cereal production in cold regions of Iran where cereals are exposed to variable cold

[1] Mohsen JANMOHAMMADI Department of Agronomy and Plant Breeding, Agriculture College, University of Maragheh, Iran; Siroos MAHFOOZI (corresponding author: siroosmahfoozi@yahoo.com) Department. of Cereals Research, Seed and Plant Improvement Institute (SPII), Karaj, Iran; Reza TVAKKOL-AFSHARI Department of Agronomy and Plant Breeding, Collage University of Agricultural and Natural Resources, Tehran University

temperatures in the vernalization range (5 to 10 °C) in early autumn and sub-zero temperatures during winter season (Mahfoozi et al, 2006). Freezing tolerance in cereals is dependent upon a highly integrated system of inducible genes (Fowler et al, 2001). It has been shown that developmental traits such as vernalization requirement and timing of vegetative to reproductive transition affect the cold acclimation process and expression of FT in wheat (Fowler et al, 1999, Limin & Fowler 2006; Sarhadi et al, 2010).

Winter cereals have developed several mechanisms to sense their surrounding environment and to adjust their phenological development consequently. One instance is vernalization, the process by which heading is promoted as plants sense exposure to the cold temperatures of winter (Kim et al, 2009). Vernalization requirement is a key adaptive trait that helps prevent heading before winter. It operates through postponing the transition from the vegetative to the reproductive phase and allows heading during more optimal circumstances in the spring (Fowler et al,, 1996, Mahfoozi et al, 2000, Mahfoozi et al, 2006). It has also been shown that low temperature and photoperiod are significant environmental factors which influence the apical morphogenesis, leaf production, and accordingly length of the vegetative growth stage in cereals (Mahfoozi et al, 2001). Based on the hypothesis of developmental regulation of FT gene expression (Fowler et al, 1999, Mahfoozi et al, 2001, Sarhadi et al, 2010), degree of FT is strictly dependent on the intensity and duration of cold-regulated gene expression. It is thought that developmental genes (vernalization Vrn, photoperiod Ppd), which control the transition from the vegetative to the reproductive phase, furthermore can determine the duration of FT genes expression and affect level of FT (Fowler et al, 2001, Danyluk et al, 2003). In addition, earliness per se genes regulate flowering time independently of vernalization and photoperiod, and are important for the fine tuning of flowering time and for the wide adaptation of wheat to different environments (Lewis et al, 2008). It has been confirmed that the full level of FT is achievable at the vegetative stage and plants may lose their ability to cold acclimation by initiation of the reproductive phase (Mahfoozi et al, 2001; Fowler and Limin 2004).

Winter-hardiness or FT can be enhanced by experience the low non-freezing temperatures which known as cold acclimation (Hughes and Dunn 1996). Cold acclimation permits winter cereal plants to engage freezing tolerance mechanisms required for winter endurance. However, the duration of exposing to low temperature as well as the temperatures that has been experienced by plant during acclimation can affect FT. During the acclimation, abundant molecular, physiological, biochemical, and metabolic processes are modified in plants (Janska et al, 2010). Despite the fact that the cereal plants display changes in the composition and accumulation of protecting compounds, i.e., carbohydrates, antioxidants, free amino acids (e.g. proline) and soluble proteins in response to cold, (Javadian et al, 2010, Janmohammadi et al, 2012), it seems that in different regions due to particular environmental conditions biological processes associated with cold acclimation will be carried out with special intensities

(Janmohammadi 2010). Hence, this may lead to different expressions of cold hardiness in a given winter wheat genotype in various environments, since some agronomical decisions like cultivar selection or sowing date which can affect the success or failure of crop production.

Proline is a principal organic molecule, which enhances appreciably under stresses conditions (Rhodes et al, 1999). Proline contributes to the protection of functional proteins from denaturation, interacts with plasma membranes, regulates cytosolic pH, balances redox potential and assists in scavenging of ROS (Konstantinova et al, 2002). Free proline concentration is used as a biochemical marker for frost tolerance as there is a positive correlation between frost resistance and proline content in plants grown under controlled or field conditions (Petcu et al, 2000, Burbulis et al, 2011).

Although a decrease in FT after the beginning of reproductive stage has been shown in several studies under controlled conditions (Fowler et al, 1996, Fowler et al, 2001; Mahfoozi et al, 2001, Limin and Fowler 2002, Limin and Fowler 2006, Prasil et al, 2004), the vegetative/reproductive transition process and expression of FT in wheat under field conditions of both cold and temperate climates has not been systematically studied. While under natural situation this process is strongly influenced by unpredictable environmental conditions like temporary warm spells and return of cold waves (Kalberer et al, 2006), as well as by genotype ability to maintain FT for a longer period. The objective of the present study was to learn how the progress of developmental stages affect the expression of FT and changes in total leaf soluble proteins and proline content during the cold acclimation and vegetative/reproductive transition in winter and spring wheat (*Triticum aestivum* L.) grown under field conditions in different regions with temperate and cold winters..

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of three wheat cultivars (Triticum aestivum L., 2n= 6x=42) including CDC Osprey (Canadian hardy winter wheat with long vernalization requirement), Pishgam (Iranian semi hardy facultative wheat with relatively medium vernalization requirement) and Pishtaz (Iranian non-hardy spring wheat with no vernalization requirement) were obtained from Seed and Plant Improvement Institute (SPII), Karaj. The cultivars were chosen based on their reputed differences in reproductive strategies and frost tolerances. For investigating the phenological development and FT, the experiments were conducted at two locations. The first was Cereals Research farm of SPII, Karaj, in centre-north Iran (35° 55' N, 50° 54' E; 1312 m) with temperate climate and a mean air temperature of 5.36°C for the November till March period. Second location was agricultural research stations of Kheyrabad, in Zanjan Province of north-western part of Iran (36° 31' N, 48° 47' E; 1770 m) in the with long cold wintersand a mean air temperature of 0.73°C for the November till March period. In both regions the cultivars were planted simultaneously on October 8 in 2008.

Mean air temperature and soil temperature at 5 cm depth was recorded from October 8 to March 16 (Fig. 1) using on-location weather stations.

Plants for vernalization response, apical development analysis, proline, protein and FT evaluates were grown in the soil in the field. The experimental design for the FT study at each region was a 3 (cultivar) by 6 (acclimation period or sampling date) factorial in a three replicate randomized complete block design. Plants of each cultivar were sampled from November 4 to March 16 in 2008–2009 at both regions to determine their FT and stage of phenological development.

Freezing tolerance

LT50 (temperature at which 50% of the plants are killed by freezing stress) was determined according to Limin and Fowler (1988). For this purpose, during the six LT acclimation experiments (from November 4 to March 16 in 2008–2009) near 100 plants of each genotype were harvested and transferred to the laboratory where whole leaves were removed and plant crowns were covered in moist sand in aluminium-weighing cans and placed in a programmable freezer and held at -3°C for 12 h. After 12 h they were cooled at a rate of 2°C h-1 down to -29°C. Five crowns were removed at 2°C intervals for each of five test temperatures selected for each cultivar in each treatment and region. Samples were thawed overnight at 4°C. Thawed crowns were planted into flats containing peat soil that was kept moist. The flats were placed in a glasshouse at 20°C. After 14 d, the numbers of living and regenerating plants were determined for each test temperature.

Phenological assessment

Stage of phenological development was determined through dissection of plant crown and rating of the shoot apex morphology according to decimal code (DC) described by Natrova and Jokes (1993). The decimal code of the plants was scored as shown in Table 1. To eliminate the inhibitory effects of cold on shoot apex development and for the correct diagnosis of vernalization fulfilment plants were transferred to glasshouse (20 °C) for 10 days after each sampling dates then crown were dissected. However, apex developments of ten plants were monitored immediately after harvesting at each sampling dates.

The fulfilment of the vernalization requirement was assessed by the Final Leaf Number (FLN) procedure described by Wang et al, (1995) and Mahfoozi et al, (2001). For determination of FLN, at each sampling date five plants along with field soil were sampled from each replicate in both regions and then planted in pots filled with soil. Pots were moved into a glasshouse at 20 °C with a 16 h day length and leaves on the main stem of each plant were numbered until the flag leaf emerged. The commencement and ending for these assessments are marked by the preliminary FLN (planted directly in a glasshouse at 20 °C i.e. non-acclimated) and the point at which lowest FLN, or vernalization fulfilment, is attained by plants that harvested from the fields during the sampling dates.

FLN was used to predict initiation of floral primordial (Hay and Ellis 1998), since vernalization fulfilment coincides with vegetative/reproductive transition and additional cold exposure cannot reduce FLN further (Mahfoozi et al, 2001). On low-temperature exposure, vernalization-requiring cultivars reduce their final leaf number (FLN) until they reach a minimum leaf number, which is the point of vernalization saturation (Berry et al, 1980, Wang et al, 1995, Fowler et al,, 1996, Mahfoozi et al, 2001). Consequently, transition from the vegetative to the reproductive phase was considered complete when the FLN for consecutive sampling dates became near constant.

Days to heading were evaluated for five plants taken from each sampling dates and transferred to glasshouse as described for FLN. The rest of the harvested plants have been used for other analysis. The vernalization requirement was defined as the minimum number of weeks required for full vernalization (i.e. when time of heading was not considerably changed by extra cold experiencing under field conditions).

Determination of compable solutes

In order to measure the proline and protein contents, fully expanded upper leaves from twenty plants of each cultivar were harvested at each sampling date and stored at -80 °C. After the completion of sampling and revealing the results of freezing tolerance, proline and protein contents was evaluated for three sampling dates including 23 Nov (initiation of cold acclimation), 26 Dec (vernalization fulfilment) and 21 Feb (reproductive growth stage). Soluble protein content was analysed by the method based on the protocol established by Bradford (1976). Proline content was determined according to the method of Bates et al, (1973).

Analysis of variance was conducted on the data using software package of SPSS, version 15 and significant differences among treatment means were calculated by LSD test.

RESULTS

Average daily air and soil temperature recorded at Karaj temperate climate and Zanjan cold region revealed that there was significant difference in frost dates (period between the first fall and last spring frost) and severity of winter between regions. At Zanjan research station, temperatures were sub-zero in much of the winter while plants in Karaj experienced cold and non-freezing temperature during the greater part of winter (Fig. 1). The trend of temperature rising during the late winter in Zanjan was very slow while the temperatures rapidly increased in Karaj. Day length in October was about 12 h and was shorten to 8 h in January. However, the difference in day length between the two regions was insignificant due to the small distance between the sites.

Freezing tolerance

Analyses of variance for LT_{50} trait indicated that main effects of region, cultivar, cold acclimation periods (sampling dates), the two-way interactions of cultivar × region, cultivar × sampling period, sampling period × region and the

three-way interaction for sampling period × region × cultivar were significantly different. Investigation the trend of FT during the sampling dates showed that increasing the cold acclimation time resulted in significant decrease in the LT_{50} (increasing freezing tolerance) and this trend continued through 26 December. After achieving maximum FT, a significant loss of FT was detected in 'Pishgam' and 'Pishtaz' plants grown in the temperate climate of Karaj. However, when the two lines acclimated in the colder climate of Zanjan, Pishtaz was able to retain its maximum FT for three weeks (Fig. 2) and Pishgam showed only a slight loss of FT. During the first 3 weeks of acclimation, CDC-Osprey hardy winter wheat showed a quicker decrease in LT_{50} (increasing FT) compared with other cultivars, especially in the cold region (Zanjan). The winter cultivars showed increased FT by 23 November, whereas no improvement in FT was noted for Pishtaz spring wheat at this time point. Rather, the spring line demonstrated a delayed onset of cold acclimation when compared to the winter lines.

Phenological development

Result of variance analyses showed that cultivar, sampling period, as well as the three-interactions for cultivar × region, sampling period × region and cultivar × sampling period interaction were highly significant for FLN. Final leaf number measurements indicated that vernalization fulfilment was achieved on 26 December for CDC-Osprey in both regions (Fig. 3). However, rate of FT improvement during the sampling dates was faster in Zanjan compared to Karaj. Result showed that vernalization fulfilment point was around November 4 for Pishgam in Zanjan region while it occurred somewhat later in Karaj region (around November 23). Evaluation of the FLN in Pishtaz spring wheat showed that a very little reduction in FLN occurred during sampling dates. However comparison of FLN in winter cultivars between the regions showed accelerated decrease for plants that acclimated in Zanjan cold region.

Assessment of shoot apex during sampling dates revealed that apical development of cultivars planted in Zanjan cold region was considerably prolonged when compared with Karaj temperate region and this status was very prominent for winter cultivars (Fig. 4). Pishtaz spring cultivar reached to the double-ridge stage (initiation of reproductive phase) between 23 Nov to 26 Dec when it coincided with maximum FT.

However, immediate crown dissection after sampling dates showed that in all investigated cultivars and regions maximum FT were observed during vegetative phase (i.e. DC 11 to 19). Results would suggest that in Karaj temperate climate, the milder climate was sufficient for both vernalization fulfilment and rapid development of shoot apex. In contrast, As the Zanjan sub-zero temperatures during autumn and winter seasons were very restrictive, phase transition in hardy winter wheat was delayed near 40 days as compared to the milder Karaj climate. Although during the second to fourth sampling dates phenological differences between cultivars and regions was very high, after the phase transition differences were relatively diminutive.

The days to heading profiles (Fig. 5) were relatively similar to those of FLN (Fig. 3). With increased acclimation period, days to heading in cv. CDC-Osprey was strictly reduced. However, slower slope of decline was observed for cv. Pishgam and it was relatively constant in Pishtaz spring cultivar in both regions. With increased duration of cold acclimation, decrease in days to heading was more severe for cultivars planted in Karaj compared to Zanjan. The amount of the changes in day to heading after 26 December was relatively insignificant, and thus vernalization requirement for all cultivars was satisfied at this time point.

Compatible solutes

Evaluation of the proline content showed that there were significant differences between cultivars ($P<0.05$), sampling dates ($P<0.01$), region ($P<0.01$) and their three-way interactions ($P<0.01$). The lowest proline concentration was recorded for non-acclimated plants with no significant difference between cultivars. Proline content increased swiftly during the first six weeks of cold acclimation in the leaves of hardy winter cultivar (Fig. 6) in both environments, whereas for the facultative and spring wheat cultivars showed an increase when grown in Zanjan. This superiority was sustained over the next 4 weeks of cold acclimation in all cultivars in both regions, since the maximum proline content in both regions was recorded on 26 December which coincided with the maximum FT. A significant reduction was observed in proline content of the all cultivars during third sampling date. The CDC-Osprey winter wheat cultivar sustained the high proline content better after vegetative/reproductive transition when was compared with both spring and facultative wheat cultivars in both regions.

The effect of vernalization fulfilment and phenological development on total soluble protein concentration is shown in Fig 7. Considering all samplings dates, the protein concentration in the different cultivars showed different profiles during cold acclimation and vegetative/reproductive transition. The results revealed that the concentration of total soluble proteins in spring cultivar was very little affected by cold acclimation or by vegetative/reproductive transition. However, change in protein concentration was considerable in hardy winter wheat during the investigated period. Cold acclimation induced a higher accumulation of soluble protein in winter and facultative wheat cultivars compared to the control (non-acclimation). Protein concentration in CDC-Osprey winter wheat cultivar acclimated under Karaj condition significantly was higher than the spring and facultative wheat cultivars during all investigated conditions. However, it was not found in Zanjan cold region as Pishgam showed the highest protein concentration. Initiation of reproductive growth induced significant decrease in protein concentration of winter and facultative wheat cultivars. The amount of this reduction in Karaj was more extensive than Zanjan cold region.

DISCUSSION

It have been revealed that during the cold acclimation of wheat under controlled environments, initially FT gradually increases, which is more rapid and lasts longer in hardy winter cultivars. However, after a definite period of time, FT reaches its utmost value followed by a slow decrease (Fowler et al, 1996, Mahfoozi et al, 2001, Prasil et al, 2004, Limin and Fowler 2006, Sasani et al, 2009, Sarhadi et al, 2010). Similarly, in the present study, the FT of 'CDC-Osprey' winter wheat increased rapidly during the eight weeks of cold acclimation and then was maintained at the highest level for the next 4 weeks before decrease occurred. However, a novel result of the present research was that the less hardy 'Pishtaz' spring wheat reached its highest FT at the same time at both location but loos of FT started earlier in the Karaj temperate region as compared to the colder climate (Fig. 2).

Phenological assessments in different climates revealed that primordial formation or phase transition is strictly affected by acclimation conditions, so that cold or sub-zero temperatures during or after vernalization fulfilment may retain maximum expression of FT which result in continuance of FT. However, severity and duration of the FT expression after vernalization fulfilment is dissimilar in winter and spring wheat.

It has been reported that the length of the vegetative stage affects the duration of FT expression (Mahoozi et al, 2006, Limin and Fowler 2006). Factors like vernalization requirement, increased length of the phyllochron, increased leaf number and lateness *per se* could extend the length of the vegetative phase and delay the reproductive phase visualized by `double ridge' formation and consequently extend the time FT genes are highly expressed (Limin and Fowler, 2002). However, sensitivity to vernalization during vegetative phase especially for hardy winter wheat was found in present study confirming previous study (Fowler et al, 2001, Mahfoozi et al, 2006).

Results suggest that regulation of developmental growth by internal mechanisms is crucial for the achievement of maximal FT, but environmental conditions can extend length of vegetative phase as observed in spring cultivar. The different trend of FT in winter and spring wheat can be interpreted by cold acclimation threshold induction temperatures, since winter wheat cultivars have an earlier response to decreasing temperatures and can start acclimation process once field soil temperatures dropped below approximately 10 °C and maintain acclimation well into winter (Fowler, 2008).

Results of present study demonstrate that protein and proline content increased in leaves of wheat seedlings by cold acclimation and subsequently decreased by initiation of reproductive growth. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions (Vierstra 1993). Obtained results show that duration and level of protein expression can be significantly influenced by temperature fluctuation. However, some of these changes result from progress of plant

development. Findings from other studies show that FT includes synthesis of proteins like as dehydrins (DHNs),, LEA (late embryogenesis abundant) proteins, antifreeze proteins and heat-shock proteins (HSPs) which act to stabilize both membrane phospholipids and proteins (Chen and Murata, 2008; Janska et al, 2010, Sarhadi et al, 2010).

Detoxification enzymes involved in scavenging of reactive oxygen species (ROS) are accumulated during low temperature exposure, but the activities disappear upon de-acclimation (Janmohammadi et al, 2012). In current study, the total protein concentration significantly decreased after vegetative/reproductive transition and it seems that rising temperatures in late winter or plant development may progressively decline expression or accumulation of some proteins. In et al, (2005) reported that gene expression in winter rye (*Secale cereale* L.) leaves related to RNA and protein metabolism (RNA-binding protein, UMP synthase, and a transcription elongation factor) was lower after deacclimation than following acclimation. Alternatively, gene expression during deacclimation may actually be more closely related to developmental transitions (Kalberer et al, 2006). A close association between the vernalization fulfilment and the start of a decline in the protein accumulation of cold hardy winter wheat Norstar with a long vernalization requirement has been reported by researchers (Fowler et al, 1996, Limin et al, 1997, Sarhadi et al, 2010).

Proline accumulation in plant tissues has been suggested to result from (a) a decrease in proline degradation, (b) an increase in proline biosynthesis, (c) a decrease in protein synthesis or proline utilization, and (d) hydrolysis of proteins (Charest and Phan 1990). A fundamental relationship between proline accumulation and cold acclimation has been demonstrated in *Arabidopsis thaliana* (Nanjo et al, 1999). Evidently, the accumulation of proline as a compatible solute might be causally related to the induction of FT in wheat seedlings. Our results are in line with findings of Javadian et al, (2010) who reported a significant increase in proline and protein content of wheat seedling under chilling stress. The mechanism of plant tolerance to freezing is extremely complicated.

The present study confirmed that vernalization requirements and low environmental temperatures influence the rate of phenological development and the expression of FT. The longer vernalization requirement and increased leaf number in winter habit wheat are main adaptive mechanism that delay the transition to the more sensitive reproductive phase and result in expression of FT for a longer period of time. Furthermore, low environmental temperatures in cold regions can hamper the rate of phase transition and may bring higher FT, even after vernalization fulfilment. However, the level of FT in hardy winter cultivars gradually reduces after the initiation of reproductive phase with the result that the plants lose their ability for re-hardening (Kalberer et al, 2006) and become more susceptible during the late winter and early spring when cold waves return. Obtained results indicated that assessment of FLN can be an effective method for estimating the vernalization fulfilment point under field conditions.

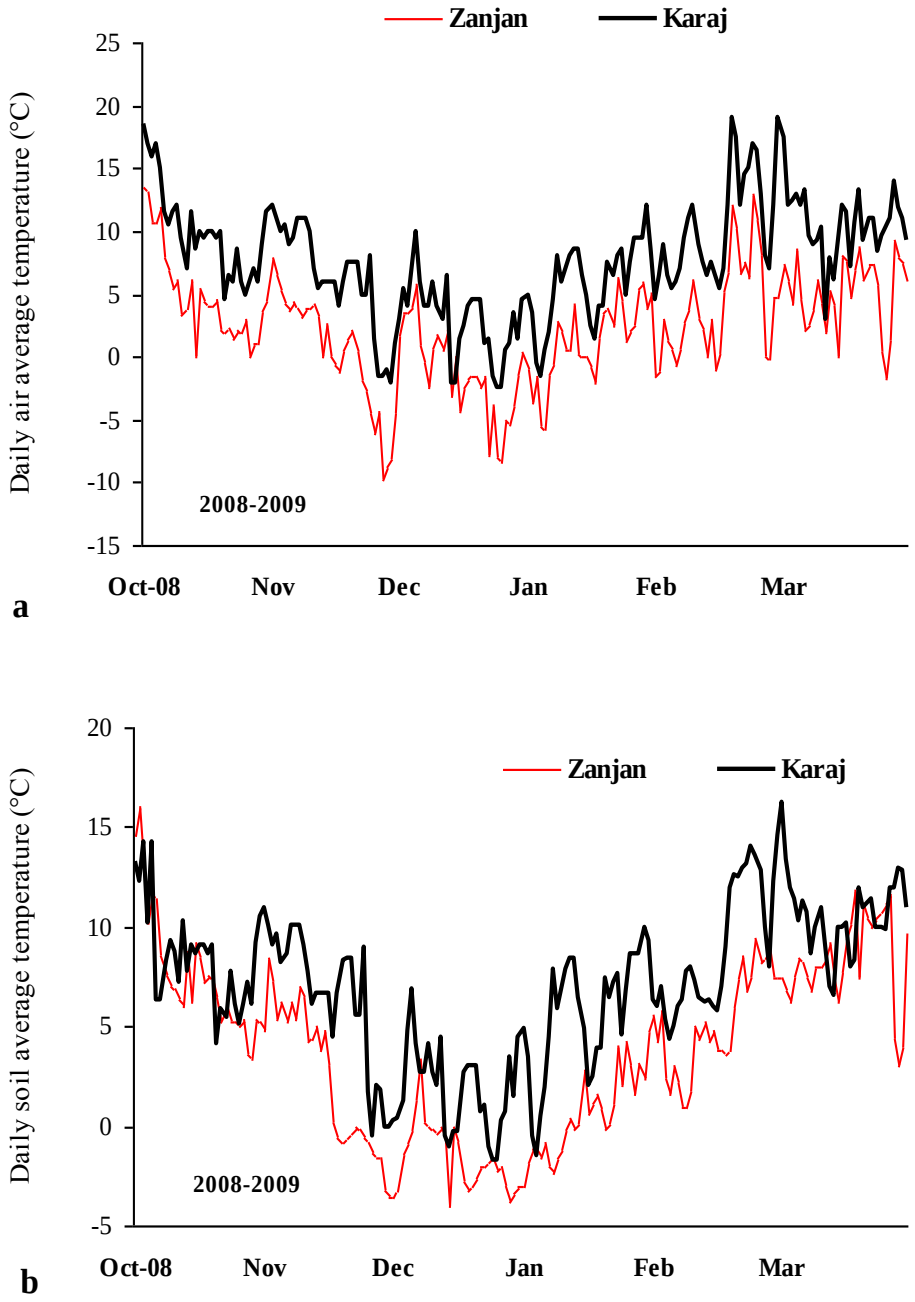

Figure 1. Average daily air (a) and soil temperature (°C) at 5 cm depth (b) as recorded at the Zanjan and Karaj Agricultural Research Station, Iran in 2008–2009.

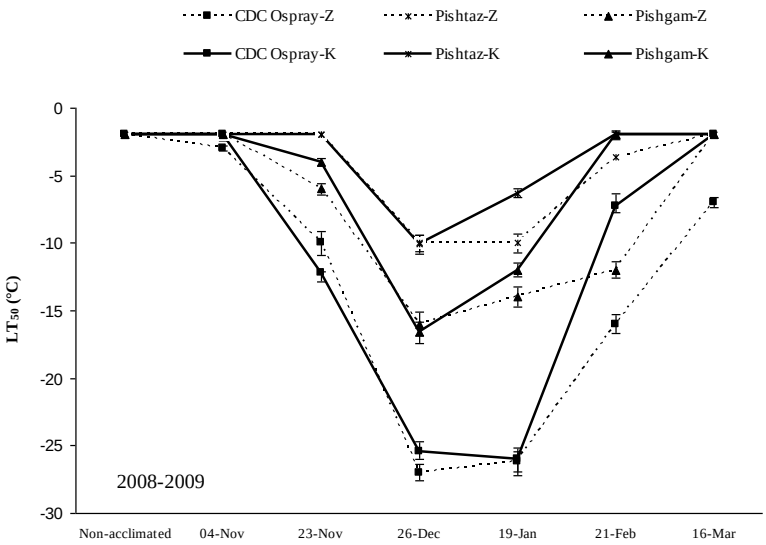

Figure 2. Freezing tolerance levels during cold season. (LT_{50}) of winter wheat cultivars CDC Osprey and Pishgam and Pishtaz spring wheat grown under field conditions at the Zanjan Agricultural Research Station (Z) and Karaj (K), Iran from November 4 to March 16, in 2008–2009. The values and standards errors (vertical bars) of three replications are shown.

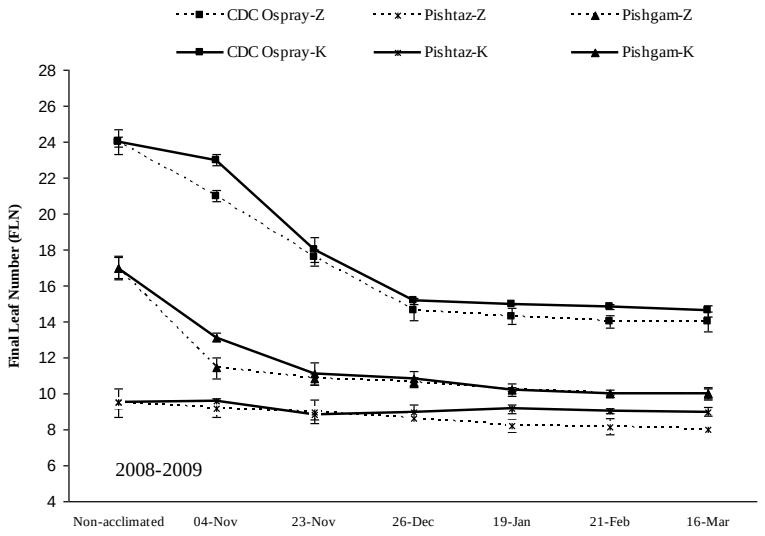

Figure 3. Final leaf number (FLN) during cold season. FLN determined for winter wheat cultivars CDC Osprey and Pishgam and Pishtaz spring wheat grown under field conditions at the Zanjan Agricultural Research Station (Z) and Karaj (K), Iran from November 4 to March 16, in 2008–2009. The values and standards errors (vertical bars) of three replications are shown.

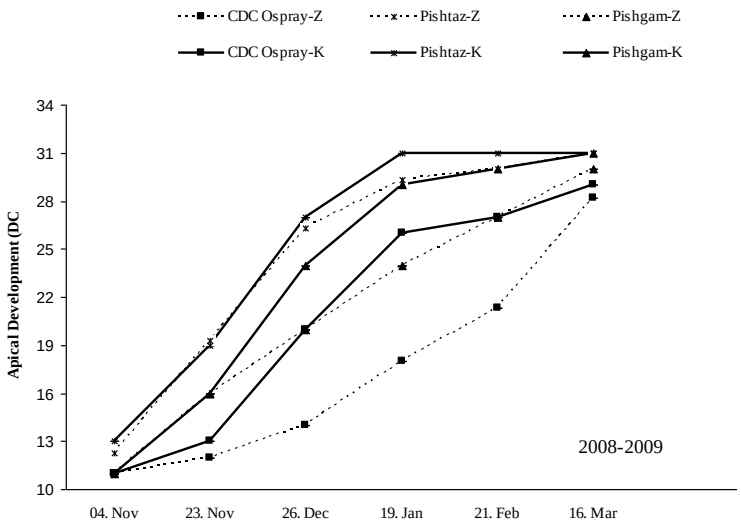

Figure 4. Shoot apex development during cold season. Morphology of shoot apical meristem expressed by decimal code (DC) for winter wheat cultivars CDC Osprey and Pishgam and Pishtaz spring wheat grown under field conditions at the Zanjan Agricultural Research Station (Z) and Karaj (K), Iran from November 4 to March 16, in 2008–2009.

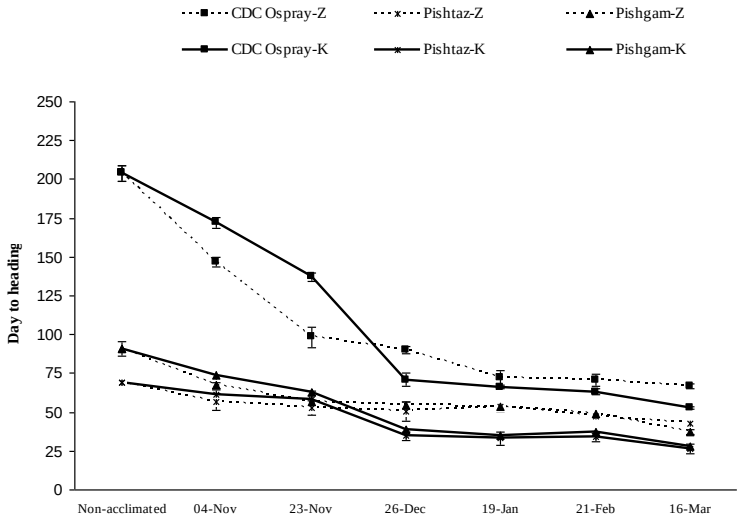

Figure 5. Effect of cold acclimation time on heading date. Day to heading for winter cultivars CDC Osprey and Pishgam and Pishtaz spring wheat. Plants were grown under field conditions at the Zanjan Agricultural Research Station (Z) and Karaj (K), Iran from November 4 to March 16, in 2008–2009 until date indicated and thereafter grown in greenhouse to maturation. The values and standards errors (vertical bars) of three replications are shown.

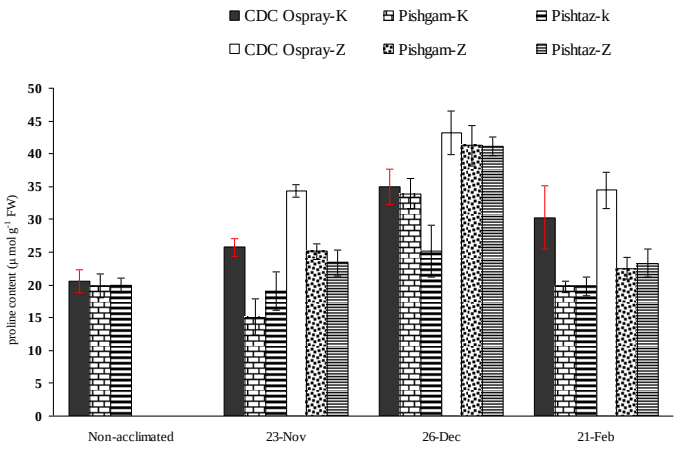

Figure 6. Proline accumulation in wheat leaves during cold season. Analysis was done on winter wheat cultivars of CDC Osprey and Pishagam and Pishtaz spring wheat grown under field conditions at the Zanjan Agricultural Research Station (Z) and Karaj (K), Iran. Samples analysed were collected during early cold acclimation (November 23), maximum frost tolerance (December 26) and early reproductive growth (February 21) in 2008–2009. The values and standards errors (vertical bars) of three replications are shown.

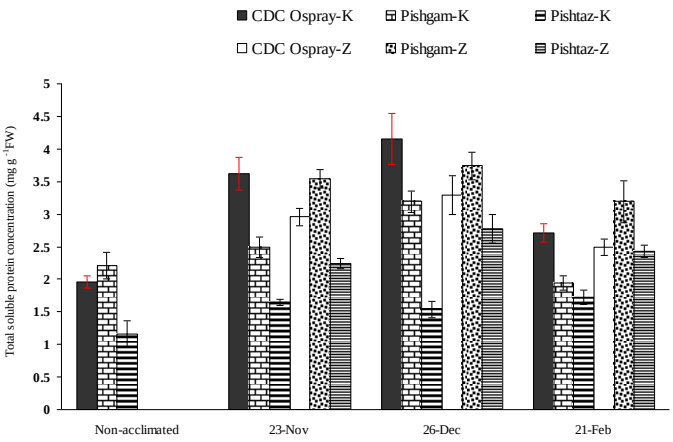

Fig 7. Soluble protein concentration in wheat leaves during cold season. Analysis was done on winter wheat cultivars CDC Osprey and Pishgam and Pishtaz spring wheat grown under field conditions at the Zanjan Agricultural Research Station (Z) and Karaj (K), Iran. Samples were collected during early cold acclimation (November 23), maximum frost tolerance (December 26) and early reproductive growth (February 21) in 2008–2009. The values and standards errors (vertical bars) of three replications are shown.

These observations indicate that the cold and sub-zero acclimation under field conditions could induce FT in wheat even after vernalization fulfilment and also during the early reproductive phase, but lower levels of tolerance are generated in temperate region.

Table 1. Description of the phenological growth stages of wheat plants which is divided into vegetative and reproductive growth stages and presented by decimal code (DC).

Vegetative phase	DC	Reproductive phase	DC
Early vegetative development of the shot apex, with one or two initiated leaves	11	Formation of double ridges DR 1 – the size of leaf primordia is bigger than that of the spikelets	20
Beginning of the shoot apex elongation	13	Formation of double ridges DR 2 – the spikelet and leaf primordia are of similar size	22
Beginning of single ridges, i.e. leaf primordia initiation on elongating shoot apex	16	Formation of double ridges DR 3 – spikelet primordial increase in size, and the growth of leaf primordia is inhibited	24
Single ridges, i.e. leaf primordia initiated along the whole shoot apex	19	Spikelet primordia are elongating; on the shoot apex only spikelet primordia are apparent	26
		Lemma initiation	29

CONCLUSION

Based on the above observations, it is concluded that cultivar development for successful production of winter crops in both high and low cold stress regions require an understanding of plant development, growth cycles and the mechanisms employed to survive periods of freezing stress. These results show that genetic and environmental interactions determine FT in wheat. Plant development toward flowering progressively reduces the ability of wheat to acclimate to low-temperatures particularly after the main shoot meristem has advanced to the reproductive growth stage.

While this work provides experimental evidence that both cold acclimation conditions of regions and vegetative/reproductive transitions have a major influence on expression of FT in wheat, further investigation is required to determine the interactions among developmental traits with the objective of identifying genetic combinations that extend the vegetative stage thereby providing the opportunity for regions with both long mild winters, like the cold areas of Iran, and a high level of freezing stress, like many parts of the northern hemisphere.

ACKNOWLEDGEMENTS

Technical assistance of Mr A. Ghorbani and A. Nazari is greatly appreciated. Financial support of Ministry of Sciences and Cereals Research Department of SPII is gratefully acknowledged.

REFERENCES

Bates, L. S. Waldren, R. P. &Teare, I. D. (1973): Rapid determination of free proline for water stress studies. *Journal of Plant Soil*, 39: 205-217.

Berry, G. J. Salisbury, P. A. &Halloran, G. M. (1980): Expression of vernalization genes in near-isogenic wheat lines: duration of vernalization period. *Annals of Botany,* 46: 235-241.

Bradford, M. M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72: 248-254.

Charest, C. & Phan, C. T. (1990): Cold acclimation of wheat (*Triticum aestivum*) properties of enzymes involved in proline metabolism. *Physiologia Plantarum,* 80: 159-168.

Chen, T. H. & Murata, N. (2008): Glycinbetaine: an effective protectant against abiotic stress in plants. *Trends in Plant Science*, 13: 499-505.

Danyluk, J. Kane, N. A. Breton, G. Limin, A. E. Fowler, D. B. & Sarhan, F. (2003): TaVRT-1, a putative transcription factor associated with vegetative to reproductive transition in cereals. *Plant Physiology*, 132: 1849-1860.

Fowler, D. B. (2008): Cold acclimation threshold induction temperatures in cereals. *Crop Science*, 48: 1147-1154.

Fowler, D. B. Breton, G. Limin, A. E. Mahfoozi, S. & Sarhan, F. (2001): Photoperiod and temperature interactions regulate low-temperature induced gene expression in barley. *Plant Physiology,* 127: 1676-1681.

Fowler, D. B. Chauvin, L. P. Limin, A. E. & Sarhan, F. (1996): The regulatory role of vernalization in the expression of low-temperature-induced genes in wheat and rye. *Theoretical and Applied Genetics*, 93: 554-559.

Fowler, D. B. & Limin, A. E. (2004): Interactions among factors regulating phenological development and acclimation rate determine low-temperature tolerance in wheat. *Annals of Botany*, 94: 717 - 724.

Fowler, D. B. Limin, A. E. & Ritchie, J. T. (1999): Low-temperature tolerance in cereals: model and genetic interpretation. *Crop Science* 39: 626-633.

Gonzalez, F. G. Slafer, G. A. & Miralles, D.J. (2002):Vernalization and photoperiod response in wheat pre-lowering reproductive phase. *Field Crop Research* 74, 183-195.

Hay, R. K. M. & Ellis, R. P. (1998): The control of flowering in wheat and barley: what recent advances in molecular genetics can reveal. *Annals of Botany,* 82: 541-554.

Hughes, M. A. & Dunn, M. A. (1996): The molecular biology of plant acclimation to low temperature. *Journal of Experimental Botany,* 47: 291-305.

In, O. Berberich, T. Romdhane, S. & Feierabend, J. (2005): Changes in gene expression during dehardening of cold-hardened winter rye (*Secale cereale* L.)

leaves and potential role of a peptide methionine sulfoxide reductase in cold-acclimation. *Planta*, 220: 941-950.

Janmohammadi, M. Enayati, V. & Sabaghnia, N. (2012): Impact of cold acclimation, de-acclimation and re-acclimation on carbohydrate content and antioxidant enzyme activities in spring and winter wheat. *Icelandic Journal of Agricultural Sciences*, 25: 3-11.

Janska, A. Marsik, P. Zelenkova, S. & Ovesna, J. (2010): Cold stress and acclimation - what is important for metabolic adjustment?. *Plant Biology*, 12: 395-405.

Javadian, N. Karimzadeh, G. Mahfoozi, S. & Ghanati, F. (2010): Cold induced changes of enzymes, proline, carbohydrates, and chlorophyll in wheat. *Russian Journal of Plant Physiology*, 57: 540-547.

Kim DH, Doyle MR, Sung S & Amasino RM 2009. Vernalization: winter and the timing of flowering in plants. *Cell and Developmental Biology* 25, 277-299.

Konstantinova, T. Parvanova, D. Atanassov, A. & Djilianov, D. (2002): Freezing tolerance tobacco transformed to accumulate osmoprotectants. *Plant Science*, 163: 157-164.

Limin, A. E. & Fowler, D. B. (1988): Cold hardiness expression in interspecific hybrids and amphiploids of the Triticeae. *Genome*, 30: 361-365.

Limin A.E. & Fowler D.B., 2002. Developmental traits affecting low-temperature tolerance response in near-isogenic lines for the Vernalization locus Vrn-A1 in wheat (*Triticum aestivum* L. em Thell). *Annals of Botany* 89, 579-85.

Limin, A. E. & Fowler, D. B. (2006): Low-temperature tolerance and genetic potential in wheat (*Triticum aestivum* L.): responses to photoperiod, vernalization and plant development. *Planta*, 224: 360-366.

1.1.1. Limin, A. E. Danyluk, J. Chauvin, L. P. Fowler, D. B. & Sarhan, F. (1997): Chromosome mapping of low-temperature induced *Wcs120* family genes and regulation of cold-tolerance expression in wheat. *Molecular and General Genetics*, 253: 720–727.

Mahfoozi, S. Limin, A. E. & Fowler, D. B. (2001): Developmental regulation of low-temperature tolerance in winter wheat. *Annals of Botany*, 87: 751-757.

Mahfoozi, S. Limin, A. E. Ahakpaz, F. & Fowler, D. B. (2006): Phenological development and expression of freezing resistance in spring and winter wheat under field conditions in north-west Iran. *Field Crops Research*, 97: 182-187.

Mahfoozi, S. Limin, A. E. Hayes, P. M. Hucl, P. & Fowler, D. B. (2000): Influence of photoperiod response on the expression of cold hardiness in wheat and barley. *Canadian Journal of Plant Science*, 80: 721-724.

Mahfoozi, S. Limin, E. & Fowler, D. B. (2001): Developmental regulation of low-temperature tolerance in winter wheat. *Annals of Botany*, 87: 751-757.

Nanjo, T. Kobayashi, T. M. Yoshida, Y. Kakubari, Y. Yamaguchi-Shinozaki, K. & Shinozaki, K. (1999): Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Letter*, 461: 205-210.

Natrova, Z. & Jokes, M. (1993): A proposal for a decimal scale of the inflorescence development of wheat. *Rostlinna vyroba*, 39: 315-328.

Prasil, I. T. Prasilova, P. & Pankova, K. (2004): Relationships among vernalization, shoot apex development and frost tolerance in wheat. *Annals of Botany*, 94: 413-418.

Rhodes, D. Verslues, P. E. & Sharp, R. E. (1999): Role of aminoacids in abiotic stress resistance, In: Sing BK (eds.) *Plant Amino acids: Biochemistry and Biotechnology*, Marcel Dekker Inc, New York, pp. 319-356

Sarhadi, E. Mahfoozi, S. Hosseini, S. A. & Hosseni-Salekdeh, G. (2010): Cold acclimation proteome analysis reveals close link between up-regulation of low-temperature associated proteins and vernalization fulfillment. *Journal* of *Proteomic Research*, 9: 5658-5667.

Sasani, S. Hemming, M. N. Oliver, S. N. Greenup, A. Tavakko-Afshari. R. Mahfoozi, S. Poustini, K. Sharifi, H. R. Dennis, E. S. Peacock, W. J. & Trevaskis, B. (2009): The influence of vernalization and day length on expression of flowering-time genes in the shoot apex and leaves of barley (*Hordeum vulgare*). *Journal of Experimental Botany*, 60: 2169-2178.

Vierstra, R. D. (1993): Protein degradation in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 44: 385-410.

Wang, S. Y. Ward, R. W. Ritchie, J. T. Fischer, R. A. & Schulthess, U. (1995): Vernalization in wheat I. A model based on the interchangeability of plant age and vernalization duration. *Field Crops Research*, 41: 91-100.

Mohsen JANMOHAMMADI,
Siroos MAHFOOZI and Reza TVAKKOL-AFSHARI

UTICAJ VEGETATIVNE/REPRODUKTIVNE TRANZICIJE NA AKUMULACIJU PROLINA I PROTEINA I NA IZRAŽAVANJE TOLERANCIJE NA SMRZAVANJE KOD SORTI PŠENICE KOJE SE UZGAJAJU U UMJERENOJ I HLADNOJ KLIMI

SAŽETAK

Ovaj projekat je pokrenut kako bi se utvrdili uticaji različitih uslova na razvojne osobine, akumulaciju kompatibilnih rastvora i toleranciju na smrzavanje (FT) kod zimskih i proljećnih kultivara uzgajanih u poljskim uslovima u Iranu. Za ovu svrhu, tokom jeseni su zasađene tri sorte sa različitim zahtjevima u odnosu na vernalizaciju (jarovizaciju), u oblasti Zanjan i Karaj, od kojih jednu karakteriše hladna, a drugu umjerena klima. Tolerancija na smrzavanje (FT) je mjerena uz pomoć LT50 vrijednosti, napredak u razvoju je procjenjen na osnovu konačnog broja listova (FLN), dok je morfologija vrška izdanaka praćena tokom hladne sezone. Rezultat je pokazao da je proljećna sorta pšenice 'Pishtaz' brzo krenula u reproduktivni rast u poređenju sa drugim sortama, mjereno minimalnim konačnim brojem listova (FLN) i vremenom za formiranje dvostrukog grebena. Fenološki razvoj zimskih sorti (CDC Osprey i Pishagam) u hladnoj regiji je znatno usporen u odnosu na regiju sa umjerenom klimom, a to kašnjenje u razvoju je bilo povezano sa većom i dugotrajnijom tolerancijom na smrzavanje. Hladna klima i temperature ispod nule, posebno u hladnoj regiji, usporile su napredovanje u ranoj fazi, nakon stadijuma vernalizacije i uticale na produžetak tolerancije na smrzavanje. Rastvorljive koncentracije proteina i prolina u listovima zimskih sorti bile su povećane tokom aklimatizacije, a zatim su vegetativnom/reproduktivnom tranzicijom umanjene. Ova zapažanja potvrđuju hipotezu da fenološki razvoj reguliše akumulaciju kompatibilnih rastvora, izražavanje tolerancije na smrzavanje, a uslovi aklimatizacije u različitim regijama određuju nivo i dužinu tolerancije na smrzavanje kod pšenice.

Ključne riječi: aklimatizacija na hladnoću, kompatibilni rastvori, razvojne osobine, vernalizacija