COLD ACCLIMATION AND VEGETATIVE/REPRODUCTIVE TRANSITION IN WINTER CEREALS

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Abstract:

Cereals display a broad capacity range to survive low temperatures (LT) and frost condition. Exposure of winter cereals to low, nonfreezing temperatures during the autumn and before freezing injury occurs can induces various changes in *genome, transcriptome, proteome, and metabolom* levels in plant cells, by which plants are able to adjust their metabolism to LT and improve their freezing tolerance (FT). The difference between the cultivars can be interpreted by hardening conditions and the different mode and severity of changes occurred in each levels during cold acclimation. FT in cereals is dependent upon phonological development and the highest level of FT only acquires during the vegetative stage and plants by initiation of the reproductive phase lose the ability for hardening. Phonological growth is controlled by the developmental genes (vernalization, photoperiod, etc.) which regulate the vegetative/reproductive transition and consequently determine the duration and level of FT expression. Furthermore under filed condition winter survival is also determined by the acclimation temperature of locations that may affect phenological development and delay the beginning of reproductive growth.

Keywords: cold acclimation, freezing tolerance, phenological development, vernalization, winter survival

1. Introduction

Low temperature (LT) and frost stress are major environmental restrictions on plant geographical distribution and performance [30, 45]. At high latitudes or hilly area, the problem of coping with LT is worsened via the need to prolong the growing season beyond the short summer. LT stress may lead to major crop losses and different phenotypic response like as poor germination, stunted seedlings, yellowing of leaves (chlorosis), reduced leaf expansion and wilting, and may lead to death of tissue which is known as necrosis [62]. Tropical and subtropical plants are not enough tolerant against LT and are simply injured through LT while overwintering plants are proficient for exhibiting sufficient levels of freezing tolerance (FT), which is achieved through experiencing the non-freezing temperatures during the autumn, which known as cold acclimation [20]. Cold acclimation permits winter cereal plants to expand qualified tolerance mechanisms required for winter endurance. Plant survival over the winter period is known as winter hardiness and it can be divided into some simpler components, one of the most imperative of which is FT [51].

Between all crop species winter cereal have the broadest adaptation. This success was partly is due, to a relatively great extent, to cereal's LT tolerance so that it has the quite high capability to withstand temperatures much lower than 1-4ºC, regarded the minimum temperature for growth [45]. But considerable variation for cold tolerance exists among cultivars of cereal species and their winter hardiness of various cultivars is extremely different. It seems that variation in FT level can be genetically created; also influenced by environmental condition, phenological developmental and physiological status at the time of exposure [15, 24, 35].

Winter-habit cereals (winter wheat, barley, oat and rye) have a vernalization requirement, which is an important adaptive feature that delays heading by postponing the transition from the vegetative to the reproductive phase, before the threat of freezing stress during winter has passed [17]. Thus, vernalization requirement allows plants to over-winter as seedlings. However, after vernalization fulfillment and at the end of the vegetative phase, the cold acclimation ability of winter cereals gradually decreases [14, 16, 26, 36]. In contrast, spring cereals (which do not have a vernalization requirement) normally develop rapidly into their reproductive phase when grown under long days and sentimental temperature is optimal [29, 25, 37].

Although researchers have intensively studied various aspects of cold acclimation, the relationship

between FT and vegetative/reproductive transition is poorly understood. Identification of the molecular basis underlying FT expression has thus become a major priority in the search for improved crop winter hardiness. This review summarizes recent work on cold acclimation, FT expression and vegetative/reproductive transition in winter cereals.

2. The genetics of freezing tolerance

Plants originated from high latitudes and altitudes, where winter season is long, have evolved the capability to control the transition from vegetative growth to flowering [13]. This evolutionary adaptation permits plants to start reproductive growth under favourable environmental conditions. The initiation and timing of this transition is determined by seasonal changes of the two main environmental cues, temperature and photoperiod. Initiation of reproductive growth in winter cereals can be accelerated by prolonged exposure to LT that are between 8 and 10 ˚C which is known as vernalization [17, 29]. A few weeks of LT are mainly enough to advance reproductive growth, but longer periods can accelerate vegetative/reproductive transition to a greater extent, up to the point when the vernalization response becomes fulfilled [33, 37].

A set of cold-regulated genes (*COR)* genes have been recognized in barley and winter wheat [6, 8]. In these species, prolonged exposure to low temperatures leads to a gradual decrease in the levels of *COR* transcripts and proteins, which is accompanied by a loss of cold tolerance. This loss of cold tolerance may be linked to the switch from the vegetative to the reproductive state as a result of vernalisation [8]. Previous studies have revealed that distinct sets of genes regulate the adaptive mechanisms allowing cereals to properly time flowering: (i) the vernalization-LT responsive genes (*Vrn-A1*, *Vrn-B1* and *Vrn-D1*) that regulate flowering and were genetically identified on chromosomes 5A, 5B and 5D; (ii) the photoperiod-responsive genes (*Ppd1*, *Ppd2* and *Ppd3*) that regulate flowering using day length; and are located on chromosomes 2D, 2B and 2A (iii) the earliness *per se* genes that influence the rate of development in fully vernalized plants grown under long-day conditions which classified in autonomous pathway and act independently of external stimuli. [5, 23, 31, 32, 34].

Alleles at the*Vrn-A1* locus appear to have a predominant effect in reducing the vernalization requirement compared to those on other *Vrn* loci [12]. For example, cultivars with *Vrn-A1* do not require vernalization at all, whereas those with *Vrn-B1* and *Vrn-D1* require vernalization for 15–30 days, and cultivars recessive for all of these genes require 45–60 days of vernalization [46]. Recently it has been revealed that isogenic line originated from spring cultivars, which containing recessive copies of all *vrn-1* loci after several backcross with hardy winter cultivars, showed a considerable FT compared to parental spring line [33]. Although there is a genetic interrelationship between vernalization requirement and FT expression, it seems that *vrn-A1* locus does not act alone in vegetative/reproductive transition and possibly other loci are involved in controlling the duration of the vegetative phase [16].

Dubcovsky et al. (1998) identified a second gene affecting vernalization response and named it *VRN2*. Contrary to the *VRN1* dominant allele for spring growth habit, *VRN2* allele for winter growth habit is dominant [56]. *VRN2* encodes a repressor of *VRN1* expression, which binds to the promoter region of the *VRN1* gene. As the vernalization process reduces the abundance of the *VRN2* gene product, *VRN1* transcription gradually increased, leading to the competence to flower [44]. Murai et al. (2003) reported that *APETALA1* (*AP1*)-like MADS box gene in wheat (*WAP1*) is as an activator of phase transition and their findings strongly suggested that *WAP1* is ortholog of *VRN1*.

In some photoperiod-sensitive winter cereals the vernalization necessity can be removed or significantly decreased through exposing the seedling to short days for several weeks and then returning them to long days [37, 44]. The short days can down-regulate *VRN2* and is expected to result in the acceleration of vegetative/reproductive transition and the elimination of the vernalization requirement [12].

3. Cold sensing and signaling

Cold acclimation involves proper perception, precise signaling and regulation of the transcriptome. The plasma membrane is recognized as the primary site of injury and is hypothesized to also be a site of perception of the LT stimulus [41]. Very little is known about cold sensors in plants. Potential sensors include Ca^{2+} influx channels, two-component histidine kinase, Receptor like kinases, Phospholipases, Photosynthetic apparatus and receptors associated with G-proteins [47, 61]. Cold stress induces transient

Ca^{2+} influx into the cytoplasm. Therefore, calcium permeable channels responsible for this Ca^{2+} influx are considered as sensors for low temperature [38]. It has been found that *AtHK,* as a well identified two-component histidine kinase in plants, up-regulated by LT [57]. Also previous study suggest that in AtHK dependent pathway stress sensing and signal traduction to the nucleus occurred through a phosphorylation cascade [58]. Dhonukshe *et al*. (2003) revealed that LT can induce accumulation of Phospholipase C and D which affect plasma membrane and lead to conformational change in the cytoskeleton or rearrangement of actin filaments. In cold-induced barley extracellular sugar concentration regulated expression of the stress-responsive genes [50]. Three different glucose signalling pathways are known in plants: one is hexokinase-dependent, the second glycolysisdependent, and the third hexokinase-independent [60]. After LT perception by sensors a variety of signalling pathways is triggered, including secondary messengers, ROS, Ca^{2+}-dependent protein kinases (CDPKs), mitogen- activated protein kinase (MAPK) cascades and the activation of transcription factors (TFs), all of which promote the production of cold-responsive proteins [27].

4. Cold acclimation under field vs. controlled conditions

Investigation of cold acclimation under controlled growth chambers and generalize the obtained result to field condition can be problematic [22]. Expression of dehydrin gene (*PpDhn1*) can be noted in this context. This gene are responsive to both short photoperiods and LT under filed conditions [2], whereas 3 weeks of short days at 20 ˚C had no impact on the gene expression under controlled condition. Perhaps the expression of the dehydrin gene is more affected by the cycling of warm and cool temperatures than by a specific low temperature which is available only in natural situations [22]. Another case that can be referred is that expression of light-responsive gene is strictly affected by light spectrum and possibly light condition in controlled environment would not be the same as in sunlight. Observed differences between the FT expression trend under controlled and natural conditions may somewhat resulted from different light spectrum [25, 35]. In growth chamber the roots of plants cool at a much quicker rate than experienced in nature, where there is a massive volume of soil. This can result to decrease in the hydraulic conductivity of the roots and consequently a transient increase in ABA may change gene expression pattern [22]. There is insufficient time for the plants grown under controlled condition to regulate gene expression during temperatures fluctuation [52]. In addition, soil or the growth medium in a pot does not possess the typical soil structure present in natural conditions, and water in pots is lost at a much more rapid rate than in field conditions. At any rate, this is just some examples that indicate how researchers must be very careful when trying to extrapolate results beyond the conditions that generated the data.

5. Structural proteins associated with freezing tolerance

With completion of genome sequencing projects and development of analytical methods for protein characterization, proteomics has become a major field of the functional genomics. Proteomics allows the global analysis of cold responsive gene products and related physiological processes. The dehydrins are a group of heat-stable, glycine-rich LEA proteins thought to be important for membrane stabilization and the protection of proteins from denaturation when the cytoplasm becomes dehydrated [27]. The most prominent dehydrin in wheat is WCS120 protein that is very efficient in the cryoprotection of lactate dehydrogenase and cell membranes stabilization [41]. COR413im was identified by Okawa et al. (2008) as an integral membrane protein targeted to the chloroplast inner envelope in response to low temperatures, where it contributes to plant freezing tolerance. Another LT-responsive gene family encodes antifreeze proteins (AFPs). In winter rye (*Secale cereale*), AFPs accumulate in response to cold, short daylength, dehydration and ethylene [21]. These proteins have a high affinity for ice and possess two typical properties: ice recrystallization inhibition and thermal hysteresis (the difference between the freezing point and the melting point). Heat Shock Proteins (HSPs) especially HSP90, HSP70, several small HSPs and chaperonins 60 and 20 increase in abundance during the cold acclimation. HSPs act as molecular chaperones that participate in membrane protection, refolding of denatured proteins and in preventing their aggregation [54]. Cho et al. (2007) reported that long period of LT (vernalization) increased the expression of ATP binding, GTP binding, translation elongation factor and glycine-rich

RNA-binding protein 7 (GRP7) in *Arabidopsis thaliana*.

It has been revealed that expression of some proteins such as Cell division control protein, asparagine synthase, RuBisCO activase, actin, heat shock protein 70, cp31BHv, serpin, fructose-bisphosphate aldolase, MADS-box transcription factor 26 and Ps16 in wheat cultivars increased by cold acclimation and significantly decreased after vegetative/reproductive transition [25]. It suggest that expression of theses proteins may are controlled by developmental regulation and they might work cooperatively to establish a new homeostasis under cold stress. Most of cold-responsive proteins are located in the chloroplasts, implying that chloroplast proteome is virtually subjective to cold stress. Cold responsive proteins mainly participate in photosynthesis, glycolysis, protein folding, redox homeostasis, transcription, translation, amino acid biosynthesis, ATP synthesis and ion transport [1, 9, 25, 43].

6. Compatible solute during cold acclimation

Plants can synthesize compatible solutes in response to desiccation, osmotic stress, salt stress or low temperature. Amino acids and some amino acid derivatives, sugars, acyclic and cyclic polyols, fructans, and quaternary amino, sulfonium compounds and potassium frequently act as compatible solutes [3, 4]. The main function of a compatible solute may be the stabilization of proteins, protein complexes or membranes under environmental stress. In in vitro experiments, compatible solutes at high concentrations have been found to reduce the inhibitory effects of ions on enzyme activity [48]. The addition of compatible solutes increased the thermal stability of enzymes [4, 18] and prevented dissociation of the oxygen evolving complex of photosystem II [42].

The sugar concentrations in transformants with an antisense inhibition of cytosolic fructose-1,6-bisphosphatase (cFBPase) and sucrose phosphate synthase (SPS) expression, or with overexpression of maize SPS, has been shown to correlate with the extent of LT tolerance after cold acclimation [49]. Cold acclimation is also associated with the accumulation of other soluble solutes like proteins, proline, and carbohydrates [26, 28]. In wheat LT led to fructan synthesis [55]. fructans are synthesised from sucrose by fructosyltransferases, and help to stabilise membranes by binding to the phosphate and choline groups of membrane lipids [59]. If sugars contribute to the stabilization of membrane structures, osmoregulation or subcellular volumes, they may act synergistically with or even as alternatives to some of the *COR* gene products. Gilmour et al. (2000) reported that constitutive expression of *CBF3* genes in *Arabidopsis* not only increased levels of cold-regulated proteins (CORs), but also enhanced levels of proline and total sugars, resulting in an increase in FT. Consequently, the *CBFs* seems to be ‘‘main keys’’ that integrate activation of multiple components of the cold acclimation response [53].

7. Conclusions and perspectives

Research on vegetative/reproductive transition has reached a most exciting stage. Vernalization and photoperiod requirement can control phonological development and by determining the length of vegetative phase may affect severity and duration of FT expression. A combined investigative approach involving physiological and biochemical analyses, aided by genomics- and proteomics-based platforms, identified a number of key basic genes and regulatory elements underlying the vegetative/reproductive transition. It has been demonstrated that three genes that control the vernalization requirement in winter cereal including *VRN1*, *VRN2* and *FT* (*VRN3*). These genes regulate not only the vernalization response but also the promotion of flowering by long days. *VRN1* is master switch that induced by vernalization and accelerates the transition to reproductive development at the shoot apex. The long days can induce the expression of *VRN3* that accelerates reproductive apex development. *VRN2* is a repressor for floral initiation and its expression suppress via vernalization. Information on the transcriptome, proteome and metabolome of vegetative/reproductive transition should be useful to improve FT through breeding programs.

8. References

1. Amme S, Matros A, Schlesier B, Mock HP: **Proteome analysis of cold stress response in *Arabidopsis thaliana* using DIGE-technology.** Journal of Experimental Botany 2006, 57: 1537-1546.
2. Artlip TS, Callahan AS, Basset CL, Wisniewski ME: **Seasonal expression of a dehydrin gene in sibling deciduous and evergreen genotypes of**

peach (*Prunus persica* L. Batsch). *Plant Molecular Biology* 1997, 33:61–70.

3. Bohnert HJ, Jensen RG: **Strategies for engineering water-stress tolerance in plants**. *Trends* in *Biotechnology* 1996, 14, 89-97.

4. Bohnert HJ, Shen B: **Transformation and compatible solutes**. Scientia Horticulturae 1999, 78: 237-260.

5. Bullrich L, Appendino L, Tranquilli G, Lewis S, Dubcovsky J: **Mapping of a thermo-sensitive earliness *per se* gene on Triticum monococcum chromosome 1A(m).** Theoretical and Applied Genetics 2002, 105: 585-593.

6. Chinnusamy V, Zhu J, Zhu JK: **Gene regulation during cold acclimation in plants**. Physiologia Plantarum 2006, 126: 52– 61.

7. Cho MR, Lee KH, Hyun Y, Lee I, Kim HJ: **Proteome analysis of vernalization-treated *Arabidopsis thaliana* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.** *Bulletin of the Korean Chemical Society* 2007, 28: 427-431.

8. Crosatti C, Pagani D, Cattivelli L, Stanca AM, Rizza F: **Effects of growth stage and hardening conditions on the association between frost resistance and the expression of the cold induced protein COR14b in barley**. Environmental and Experimental Botany 2008, 62: 93-100

9. Cui S, Huang F, Wang J, Ma X, Cheng Y, Liu J: **A proteomic analysis of cold stress responses in rice seedlings.** Proteomics 2005, 5: 3162-3172.

10. Dhonukshe P, Laxalt AM, Goedhart J, Gadella TW, Munnik T: **Phospholipase D activation correlates with microtubule reorganization in living plant cells**. Plant Cell 2003, 15: 2666-2679.

11. Dubcovsky J, Lijavetzkyu D, Appendino L, Tranquilli G: **Comparative RFLP mapping of *Triticum monococcum* genes controlling vernalization requirement**. *Theoretical and Applied Genetics* 1998, 97: 968–975.

12. Dubcovsky J, Loukoianov A, Fu D, Valarik M, Sanchez A, Yan L: **Effect of photoperiod on the regulation of wheat vernalization genes *VRN1* and *VRN2***. Plant Molecular Biology 2006, 60: 469-480.

13. Flood RG, Halloran GM: **The nature and duration of gene action for vernalization response in wheat**. *Annals of Botany*1984, 53: 263-368

14. Fowler DB, Breton G, Limin AE, Mahfoozi S, Sarhan F: **Photoperiod and temperature interactions regulate low-temperature induced gene expression in barley**. Plant Physiology 2001, 127: 1676-1681.

15. Fowler DB, Limin AE, Ritchie JT: **Low-temperature tolerance in cereals: model and genetic interpretation**. Crop Science 1999, 39: 626-633.

16. Fowler DB, Limin AE: **Interactions among factors regulating phenological development and acclimation rate determine low-temperature tolerance in wheat**. Annals of Botany 2004, **94**: 717 - 724.

17. Fowler DB, Limin AE, Wang SY, Ward RW: **Relationship between low-temperature tolerance and vernalization response in wheat and rye**. Canadian Journal of Plant Sciences 1996, 76: 37-42.

18. Galinski EA: **Compatible solutes of halophilic eubacteria: molecular principles, water-solute interaction, stress protection**. Experientia 1993, 49, 487-496.

19. Gilmour SJ; Sebolt AM; Salazar MP; Everard, JD,Thomashow MF: **Overexpression of the Arabidopsis *CBF3* transcriptional activator mimics multiple biochemical changes associated with cold acclimation**. Plant Physiology 2000, 124: 1854-1865.

20. Gordon RG, Chauvin L, Sarhan F, Huner NP: **Cold acclimation and freezing tolerance, a complex interaction of light and temperature**. Plant Physiology 1997, 114: 467-474.

21. Griffith M, Yaish MW: **Antifreeze proteins in overwintering plants: a tale of two activities**. Trends in Plant Science 2004, 9: 399-405.

22. Gusta LV, Trischuk R, Weiser CJ: **Plant cold acclimation: the role of abscisic acid**. *Journal of Plant Growth Regulation* 2005, 24: 308-318

23. Iwaki K, Nishida J, Yanagisawa T, Yoshida H, Kato K: **Genetic analysis of *Vrn-B1* for vernalization requirement by using linked dCAPS markers in bread wheat (*Triticum aestivum* L.)**. *Theoretical and Applied Genetics* 2002, 104: 571-576.

24. Janmohammadi M, Tavakol-Afshari R, Mahfoozi S, Alizadeh H, Kamel M, Khiavi M: **Relationship among phenological development, physiological indices and freezing tolerance in winter wheat and rye under field conditions in moderate and cold regions**. Elecronic Journal of Crop Production 2010, 3: 115-137.

25. Janmohammadi M: **Study of interrelationship between vegetative/reproductive transition stage and cold induced proteins expression using proteomics analysis in wheat grown under field conditions**. P.hD. *University* of *Tehran, Faculty of Agricultural Sciences and Engineering*. *Tehran,* 2010, *236 pp.*

26. Janmohammadi M, Enayati V, Sabaghnia N: **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 2012, 25: 3-11.

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

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

29. Kirby KJM, Appleyard M: **Effects of photoperiod on the relation between development and yield per plant of a range of spring barley varieties**. Z. Pflanzenzuchtg 1980, 85: 226-239.

30. Larcher W: 1995. **Physiological Plant Ecology edn** 3. Berlin: Springer 506 *p*.

31. Laurie DA, Pratchett N, Bezant JH, Snape JW: **RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross**. Genome 1995, 38: 575-585.

32. Law CN, Sutka J, Worland AJ: **A genetic study of day-length response in wheat**. Heredity 1978, 41: 185-191.

33. Limin AE, Fowler DB: **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 2002, 89: 579-585.

34. Loukoianov A, Yan L, Blechl A, Sanchez A, Dubcovsky J: **Regulation of *VRN-1* vernalization genes in normaland transgenic polyploid wheat**. Plant Physiology 2005, 138: 2364-2373.

35. Mahfoozi S, Limin AE, Ahakpaz F, Fowler DB: **Phenological development and expression of freezing resistance in spring and winter wheat under field conditions in north-west Iran**. Field Crops Research 2006, 97: 182-187.

36. Mahfoozi S, Limin AE, Fowler DB: **Developmental regulation of low-temperature tolerance in winter wheat.** Annals of Botany 2001, 87: 751-757.

37. Mahfoozi S, Limin AE, Hayes PM, Hucl P, Fowler DB: **Influence of photoperiod response on the expression of cold hardiness in wheat and barley**. *Canadian Journal of Plant Science* 2000, 80: 721-724.

38. Monroy AF, Dhindsa RS: **Low-temperature signal transduction: induction of cold acclimation-specific genes of alfalfa by calcium at 25 degrees C**. Plant Cell 1995, 7:321-331.

39. Murai K, Miyamae M, Kato H, Takumi S, Ogihara Y: ***WAP1*, a wheat *APETALA1* homolog, plays a central role in the phase transition from vegetative to reproductive growth**. Plant Cell Physiology 2003, 44: 1255-1265.

40. Okawa K, Nakayama K, Kakizaki T, Yamashita T, Inaba T: **Identification and characterization of Cor413im proteins as novel components of the chloroplast inner envelope**. Plant, Cell and Environment 2008, 31: 1470-1483.

41. Ouellet F: **Cold acclimation and freezing tolerance in plants**. Encyclopedia of Life Sciences, John Wiley & Sons, Ltd. 2007.

42. Papageorgiou G, Murata N: **The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving photosystem II complex**. *Photosynthesis* Research 1995, 44: 243-252.

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

44. Sasani S, Hemming MN, Oliver SN, Greenup A, Tavakko-Afshari R, Mahfoozi S, Poustini K, Sharifi HR, Dennis ES, Peacock WJ, Trevaskis B: **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 2009, 60: 2169-2178.

45. Sãulescu NN, Braun HJ: Breeding for adaptation to environmental factors. **Cold tolerance**. *In* Reynolds MP, Ortiz-Monasterio JI & McNab A (eds.) *Application of physiology in wheat breeding*, Mexico, 2001, CIMMYT, pp. 111-123.

46. Shindo C, Tsujimoto H, Sasakuma T: **Segregation analysis of heading traits in hexaploid wheat utilizing recombinant inbred lines**. Heredity 2003, 90: 56-63.

47. Solanke AU, Sharma AK: **Signal transduction during cold stress in plants**. *Physiology and Molecular Biology of Plants* 2008, 14: 69-79.

48. Solomon A, Beer S, Waisel Y, Jones GP, Paleg LG: **Effects of NaCl on the carboxylating activity of Rubisco from Tamarix jordanis in the presence and absence of prolinerelated compatible solutes**. Plant Physiology 1994, 90: 198-204.

49. Stitt M, Hurry V: **A plant for all seasons: Alterations in photosynthetic carbon metabolism during cold acclimation in Arabidopsis**. Current Opinion in Plant Biology 2002, 5, 199-206.

50. Tabaei-Aghdaei SR, Pearce RS, Harrison P: **Sugars regulate cold-induced gene expression and freezing-tolerance in barley cell cultures**. Journal of Experimental Botany 2003, 54: 1565-1575.

51. Tantau H, Balko C, Brettschneider B, Melz G, Do¨rffling K: **Improved frost tolerance and winter survival in winter barley (Hordeum vulgare L.) by in vitro selection of proline overaccumulating lines**. Euphytica 2004, 139: 19–32.

52. Thomashow MF: **Plant cold acclimation: freezing tolerance genes and regulatory mechanisms**. *Annual Review of Plant Physiology* and *Plant Molecular Biology* 1999, 50:571-599.

53. Thomashow MF: **So what's new in the field of plant cold acclimation? Lots**!. Plant Physiology 2001, 125: 89-93.

54. Timperio AM, Egidi MG, Zolla L: **Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP)**. *Journal of Proteomics* 2008, 71: 391-411.

55. Tognetti JA, Calderon PL, Pontis HG: **Fructan metabolism: Reversal of cold acclimation**. Journal of Plant Physiology 1989, 134: 232-236.

56. Tranquilli G, Dubcovsky J: **Epistatic interaction between vernalization genes *Vrn-Am1* and *Vrn-Am2* in diploid wheat**. *Journal of Heredity* 2000, 91: 304–306.

57. Urao T, Miyata S, Yamaguchi-Shinozaki K, Shinozaki K: **Possible His to Asp phosphorelay signaling in an *Arabidopsis* two-component system**. FEBS Letter 2000, 478: 227-232.

58. Urao T, Yakubov B, Satoh R, Yamaguchi-Shinozaki K, Seki M, Hirayama T, Shinozaki K: **A transmembrane hybrid-type histidine kinase in *Arabidopsis* functions as an osmosensor**. Plant Cell 1999, 11: 1743-1754.

59. Valluru R, Van den Ende W: **Plant fructans in stress environments: emerging concepts and future prospects**. Journal of Experimental Botany 2008, 59: 2905-2916.

60. Xiao W, Sheen J, Jang JC: **The role of hexokinase in plant sugar signal transduction and growth and development**. Plant Molecular Biology 2000, 44: 451-461.

61. Xiong L, Schumaker KS, Zhu JK: **Cell signaling during cold, drought, and salt stress**. The Plant Cell 2002, 14: S165-183.

62. Yadav SK: **Cold stress tolerance mechanisms in plants**. *Agronomy for Sustainable Development* 2010, 30: 515-527.