

REVIEW ARTICLE

Cold stress and acclimation – what is important for metabolic adjustment?

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ABSTRACT

As sessile organisms, plants are unable to escape from the many abiotic and biotic factors that cause a departure from optimal conditions of growth and development. Low temperature represents one of the most harmful abiotic stresses affecting temperate plants. These species have adapted to seasonal variations in temperature by adjusting their metabolism during autumn, increasing their content of a range of cryo-protective compounds to maximise their cold tolerance. Some of these molecules are synthesised *de novo*. The down-regulation of some gene products represents an additional important regulatory mechanism. Ways in which plants cope with cold stress are described, and the current state of the art with respect to both the model plant *Arabidopsis thaliana* and crop plants in the area of gene expression and metabolic pathways during low-temperature stress are discussed.

INTRODUCTION

Environmental variables, especially those affecting water availability and temperature, are the major determinants of plant growth and development. Oceans and the low-temperature Polar regions occupy almost 80% of the Earth's surface. Only one-third of the total land area is free of ice, and some 42% of this land regularly experiences temperatures below $-20\text{ }^{\circ}\text{C}$ (Juntilla & Robberecht 1999). In such areas, plants require specialised mechanisms to survive exposure to low temperature. Most temperate plant species have evolved a degree of cold tolerance, the extent of which is typically dependent on a combination of the minimum temperature experienced and the length of exposure to cold stress. Variation in tolerance level can be genetically determined, as well as being affected by plant developmental stage and physiological status at the time of exposure. A number of major temperate crop species are sown in the autumn and reach maturity in the following summer. Plant survival over the winter period – termed winter hardiness – can be broken down into a number of simpler components, one of the most important of which is frost tolerance (Tantau *et al.* 2004). Identification of the key genes underlying cold stress has thus become a major priority in the search for improved crop winter hardiness. A deeper understanding of the regulation of these genes, and of their response to low-temperature stress, would allow clarification of the ways in which plants adjust to the stress. Knowledge of this type is widely expected to

provide opportunities for the manipulation of gene expression in crop plants, with a view to engineering higher levels of cold tolerance.

COLD ADAPTATION

Species adapted by natural selection to cold environments have evolved a number of physiological and morphological means to improve survival in the face of extended cold periods (Guy 1999). Typically, these species – herbs, grasses and ground shrubs – are of short stature, have a low leaf surface area and a high root/shoot ratio. Their growth habit takes full advantage of any heat emitted from the ground during the day and minimises night chilling, since air temperature is maintained most effectively near the soil surface (Nilsen & Orcutt 1996). Cold-adapted plants tend to be slow growing, have the C3 mode of photosynthesis and store sugars in underground tissues. Plants well adapted to cool environments have evolved an efficient respiration system, which allows them to rapidly mobilise stored reserves during the short growing season. The timing of developmental and physiological responses to environmental stress is under strict genetic control (Guy 1999).

STRESS AVOIDANCE AND TOLERANCE

The two distinct strategies taken by plants to combat low-temperature stress are avoidance and tolerance. Stress avoid-

ance entails preventing the freezing of sensitive tissues. Some succulent species (with thick tissue mass and abundant water content) are able to accumulate residual heat during the day and dissipate it slowly during the cold night (Nilsen & Orcutt 1996); many annual herbs survive in the form of dormant organs or seed; others protect the shoot meristem with leaves (Kacperska 1999). A more elaborate avoidance strategy involves supercooling, in which endogenous ice nucleation is prevented by inhibiting the formation of ice nucleators, even where the temperature falls as low as -40 °C. Extremely winter hardy species can generate within their cells so-called 'liquid glass', a highly viscous solution that prevents ice nucleation even at -196 °C. Such cells become osmotically, thermally and mechanically de-sensitised to the presence of external ice (Wisniewski & Fuller 1999). Where the severity of the stress is more progressive, tolerant plants have evolved the ability to acclimatise, defined by Kacperska (1999) as the non-heritable modification of structure and function in response to stress, in a way that reduces harm and thereby improves fitness.

The plant response to low-temperature stress can be divided into three distinct phases. The first is cold acclimation (pre-hardening), which occurs at low, but above zero temperatures. The second stage (hardening), during which the full degree of tolerance is achieved, requires exposure to a period of sub-zero temperatures. The final phase is plant recovery after winter (Li *et al.* 2008). Some plants (especially trees) need a combination of short photoperiod and low temperature to fully develop their cold tolerance. In these cases, tolerance can be lost if the temperature is raised above zero and the photoperiod is lengthened (Juntilla & Robberecht 1999; Kacperska 1999). Plant organs differ in their level of tolerance – typically the roots are much more sensitive than the crown (McKersie & Leshem 1994), which is understandable given that the crown is the site of the major meristem responsible for production of new roots and shoots at the end of the cold period.

COLD ACCLIMATION

Overwintering temperate plant species acclimatise during autumn, during which their metabolism is redirected towards synthesis of cryoprotectant molecules such as soluble sugars (saccharose, raffinose, stachyose, trehalose), sugar alcohols (sorbitol, ribitol, inositol) and low-molecular weight nitrogenous compounds (proline, glycine betaine). These, in conjunction with dehydrin proteins (DHNs), cold-regulated proteins (CORs) and heat-shock proteins (HSPs), act to stabilise both membrane phospholipids and proteins, and cytoplasmic proteins, maintain hydrophobic interactions and ion homeostasis, and scavenge reactive oxygen species (ROS); other solutes released from the symplast serve to protect the plasma membrane from ice adhesion and subsequent cell disruption (Hare *et al.* 1998; Iba 2002; Wang *et al.* 2003; Gusta *et al.* 2004; Chen & Murata 2008). The process of solute release, especially of vacuolar fructans, to the extracellular space is a vesicle-mediated, tonoplast-derived exocytosis (Valluru & Van den Ende 2008). Fructans are transported to the apoplast by post-synthesis mechanisms, probably in response to cold stress (Valluru *et al.* 2008). The activity of fructan exohydrolase, which generates increased sugar (glu-

cose, fructose, sucrose) content, is an important part of the hardening process. Symplastic and apoplastic soluble sugar – not only fructan precursors, but also trehalose, raffinose, as well as fructo- and gluco-oligosaccharides – contributes directly to membrane stabilisation (Livingston *et al.* 2006). Also important is the increased activity of the antioxidative enzymes superoxide dismutase, glutathione peroxidase, glutathione reductase, ascorbate peroxidase and catalase, as well as the presence of a series of non-enzymatic antioxidants, such as tripeptidthiol, glutathione, ascorbic acid (vitamin C) and α -tocopherol (vitamin E) (Chen & Li 2002).

In addition to the production of protective compounds that participate in membrane stabilisation, cold acclimation also affects cell lipid composition, which is necessary for the maintenance of plasma membrane functionality. In particular, the proportion of unsaturated fatty acids making up the phospholipids is increased (Rajashekar 2000). De Palma *et al.* (2008) suggested that both the composition of the phospholipids and their ability to interact with other protective proteins are important for generating a higher level of freezing resistance. Phospholipase D, in particular, participates in the degradation of phospholipids, and its suppression may therefore be relevant in improving freezing tolerance (Rajashekar 2000). Some plants respond to cold stress by the synthesis of proteins that inhibit the activity of ice nucleators. Some of these so-called 'anti-freeze' proteins are highly similar in sequence to plant pathogen-related (PR) proteins (particularly in winter rye), and accumulate in response to cold, drought or the exogenous supply of ethylene (Moffatt *et al.* 2006). These proteins assemble as oligomers, which can bind to the surface of a newly formed ice crystal, and thereby influence its subsequent shape and growth. Their antifreeze activity is modulated by Ca^{2+} , which is either released from pectin or bound to specific proteins. An altered ratio of abscisic acid (ABA) to gibberellin content, in favour of ABA, results in the retardation of growth required for pre-hardening (Juntilla *et al.* 2002). Gibberellin content is regulated by a family of nuclear growth-repressing proteins called DELLAs, and these are components of the C-repeat (CRT) binding factor 1 (CBF1)-mediated cold stress response. However, the degradation of DELLAs is stimulated by gibberellins (Achard *et al.* 2008).

COLD SENSING AND SIGNALLING

The identity of the plant sensors of low temperature remains as yet unknown (Chinnusamy *et al.* 2006). Multiple primary sensors may be involved, with each perceiving a specific aspect of the stress, and each involved in a distinct branch of the cold signalling pathway (Xiong *et al.* 2002). Potential sensors include Ca^{2+} influx channels, two-component histidine kinase and receptors associated with G-proteins (Xiong *et al.* 2002). Certain cytoskeletal components (microtubules and actin filaments) participate in cold sensing by modulating the activity of Ca^{2+} channels following membrane rigidification (Nick 2000; Örvär *et al.* 2000; Abdrakhamanova *et al.* 2003). Because of its basic role in separating the internal from the external environment, the plasma membrane has been considered as a site for the perception of temperature change (Sangwan *et al.* 2002; Uemura *et al.* 2006; Vaultier *et al.* 2006; Wang *et al.* 2006), with its rigidification representing an early response (Vaultier *et al.* 2006). The phosphorylation

of proteins, together with the suppression of protein phosphatase activity, may also provide a means for the plant to sense low temperatures (Rajashekar 2000). Thus, a variety of signalling pathways is triggered, including secondary messengers, ROS, Ca²⁺-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. These products can be divided into two distinct groups: regulatory proteins controlling the transduction of the cold stress signal, and proteins functionally involved in the tolerance response. The latter include LEA (late embryogenesis abundant) proteins, antifreeze proteins, mRNA-binding proteins, chaperones, detoxification enzymes, proteinase inhibitors, transporters, lipid-transfer proteins and enzymes required for osmoprotectant biosynthesis (Shinozaki & Yamaguchi-Shinozaki 2000; Xiong & Zhu 2001; Wang *et al.* 2003; Grennan 2006; Nakashima & Yamaguchi-Shinozaki 2006; Yamaguchi-Shinozaki & Shinozaki 2006). An outline of these processes is given in Fig. 1.

THE CBF/DREB RESPONSIVE PATHWAY

The CBF/DREB responsive pathway provides one of the most important routes for the production of cold responsive pro-

teins. The major cis-acting element involved in CBF/DREB is DRE (dehydration-responsive element)/CRT. Two major groups of transcription factors bind to DRE/CRT sequences, namely CBF/DREB1 (CRT-binding factor/DRE-binding protein) in low-temperature signalling, and DREB2 during osmotic stress (Nakashima & Yamaguchi-Shinozaki 2006). CBF1, 2 and 3 are all responsive to low temperature, and their encoding genes are present in tandem on *Arabidopsis thaliana* chromosome 4. The genes carry the conserved AP2/ERF domain DNA-binding motif (Riechmann & Meyerowitz 1998). CBF2/DREB1C is a negative regulator of both CBF1/DREB1B and CBF3/DREB1A. CBF3 is thought to regulate the expression level of CBF2 (Novillo *et al.* 2004; Chinnusamy *et al.* 2006). Thus, the function(s) of CBF1 and CBF3 differ from those of CBF2, and act additively to induce the set of CBF-responsive genes required to complete the process of cold acclimation (Novillo *et al.* 2007). Upstream of CBF lie both ICE1 (inducer of CBF expression), a positive regulator of CBF3, and HOS1 (high expression of osmotically sensitive), a negative regulator of ICE1. The HOS1 product is a RING E3 ligase targeting ICE1 for degradation in the proteasome (Dong *et al.* 2006). Because of the rapid (within a few minutes) induction of CBF transcripts following plant exposure to low temperature, ICE1 is unlikely to require *de novo* synthesis, but rather is already present in the absence of cold stress and is only activated when the temperature decreases (Chinnusamy *et al.* 2003). The LOS1 (low expression of osmotically responsive genes) product is a translation elongation factor 2-like protein, which negatively regulates CBF expression.

The likely regulators of CBF1 and CBF2 are bHLH proteins other than ICE1. To obtain transient expression of CBFs, the levels of CBF1 and CBF3 transcript, after their induction by ICE1, are subsequently lowered by CBF2. Accordingly, the peak expression of CBF2 in response to low temperature has been shown to occur about 1 h later than that of either CBF1 or CBF3 (Novillo *et al.* 2004). In addition to ICE1, a further positive regulator of CBF expression is LOS4, an RNA helicase-like protein (Gong *et al.* 2002) (Fig. 2). However, CAX1 (cation exchanger), which plays a role in returning cytosolic

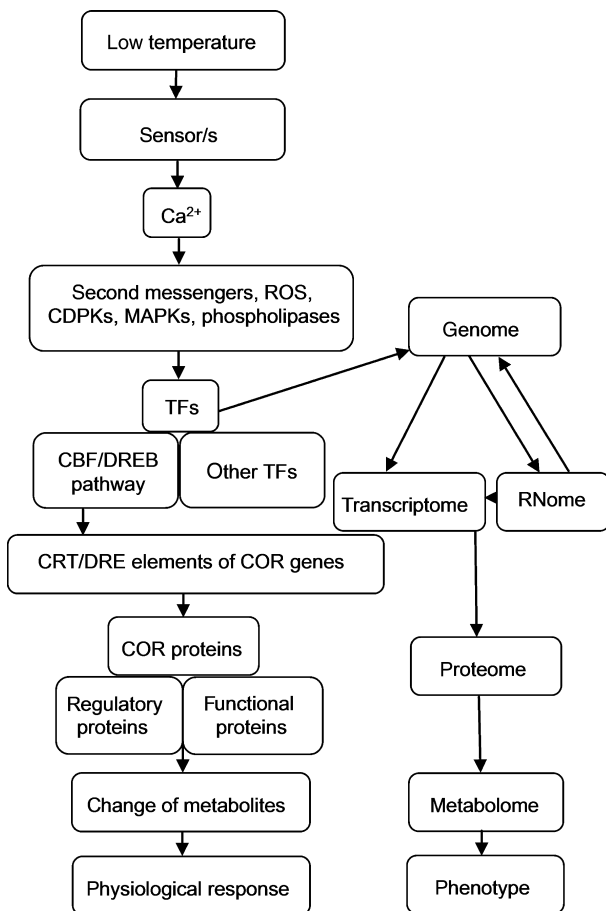


Fig. 1. Scheme illustrating the molecular response of plants to low-temperature stress, realised as changes in transcriptome, proteome, metabolome and phenotype.

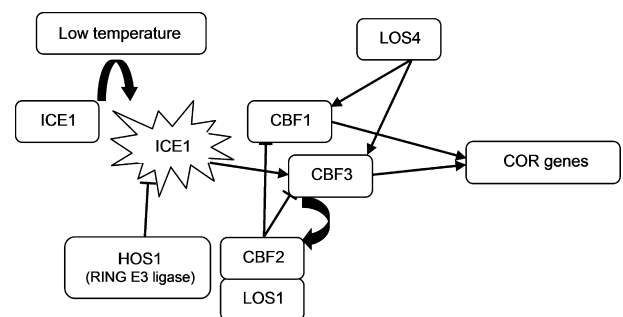


Fig. 2. The CBF (C-repeat-binding factor) pathway in plants. ICE1 (inducer of CBF expression) is activated by low temperature and is inhibited by HOS1 (high expression of osmotically sensitive). This triggers the expression of CBF3, which promotes the accumulation of COR (cold regulated) gene products. CBF3 expression also positively regulates the expression of CBF2, which, in turn, leads to the down-regulation of CBF3 and CBF1. LOS4 (low expression of osmotically responsive genes) is a positive regulator of CBF expression, and LOS1 a negative regulator.

Ca²⁺ concentrations to basal levels following a transient increase in response to low-temperature stress, is a negative regulator of *CBF1*, 2 and 3 (Hirschi 1999; Catalá *et al.* 2003). The ICE1-CBF pathway provides positive regulation of the expression of certain Zn finger transcriptional repressors and the SFR6 (sensitivity to freezing) protein, which is required for CBF function. SFR6 may be part of an adaptor complex required for CBF action, or alternatively, may be involved in the translation machinery of CBF transcripts and in CBF protein stability (Shinozaki & Yamaguchi-Shinozaki 2000; Zarka *et al.* 2003; Fowler *et al.* 2005; Lee *et al.* 2005; Chinnusamy *et al.* 2006; Dong *et al.* 2006; Van Buskirk & Thomashow 2006). Further pathways, not under CBF control, are also involved in the regulation of cold-responsive genes (Fowler & Thomashow 2002; Chen & Zhu 2004; Chinnusamy *et al.* 2006; Van Buskirk & Thomashow 2006). In particular, Chinnusamy *et al.* (2006) have shown that both HOS9 and HOS10 transcription factors play a role in the regulation of freezing tolerance in a CBF-independent manner.

THE CBF/DREB RESPONSIVE PATHWAY IN CROPS

Knowledge gained from the study of the model plant *A. thaliana* has proven to be largely, but not completely, transferable to crop plants. Unfortunately, many key stress responses are not transferable, *e.g.* given Oh *et al.*'s (2007) experience with over-expression of the barley low temperature induced gene *HvCBF4* in rice, which resulted in the up-regulation of a set of genes not predicted from the heterologous expression of *AtCBF3* in rice. For cereal species, this disadvantage is to some extent balanced by the availability of the rice genome sequence, and its increasing level of annotation, since the cereal species genomes are all closely related to one another (Moore *et al.* 1995). In barley, the CBF genes *HvCBF3*, *HvCBF4* and *HvCBF8* are all components of the frost resistance quantitative trait locus (QTL) located on chromosome 5H (Francia *et al.* 2004). A contrasting approach has targeted comparisons between spring- and winter-sown cereal cultivars. Thus, for example, Monroy *et al.* (2007) observed that spring and winter wheats share the same initial rapid expression of cold-inducible genes, but that their transcriptional profiles diverge widely during cold acclimation. While in winter cultivars the expression of cold acclimation genes continues over time, in spring cultivars, their levels of expression decline and the cold acclimation process is overridden by the transition from the vegetative to the reproductive stage.

Although the regulation of genes in the CBF-responsive pathway has been only marginally explored to date in woody plants, there are some indications that their regulation is more complex than in herbaceous species. For example four *Eucalyptus gunnii* CBF1 genes displayed differential expression in response to cold treatment (Kayal *et al.* 2006; Navarro *et al.* 2009).

SIGNALLING CROSS-TALK

The signalling pathway associated with cold stress is believed to be rather less dependent on ABA than those involved in the response to either moisture or salinity stress (Shinozaki & Yamaguchi-Shinozaki 2000; Shinozaki *et al.* 2003; Zhang *et al.* 2004). However, it is clear that some cross-talk does occur

between the various abiotic stress signalling pathways, as the transcription of members of identical gene families is induced, and rather similar products are accumulated (Shinozaki & Yamaguchi-Shinozaki 2000; Shinozaki *et al.* 2003; Chen & Zhu 2004; Chinnusamy *et al.* 2004). For instance, in *A. thaliana* the hydrophilic proteins COR15a and COR78 are accumulated as a response to both cold and moisture stress (Rajashekar 2000). Because many abiotic stresses – including freezing, drought and salinity – result in cellular dehydration, it is hardly unexpected to find some overlap in the various signalling pathways that all deliver protection against cellular dehydration. This commonality presumably lies behind the involvement of the CBF regulon in the abiotic stress response (Fowler *et al.* 2005). A key link between the various pathways is the ROS network, which balances scavenging with production (Torres & Dangl 2005). Plants are thought to have evolved a high degree of control over ROS toxicity, to the extent that ROS are exploited as signalling molecules (Timperio *et al.* 2008). The plant cell senses ROS *via* redox-sensitive transcription factors (*e.g.* nitrogen permease reactivator or heat-shock factors), which activate functional proteins involved in the re-establishment of cellular homeostasis (Mittler *et al.* 2004).

Freezing tolerance has been identified as a multigene trait. Some interspecific variation has been identified among relevant gene products (Rajashekar 2000), but the regulation of many of the genes induced during cold acclimation are conserved between species (Cattivelli *et al.* 2002; Chen & Zhu 2004; Chinnusamy *et al.* 2006; Nakashima & Yamaguchi-Shinozaki 2006). Zhu *et al.* (2008) have suggested that histone acetylation/deacetylation is an important player in gene activation and repression during cold acclimation, and in particular showed that the *HOS15* gene product, a nuclear-localised repressor protein that functions as a histone deacetylase, specifically interacts with histone H4. A recent research focus has centred on the role of microRNAs (miRNAs) as regulators of stress responses. Several stress-related elements are present in the promoter regions of certain miRNAs, and some miRNAs are known to be inducible by abiotic stress. miRNA expression profiling has also been used to demonstrate the existence of cross-talk between the salinity, cold and drought stress signalling pathways (Liu *et al.* 2008).

FUNCTIONAL PROTEINS INVOLVED IN COLD ACCLIMATION

The level of cold hardiness has been successfully correlated with the level of expression of barley *COR* genes (Pearce *et al.* 1996). During the cold acclimation process, *COR* genes such as *COR tmc-ap3* and *COR14b* are up-regulated, as are *BLT* (barley low temperature) genes (*BLT14*, *BLT63*, *BLT801*, *BLT4*) and *ELIP* (early light inducible protein) genes (Cattivelli *et al.* 2002). Other genes are down-regulated, most typically those associated with photosynthesis, such *LHC* (light harvesting complex) and plastocyanin (Atienza *et al.* 2004). Atienza *et al.* (2004) investigated the induction of the three genes, *DHN5*, *DHN8* and *COR14b*, in barley, and found them to be cold specific. Other up-regulated genes included those encoding enzymes involved in amino acid metabolism (chloroplast-dependent, except for proline, which was chloroplast-independent).

LEA PROTEINS

The dehydrins are a group of heat-stable, glycine-rich LEA proteins thought to be important for membrane stabilisation and the protection of proteins from denaturation when the cytoplasm becomes dehydrated. Nakayama *et al.* (2008) have suggested that some of them, especially COR15am, function as a protectant by preventing protein aggregation. The dehydrins ERD10 (early response to dehydration) and ERD14 function as chaperones and interact with phospholipid vesicles through electrostatic forces (Kovacs *et al.* 2008). Several dehydrins are significantly accumulated during cold stress (Allagulova *et al.* 2003; Kawamura & Uemura 2003; Renaut *et al.* 2004). Microarray experiments have shown that the expression profile of specific combinations of dehydrin genes can provide a reliable indication of low temperature and drought stress (Tommasini *et al.* 2008). 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. However, the SFR2 protein, which is protective of the chloroplast during freezing, is localised in the chloroplast outer envelope membrane (Fourrier *et al.* 2008).

HSP PROTEINS

Osmotic, cold and salt stresses are the strongest inducers of HSP expression in plants (Timperio *et al.* 2008). Some HSPs (in particular HSP90, HSP70, several small HSPs and chaperonins 60 and 20) increase in abundance following exposure to low temperature, and unlike the HSPs produced in response to high temperature stress, which function as molecular chaperones, these have a strong cryoprotective effect, participating in membrane protection, in the refolding of denatured proteins and in preventing their aggregation (Renaut *et al.* 2006; Timperio *et al.* 2008). Small HSPs are not themselves able to refold non-native proteins, but do facilitate refolding effected by HSP70 and HSP100 (Sun *et al.* 2002; Mogk *et al.* 2003).

PR PROTEINS – THE CONNECTION BETWEEN BIOTIC AND ABIOTIC STRESS

Some PR proteins are also responsive to low-temperature stress. Among these are PR-2 (β -1,3-glucanase), PR-3, PR-4, PR-5 (thaumatin-like proteins), PR-8, PR-10 (Bet v-1 homologues), PR-11 (chitinases) and PR-14 (lipid transfer proteins). The β -1,3-glucanases, chitinases and thaumatin-like proteins have antifreeze activity in the apoplastic space, which means that they are able to inhibit the recrystallisation of intercellular ice, and even prevent the formation of intracellular ice (Wisniewski *et al.* 1999; Griffith & Yaish 2004; Renaut *et al.* 2006). Because extracellular freezing can cause cell dehydration, the down-regulation of aquaporins (water channels that regulate water efflux) may well be important for cold acclimation (Peng *et al.* 2008).

ENZYMATIC AND METABOLIC RESPONSE

Many enzymes are involved in the cold response machinery. In addition to those associated with osmolyte metabolism,

detoxification cascades and photosynthesis, lignin metabolism (caffeic acid 3-O-methyltransferase), secondary metabolism, cell wall polysaccharide remodelling, starch metabolism, sterol biosynthesis and raffinose family oligosaccharide (myo-inositol-1-phosphate synthase and galactinol synthase) synthesis are all participants in the global response to cold stress (Fowler & Thomashow 2002; Renaut *et al.* 2006). Whereas the transcription level of genes involved in photosynthesis, tetrapyrrole synthesis, cell wall, lipid and nucleotide metabolism is negatively correlated with freezing tolerance, the level of transcription of genes associated with carbohydrate, amino acid and secondary metabolism (*e.g.* flavonoids) is positively correlated with freezing tolerance (Hannah *et al.* 2006).

Much attention has been paid to studying the response of saccharide metabolism to low-temperature conditions. The transcriptional up-regulation of the raffinose oligosaccharide pathway results in accumulation of monosaccharides and disaccharides (including glucose, fructose, sucrose, galactinol, melibiose and raffinose) (Cook *et al.* 2004; Hannah *et al.* 2006; Usadel *et al.* 2008). A key enzyme in the synthesis of raffinose oligosaccharides is galactinol synthase, which catalyses the first committed step in raffinose synthesis. Transcription of this enzyme, along with that of raffinose synthase and to some extent that of a number of enzymes involved in the synthesis of precursors for the raffinose pathway, such as members of the myoinositol phosphate synthase family, is induced by a fall in temperature (Usadel *et al.* 2008). Raffinose accumulation on its own, however, is neither sufficient nor necessary for the induction of freezing tolerance or cold acclimation in *A. thaliana* (Zuther *et al.* 2004). Nishizawa *et al.* (2008) have also suggested the possibility that both galactinol and raffinose are ROS scavengers. The drought, salinity and cold tolerance of rice transformed with an over-expressed *Escherichia coli* trehalose biosynthetic gene were all significantly better than the wild type (Garg *et al.* 2002). In addition to those in the raffinose pathway, other sugar metabolism enzymes are also involved in the cold response. In *A. thaliana*, sucrose synthesis genes, among which are those encoding sucrose phosphate synthase (SPS), are known to be induced by low temperature (Usadel *et al.* 2008), while transcript levels of several members of the invertase family (along with the overall level of invertase activity in the plant) are suppressed. In other plant species, such as wheat and tomato for example, invertase activity is up-regulated by a fall in temperature, although this effect is weak in accessions that are chilling tolerant (Artuso *et al.* 2000; Vargas *et al.* 2007). Recent research has underlined a key role for metabolite transporters in carbohydrate metabolism under low temperature conditions, as well as their partitioning between the chloroplast and the cytosol (Kaplan *et al.* 2007; Guy *et al.* 2008).

The metabolism of nitrogenous compounds is also responsive to low-temperature stress (Kaplan *et al.* 2004; Usadel *et al.* 2008), in particular that of certain amino acids and polyamine compounds (Davey *et al.* 2009). Transcript signal levels of *A. thaliana* enzymes involved in amino acid metabolism are notably affected by cold stress, with some being increased (especially those associated with proline biosynthesis, those within the glutamate and ornithine pathways, and those encoding cysteine and polyamine synthesis), and

others, such as the genes responsible for branched-chain amino acid degradation, tending to be repressed (Kaplan *et al.* 2007; Usadel *et al.* 2008). The metabolic fingerprinting of several ecotypes of *A. lyrata* ssp. *petraea* has suggested a significant influence of cold stress on the expression level of genes within the glutamine-associated pathways (e.g. an increase in glutamine synthetase and suppression of asparagine synthetase), which are important for the metabolism of nitrogen (Davey *et al.* 2009). GABA (γ -aminobutyric acid) is an important amine-containing metabolite associated with cryoprotection in barley and wheat (Mazzucotelli *et al.* 2006). It is synthesised in the cytosol *via* the decarboxylation of glutamine by glutamate decarboxylase (GAD). In *A. thaliana*, GAD genes are rapidly up-regulated by the imposition of cold stress, well before any observable increase in GABA content (Kaplan *et al.* 2007). These data support the notion that glutamate availability and GAD activity are associated with freezing tolerance and GABA biosynthesis (Guy *et al.* 2008).

Transcript levels associated with lipid metabolism genes are generally suppressed by a decrease in temperature (Hannah *et al.* 2006). However, some evidence derived from *A. thaliana* shows that a number of lipid catabolism enzymes (in particular, phospholipase A and D) are activated by a fall in temperature, and this is followed by a rise in the amount of free fatty acids present (Wang *et al.* 2006; Usadel *et al.* 2008). Another important group of hydrolases, the galactolipases, are less markedly cold-induced than the phospholipases, although an increase in their activity is thought to contribute significantly to chilling susceptibility in plants (Kaniuga 2008). Their lipo-hydrolytic activity is more likely to be linked to freezing tolerance than to cold acclimation (Wang *et al.* 2006).

The expression of secondary metabolism genes is generally well correlated with freezing tolerance (Hannah *et al.* 2006; Usadel *et al.* 2008). In *A. thaliana*, cold stress induces the biosynthesis of flavonoids and anthocyanins, glucosinolates, terpenoids and phenylpropanoids (Kaplan *et al.* 2007). Anthocyanin content is also positively correlated with cold tolerance in some ecotypes, and its level in the leaf has been observed to rise significantly during cold acclimation (Marczak *et al.* 2008). This response is also widespread among other plant species (Chalker-Scott 1999). Above normal levels of anthocyanin and the blue light absorbing flavonols in the leaf ensure that chlorophyll is not over-excited under conditions of extreme cold (Hannah *et al.* 2006; Korn *et al.* 2008). The presence of salicylic acid, which plays an important role in plant defence against pathogens, can also be heightened by cold stress, together with that of a range of secondary metabolism precursors, such as phenylpropanoids, free fatty acids and branched or aromatic amino acids. This suggests, perhaps, a link between the plants' defence machinery and protection from cold-induced damage. Nevertheless, the biosynthesis of certain secondary metabolites (e.g. terpenoid indole alkaloids in *Catharanthus roseus*) is suppressed by low temperature (Dutta *et al.* 2007). Transcriptome profiling studies have also demonstrated increases in the expression level of genes associated with ABA signalling, as well as of ABA responsive genes (Usadel *et al.* 2008). Finally, genes regulating other metabolic pathways, along with signalling genes associated with secondary metabolism products (such as jasmonic acid and ethylene) are down-regulated by cold.

Overall, these observations underline that the regulation of secondary metabolism is highly complex in plants.

SUGARS AS SIGNALLING MOLECULES

Sugars represent not just an energy source, but are also carbon precursors, substrates for polymers, storage and transport compounds and signalling molecules (Rolland *et al.* 2006; Wormit *et al.* 2006). In cold-induced barley cell cultures, the extracellular sugar concentration regulates expression of the stress-responsive genes *BLT4.9* (non-specific lipid transfer protein) and *DHN1* (Tabaei-Aghdai *et al.* 2003). Three different glucose signalling pathways are known in plants: one is hexokinase-dependent, the second glycolysis-dependent, and the third hexokinase-independent (Xiao *et al.* 2000). Hexokinase functions as an intracellular glucose sensor, while some membrane receptors probably act as extracellular sensors (Moore *et al.* 2003; Rolland *et al.* 2006). It is also believed that plants have a disaccharide sensing system, involving sucrose and trehalose. Sucrose transport to the cell and its subsequent cleavage by invertase or sucrose synthase is the source of the signal (Koch 2004; Roitsch & Gonzalez 2004; Rolland *et al.* 2006; Sauer 2007; Iordachescu & Imai 2008). Trehalose, a disaccharide confined mostly to organisms adapted to situations of extreme desiccation, where its role is to protect proteins and membranes, plays, together with its precursor trehalose-6-phosphate, an important regulatory role in sugar metabolism and plant development (Iordachescu & Imai 2008). In barley, trehalose induces the expression and activity of fructan biosynthesis enzymes. However, for fructan accumulation, glucose or mannitol is also required (Wagner *et al.* 1986; Müller *et al.* 2000). From a microarray analysis following trehalose treatment, Bae *et al.* (2005) showed that the expression of a wide range of other genes was also influenced by trehalose. A role for trehalose and trehalose-6-phosphate in abiotic and biotic stress signalling has been confirmed by the observation that coordinated changes occur in transcript levels of the enzymes involved in their metabolism, especially after exposure to cold, osmotic and salinity stresses and in response to *Pseudomonas syringae* infiltration (Iordachescu & Imai 2008).

Fructose-based polymers (fructans) also contribute to the cold and drought tolerance of several plant families. These molecules are synthesised from sucrose by fructosyltransferases, and help to stabilise membranes by binding to the phosphate and choline groups of membrane lipids. This stabilisation results in reduced water loss from the dry membranes (Valluru & Van den Ende 2008). In addition, fructans are suspected of stimulating the production of alternative cryoprotectants (Valluru *et al.* 2008). Both size- and species-dependent differences are thought to exist among cereal fructans (Hincha *et al.* 2007). Some Poaceae species can accumulate fructans (*Triticum*, *Hordeum*, *Avena*, *Poa*, *Lolium*), but others cannot (*Oryza*). This difference was supposed by Ji *et al.* (2006) to reflect an evolutionary event that separated the Panicoideae (rice, sorghum, maize, etc.) from the Pooideae (wheat, barley, rye, etc.). Although all cereal species have invertases (from which fructan biosynthesis enzymes evolved), the fructan non-accumulators lack fructan biosynthesis enzymes. Some data have also been generated to suggest a role for β -amylase during cold and other abiotic stresses. The

hypothesis put forward by Kaplan *et al.* (2006) was that this enzyme provides some protection to photosystem II photochemical efficiency by catalysing the synthesis of maltose.

CONCLUSION

Plants, and in particular crop species, can withstand various stresses to a certain extent. 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 response to cold stress. Spring-sown crop cultivars differ from winter-sown cultivars in the patterns of expression of various transcription factors, with the consequent induction of a different spectrum of stress responsive genes. Although many genes and their alleles have been described and their functions identified, much remains to be elucidated in the area of gene networking and the interactions between genes, proteins and metabolites.

There is as yet no simple general model that has captured the complexity of the signalling pathways underlying the effectiveness of cold acclimation in strengthening freezing tolerance. Some metabolites are clearly multifunctional, as for example certain sugars, which not only have a role in osmoprotection, but also in signalling; or certain proteins, which are able to adjust their function according to the nature of the stress (for example PR-2 and PR-11, which inhibit their glucanase and chitinase activity during cold stress, and induce antifreeze activity). The function of some other proteins is controlled by the composition of isoforms present, as for example alternative oxidase, where one form (AOX1) is induced by stress, while the other (AOX2) is constitutively expressed. In certain gene families, the function of some members is stress-specific, but others respond to many stresses (a good example of this is provided by the dehydrins, some of which are induced specifically by cold stress, others are drought-induced, and still others are salinity-responsive).

What then should be the major focus of abiotic stress research? One possibility is to view the topic as a systems biology problem, in which an attempt is made to target all possible levels of the plant response to its environment, along with the manifold interactions between various stresses. The alternative view is reductionist, in which individual genes or pathways are targeted by gene knockout methods (such as RNAi, or site-directed mutagenesis). Of particular interest would be to understand the processes of sugar sensing and signalling, and the role(s) and regulation of alternative oxidase in the face of abiotic stress. The signalling of a number of environmental and developmental stimuli is effected by linking receptors with downstream targets, a phenomenon in which MAP kinases are intimately involved. The specific expression of various MAPK components is key to the specificity of many responses and thus a study of these components may represent a fruitful source of information as the science behind the stress (both abiotic and biotic) continues to develop.

High throughput genomic and transcriptomic technologies have been developed so far for rather a limited number of plant species. The number of plant species whose genomes have been fully sequenced is, however, growing apace ([\[www.phytozome.net/\]\(http://www.phytozome.net/\)\) as the cost of sequencing technology falls. At the same time, advances in transcript capture and resequencing are opening new perspectives for unravelling the molecular mechanisms underlying the many and complex plant responses to the environment.](http://</p>
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REFERENCES

- Abdrakhamanova A., Wang Q.Y., Khokhlova L., Nick P. (2003) Is microtubule disassembly a trigger for cold acclimation? *Plant & Cell Physiology*, **44**, 676–686.
- Achard P., Gong F., Cheminant S., Alioua M., Hedden P., Genschik P. (2008) The cold-inducible CBF1 factor-dependent signaling pathway modulates the accumulation of the growth-repressing DELLA proteins via its effect on gibberellin metabolism. *The Plant Cell*, **20**, 2117–2129.
- Allagulova C.R., Gimalov F.R., Shakirova F.M., Vakhitov V.A. (2003) The plant dehydrins: structure and putative functions. *Biochemistry (Moscow)*, **68**, 945–951.
- Artuso A., Guidi L., Soldatini G.F., Pardossi A., Tognoni F. (2000) The influence of chilling on photosynthesis and activities of some enzymes of sucrose metabolism in *Lycopersicon esculentum* Mill. *Acta Physiologiae Plantarum*, **22**, 95–101.
- Atienza S.G., Faccioli P., Perrotta G., Dalfino G., Zschiesche W., Humbeck K., Stanca A.M., Cattivelli L. (2004) Large-scale analysis of transcript abundance in barley subjected to several single and combined abiotic stress conditions. *Plant Science*, **167**, 1359–1365.
- Bae H.H., Herman E., Bailey B., Bae H.J., Sicher R. (2005) Exogenous trehalose alters *Arabidopsis* transcripts involved in cell wall modification, abiotic stress, nitrogen metabolism, and plant defense. *Physiologia Plantarum*, **125**, 114–126.
- Catalá R., Santos E., Alonso J.M., Ecker J.R., Martínez-Zapater J.M., Salinas J. (2003) Mutations in the Ca²⁺/H⁺ transporter CAX1 increase CBF/DREB1 expression and the cold-acclimation response in *Arabidopsis*. *The Plant Cell*, **15**, 2940–2951.
- Cattivelli L., Baldi P., Crosatti C., Di Fonzo N., Faccioli P., Grossi M., Mastrangelo A.M., Pecchioni N., Stanca A.M. (2002) Chromosome regions and stress-related sequences involved in resistance to abiotic stress in Triticeae. *Plant Molecular Biology*, **48**, 649–665.
- Chalker-Scott L. (1999) Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology*, **70**, 1–9.
- Chen W.P., Li P.H. (2002) Attenuation of reactive oxygen production during chilling in ABA-treated maize cultured cells. In: Li C., Palva E.T. (Eds), *Plant cold hardiness*. Kluwer Academic Publishers, Dordrecht, The Netherlands: pp. 223–233.
- Chen T.H., Murata N. (2008) Glycinbetaine: an effective protectant against abiotic stress in plants. *Trends in Plant Science*, **13**, 499–505.

- Chen W.J., Zhu T. (2004) Networks of transcription factors with roles in environmental stress response. *Trends in Plant Science*, **9**, 591–596.
- Chinnusamy V., Ohta M., Kanrar S., Lee B., Hong X., Agarwal M., Zhu J.-K. (2003) ICE1: a regulator of cold-induced transcriptome and cold tolerance in *Arabidopsis*. *Genes & Development*, **17**, 1043–1054.
- Chinnusamy V., Schumaker K., Zhu J.-K. (2004) Molecular genetic perspectives on cross-talk and specificity in abiotic stress signaling in plants. *Journal of Experimental Botany*, **55**, 225–236.
- Chinnusamy V., Zhu J., Zhu J.-K. (2006) Gene regulation during cold acclimation in plants. *Physiologia Plantarum*, **126**, 52–61.
- Cook D., Fowler S., Fiehn O., Thomashow M.F. (2004) A prominent role for the CBF cold response pathway in configuring the low-temperature metabolome of *Arabidopsis*. *Proceedings of the National Academy of Sciences USA*, **101**, 15243–15248.
- Davey M.P., Woodward F.I., Quick W.P. (2009) Intraspecific variation in cold-temperature metabolic phenotypes of *Arabidopsis lyrata* ssp. *petraea*. *Metabolomics*, **5**, 138–149.
- De Palma M., Grillo S., Massarelli I., Costa A., Balogh G., Vigh L., Leone A. (2008) Regulation of desaturase gene expression, changes in membrane lipid composition and freezing tolerance in potato plants. *Molecular Breeding*, **21**, 15–26.
- Dong C.-H., Agarwal M., Zhang Y., Xie Q., Zhu J.-K. (2006) The negative regulator of plant cold responses, HOS1, is a RING E3 ligase that mediates the ubiquitination and degradation of ICE1. *Proceedings of the National Academy of Sciences USA*, **103**, 8281–8286.
- Dutta A., Sen J., Deswal R. (2007) Downregulation of terpenoid indole alkaloid biosynthetic pathway by low temperature and cloning of a AP2 type C-repeat binding factor (CBF) from *Catharanthus roseus* (L.) G. Don. *Plant Cell Reports*, **26**, 1869–1878.
- Fourrier N., Bédard J., Lopez-Juez E., Barbrook A., Bowyer J., Jarvis P., Warren G., Thorlby G. (2008) A role for SENSITIVE TO FREEZING2 in protecting chloroplasts against freeze-induced damage in *Arabidopsis*. *The Plant Journal*, **55**, 734–745.
- Fowler S., Thomashow M.F. (2002) *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *The Plant Cell*, **14**, 1675–1690.
- Fowler S.G., Cook D., Thomashow M.F. (2005) Low temperature induction of *Arabidopsis* CBF1, 2, and 3 is gated by the circadian clock. *Plant Physiology*, **137**, 961–968.
- Francia E., Rizza F., Cattivelli L., Stanca A.M., Galiba G., Tóth B., Hayes P.M., Skinner J.S., Pecchioni N. (2004) Two loci on chromosome 5H determine low-temperature tolerance in a “Nure” (winter) × “Tremois” (spring) barley map. *Theoretical and Applied Genetics*, **108**, 670–680.
- Garg A.K., Kim J.-K., Owens T.G., Ranwala A.P., Choi Y.D., Kochian L.V., Wu R.J. (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proceedings of the National Academy of Sciences USA*, **99**, 15898–15903.
- Gong Z., Lee H., Xiong L., Jagendorf A., Stevenson B., Zhu J.-K. (2002) RNA helicase-like protein as an early regulator of transcription factors for plant chilling and freezing tolerance. *Proceedings of the National Academy of Sciences USA*, **99**, 11507–11512.
- Grennan A.K. (2006) Abiotic stress in rice. An “omic” approach. *Plant Physiology*, **140**, 1139–1141.
- Griffith M., Yaish M.W.F. (2004) Antifreeze proteins in overwintering plants: a tale of two activities. *Trends in Plant Science*, **9**, 399–405.
- Gusta L.V., Wisniewski M., Nesbitt N.T., Gusta M.L. (2004) The effect of water, sugars, and proteins on the pattern of ice nucleation and propagation in acclimated and nonacclimated canola leaves. *Plant Physiology*, **135**, 1642–1653.
- Guy C. (1999) The influence of temperature extremes on gene expression, genomic structure, and the evolution of induced tolerance in plants. In: Lerner H.R. (Ed.), *Plants responses to environmental stresses. from phytohormones to genome reorganization*. Marcel Dekker, Inc., New York, Basel, pp 497–548.
- Guy C., Kaplan F., Kopka J., Selbig J., Hinchin D.K. (2008) Metabolomics of temperature stress. *Physiologia Plantarum*, **132**, 220–235.
- Hannah M.A., Wiese D., Freund S., Fiehn O., Heyer A.G., Hinchin D.K. (2006) Natural genetic variation of freezing tolerance in *Arabidopsis*. *Plant Physiology*, **142**, 98–112.
- Hare P.D., Cress W.A., Van Staden J. (1998) Dissecting the roles of osmolyte accumulation during stress. *Plant, Cell and Environment*, **21**, 535–553.
- Hinchin D.K., Livingston D.P. III, Premakumar R., Zuther E., Obel N., Cacela C., Heyer A.G. (2007) Fructans from oat and rye: composition and effects on membrane stability during drying. *Biochimica et Biophysica Acta*, **1768**, 1611–1619.
- Hirschi K.D. (1999) Expression of *Arabidopsis* CAX1 in tobacco: altered calcium homeostasis and increased stress sensitivity. *The Plant Cell*, **11**, 2113–2122.
- Iba K. (2002) Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. *Annual Review of Plant Biology*, **53**, 225–245.
- Iordachescu M., Imai R. (2008) Trehalose biosynthesis in response to abiotic stresses. *Journal of Integrative Plant Biology*, **50**, 1223–1229.
- Ji X., Van den Ende W., Schroeven L., Clerens S., Geuten K., Cheng S., Bennett J. (2006) The rice genome encodes two vacuolar invertases with fructan exohydrolase activity but lacks the related fructan biosynthesis genes of the Pooideae. *New Phytologist*, **173**, 50–62.
- Juntilla O., Robberecht R. (1999) Ecological aspects of cold-adapted plants with special emphasis on environmental control of cold hardening and dehardening. In: Margesin R., Schinner F. (Eds), *Cold-adapted organisms – ecology, physiology, enzymology and molecular biology*. Springer-Verlag, Berlin, Germany: pp. 57–77.
- Juntilla O., Welling A., Li C., Berhany A.T., Palva E.T. (2002) Physiological aspects of cold hardiness in northern deciduous tree species. In: Li Ch., Palva E.T. (Eds), *Plant cold hardiness*. Kluwer Academic Publishers, Dordrecht, The Netherlands: pp. 65–76.
- Kacperska A. (1999) Plant response to low temperature: signaling pathways involved in plant acclimation. In: Margesin R., Schinner F. (Eds), *Cold-adapted organisms – ecology, physiology, enzymology and molecular biology*. Springer-Verlag, Berlin, Germany: pp. 79–103.
- Kaniuga Z. (2008) Chilling response of plants: importance of galactolipase, free fatty acids and free radicals. *Plant Biology*, **10**, 171–184.

- Kaplan F., Kopka J., Haskell D.W., Zhao W., Schiller K.C., Gatzke N., Sung D.Y., Guy C.L. (2004) Exploring the temperature-stress metabolome of *Arabidopsis*. *Plant Physiology*, **136**, 4159–4168.
- Kaplan F., Sung D.Y., Guy C.L. (2006) Roles of β -amylase and starch breakdown during temperature stress. *Physiologia Plantarum*, **126**, 120–128.
- Kaplan F., Kopka J., Sung D.Y., Zhao W., Popp M., Porat R., Guy C.L. (2007) Transcript and metabolite profiling during cold acclimation of *Arabidopsis* reveals an intricate relationship of cold-regulated gene expression with modifications in metabolite content. *The Plant Journal*, **50**, 967–981.
- Kawamura Y., Uemura M. (2003) Mass spectrometric approach for identifying putative plasma membrane proteins of *Arabidopsis* leaves associated with cold acclimation. *The Plant Journal*, **36**, 141–154.
- Kayal W.E., Navarro M., Marque G., Keller G., Marque C., Teulieres C. (2006) Expression profile of CBF-like transcriptional factor genes from *Eucalyptus* in response to cold. *Journal of Experimental Botany*, **57**, 2455–2469.
- Koch K. (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Current Opinion in Plant Biology*, **7**, 235–246.
- Korn M., Peterek S., Mock H.P., Heyer A.G., Hinch D.K. (2008) Heterosis in the freezing tolerance, and sugar and flavonoid contents of crosses between *Arabidopsis thaliana* accessions of widely varying freezing tolerance. *Plant, Cell and Environment*, **31**, 813–827.
- Kovacs D., Kalmar E., Torok Z., Tompa P. (2008) Chaperone activity of ERD10 and ERD14, two disordered stress-related plant proteins. *Plant Physiology*, **147**, 381–390.
- Lee B., Henderson D.A., Zhu J.-K. (2005) The *Arabidopsis* cold-responsive transcriptome and its regulation by ICE1. *The Plant Cell*, **17**, 3155–3175.
- Li W., Wang R., Li M., Li L., Wang C., Welti R., Wang X. (2008) Differential degradation of extraplastidic and plastidic lipids during freezing and post-freezing recovery in *Arabidopsis thaliana*. *The Journal of Biological Chemistry*, **283**, 461–468.
- Liu H.H., Tian X., Li Y.J., Wu C.A., Zheng C.C. (2008) Microarray-based analysis of stress-regulated microRNAs in *Arabidopsis thaliana*. *RNA*, **14**, 836–843.
- Livingston D.P., Premakumar R., Tallury S.P. (2006) Carbohydrate partitioning between upper and lower regions of the crown in oat and rye during cold acclimation and freezing. *Cryobiology*, **52**, 200–208.
- Marczak L., Kachlicki P., Kozniowski P., Skiryicz A., Krajewski P., Stobiecki M. (2008) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry monitoring of anthocyanins in extracts from *Arabidopsis thaliana* leaves. *Rapid Communications in Mass Spectrometry*, **22**, 3949–3956.
- Mazzucotelli E., Tartari A., Cattivelli L., Forlani G. (2006) Metabolism of gamma-aminobutyric acid during cold acclimation and freezing and its relationship to frost tolerance in barley and wheat. *Journal of Experimental Botany*, **57**, 3755–3766.
- McKersie B.D., Leshem Y.Y. (1994) *Stress and stress coping in cultivated plants*. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Mittler R., Vanderauwera S., Gollery M., Van Breusegem F. (2004) Reactive oxygen gene network of plants. *Trends in Plant Science*, **9**, 490–498.
- Moffatt B., Ewart V., Eastman A. (2006) Cold comfort: plant anti-freeze proteins. *Physiologia Plantarum*, **126**, 5–16.
- Mogk A., Schlieker C., Friedrich K.L., Schönfeld H.J., Vierling E., Bukau B. (2003) Refolding of substrates bound to small Hsps relies on a disaggregation reaction mediated most efficiently by ClpB/DnaK. *Journal of Biological Chemistry*, **278**, 31033–31042.
- Monroy A.F., Dryanova A., Malette B., Oren D.H., Farajalla M.R., Liu W., Danyluk J., Ubayasena L.W.C., Kane K., Scoles G.J., Sarhan F., Gulick P.J. (2007) Regulatory gene candidates and gene expression analysis of cold acclimation in winter and spring wheat. *Plant Molecular Biology*, **64**, 409–423.
- Moore G., Devos K.M., Wang Z., Gale M.D. (1995) Grasses, line up and form a circle. *Current Biology*, **5**, 737–739.
- Moore B., Zhou L., Rolland F., Hall Q., Cheng W.H., Liu Y.-X., Hwang I., Jones T., Sheen J. (2003) Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science*, **300**, 332–336.
- Müller J., Aeschbacher R.A., Sprenger N., Boller T., Wiemken A. (2000) Disaccharide-mediated regulation of sucrose: fructan-6-fructosyltransferase, a key enzyme of fructan synthesis in barley leaves. *Plant Physiology*, **123**, 265–274.
- Nakashima K., Yamaguchi-Shinozaki K. (2006) Regulons involved in osmotic stress-responsive and cold stress-responsive gene expression in plants. *Physiologia Plantarum*, **126**, 62–71.
- Nakayama K., Okawa K., Kakizaki T., Inaba T. (2008) Evaluation of the protective activities of a late embryogenesis abundant (LEA) related protein, Cor15am, during various stresses *in vitro*. *Bioscience, Biotechnology and Biochemistry*, **72**, 1642–1645.
- Navarro M., Marque G., Ayax C., Keller G., Borges J.P., Marque C., Teulieres C. (2009) Complementary regulation of four *Eucalyptus* CBF genes under various cold conditions. *Journal of Experimental Botany*, **60**, 2713–2724.
- Nick P. (2000) Control of the response to low temperatures. In: Nick P. (Ed.), *Plant microtubules: potential for biotechnology*. Springer, Berlin, Germany: pp. 121–135.
- Nilsen E.T., Orcutt D.M. (1996) *The physiology of plants under stress – abiotic factors*. John Wiley & Sons, Inc., New York.
- Nishizawa A., Yabuta Y., Shigeoka S. (2008) Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiology*, **147**, 1251–1263.
- Novillo F., Alonso J.M., Ecker J.R., Salinas J. (2004) CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA*, **101**, 3985–3990.
- Novillo F., Medina J., Salinas J. (2007) *Arabidopsis* CBF1 and CBF3 have a different function than CBF2 in cold acclimation and define different gene classes in the CBF regulon. *Proceedings of the National Academy of Sciences USA*, **104**, 21002–21007.
- Oh S.-J., Kwon C.-W., Choi D.-W., Song S.I., Kim J.-K. (2007) Expression of barley HvCBF4 enhances tolerance to abiotic stress in transgenic rice. *Plant Biotechnology Journal*, **5**, 646–656.
- Okawa K., Nakayama K., Kakizaki T., Yamashita T., Inaba T. (2008) Identification and characterization of Cor413im proteins

- as novel components of the chloroplast inner envelope. *Plant, Cell and Environment*, **31**, 1470–1483.
- Örvar B.L., Sangwan V., Omann F., Dhindsa R. (2000) Early steps in cold sensing by plant cells: the role of actin cytoskeleton and membrane fluidity. *The Plant Journal*, **23**, 785–794.
- Pearce R.S., Dunn M.A., Rixon J.E., Harrison P., Hughes M.A. (1996) Expression of cold-induced genes and frost hardiness in the crown meristem of young barley (*Hordeum vulgare* L. cv. Igri) plants grown in different environments. *Plant, Cell and Environment*, **12**, 275–290.
- Peng Y., Arora R., Li G., Wang X., Fessehae A. (2008) *Rhododendron catawbiense* plasma membrane intrinsic proteins (RcPIPs) are aquaporins and their overexpression compromises constitutive freezing tolerance and cold acclimation ability of transgenic *Arabidopsis* plants. *Plant, Cell and Environment*, **31**, 1275–1289.
- Rajashekar C.B. (2000) Cold response and freezing tolerance in plants. In: Wilkinson R.E. (Ed.), *Plant–environment interactions*, 2nd edition. Marcel Dekker, Inc., New York, USA: pp 321–341.
- Renaut J., Lutts S., Hoffmann L., Hausman J.-F. (2004) Responses of poplar to chilling temperatures: proteomic and physiological aspects. *Plant Biology*, **6**, 81–90.
- Renaut J., Hausman J.-F., Wisniewski M.E. (2006) Proteomics and low temperature studies: bridging the gap between gene expression and metabolism. *Physiologia Plantarum*, **126**, 97–109.
- Riechmann J.L., Meyerowitz E.M. (1998) The AP2/EREBP family of plant transcription factors. *Biological Chemistry*, **379**, 633–646.
- Roitsch T., Gonzalez M.-C. (2004) Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science*, **9**, 606–613.
- Rolland F., Baena-Gonzales E., Sheen J. (2006) Sugar sensing and signalling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology*, **57**, 675–709.
- Sangwan V., Örvar B.L., Dhindsa R.S. (2002) Early events during low temperature signaling. In: Li C., Palva E.T. (Eds), *Plant cold hardiness*. Kluwer Academic Publishers, Dordrecht, The Netherlands: pp. 43–53.
- Sauer N. (2007) Molecular physiology of higher plant sucrose transporters. *FEBS Letters*, **581**, 2309–2317.
- Shinozaki K., Yamaguchi-Shinozaki K. (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Current Opinion in Plant Biology*, **3**, 217–223.
- Shinozaki K., Yamaguchi-Shinozaki K., Seki M. (2003) Regulatory network of gene expression in the drought and cold stress responses. *Current Opinion in Plant Biology*, **6**, 410–417.
- Sun W., Van Montagu M., Verbruggen N. (2002) Small heat shock proteins and stress tolerance in plants. *Biochimica et Biophysica Acta*, **1577**, 1–9.
- Tabaei-Aghdaei S.R., Pearce R.S., Harrison P. (2003) Sugars regulate cold-induced gene expression and freezing-tolerance in barley cell cultures. *Journal of Experimental Botany*, **54**, 1565–1575.
- Tantau H., Balko C., Bretschneider B., Melz G., Dörffling K. (2004) Improved frost tolerance and winter survival in winter barley (*Hordeum vulgare* L.) by *in vitro* selection of proline over-accumulating lines. *Euphytica*, **139**, 19–32.
- Timperio A.M., Egidi M.G., Zolla L. (2008) Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). *Journal of Proteomics*, **71**, 391–411.
- Tommasini L., Svensson J.T., Rodriguez E.M., Wahid A., Malatrasi M., Kato K., Wanamaker S., Resnik J., Close T.J. (2008) Dehydrin gene expression provides an indicator of low temperature and drought stress: transcriptome-based analysis of barley (*Hordeum vulgare* L.). *Functional and Integrative Genomics*, **8**, 387–405.
- Torres M.A., Dangel J.L. (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Current Opinion in Plant Biology*, **8**, 397–403.
- Uemura M., Tominaga Y., Nakagawara C., Shigematsu S., Minami A., Kawamura Y. (2006) Responses of the plasma membrane to low temperatures. *Physiologia Plantarum*, **126**, 81–89.
- Usadel B., Blasing O.E., Gibon Y., Retzlaff K., Hoehne M., Gunther M., Stitt M. (2008) Multilevel genomic analysis of the response of transcripts, enzyme activities and metabolites in *Arabidopsis* rosettes to a progressive decrease of temperature in the non-freezing range. *Plant, Cell and Environment*, **31**, 518–547.
- Valluru R., Van den Ende W. (2008) Plant fructans in stress environments: emerging concepts and future prospects. *Journal of Experimental Botany*, **59**, 2905–2916.
- Valluru R., Lammens W., Claupein W., Van den Ende W. (2008) Freezing tolerance by vesicle-mediated fructan transport. *Trends in Plant Science*, **13**, 409–414.
- Van Buskirk H.A., Thomashow M.F. (2006) *Arabidopsis* transcription factors regulating cold acclimation. *Physiologia Plantarum*, **126**, 72–80.
- Vargas W.A., Pontis H.G., Salerno G.L. (2007) Differential expression of alkaline and neutral invertases in response to environmental stresses: characterization of an alkaline isoform as a stress-response enzyme in wheat leaves. *Planta*, **226**, 1535–1545.
- Vaultier M.-N., Cantrel C., Vergnolle C., Justin A.-M., Demandre C., Benhassaine-Kesri G., Cicek D., Zachowski A., Ruelland E. (2006) Desaturase mutants reveal that membrane rigidification acts as a cold perception mechanism upstream of the diacylglycerol kinase pathway in *Arabidopsis* cells. *FEBS Letters*, **580**, 4218–4223.
- Wagner W., Wiemken A., Matile P. (1986) Regulation of fructan metabolism in leaves of barley (*Hordeum vulgare* L. cv. Gerbel). *Plant Physiology*, **81**, 444–447.
- Wang W., Vinocur B., Altman A. (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, **218**, 1–14.
- Wang X., Li W., Li M., Welti R. (2006) Profiling lipid changes in plant response to low temperatures. *Physiologia Plantarum*, **126**, 90–96.
- Wisniewski M., Fuller M. (1999) Ice nucleation and deep supercooling in plants: new insights using infrared thermography. In: Margesin R., Schinner F. (Eds), *Cold-adapted organisms – ecology, physiology, enzymology and molecular biology*. Springer-Verlag, Berlin, Germany: pp. 105–118.
- Wisniewski M., Webb R., Balsamo R., Close T.J., Yu X.-M., Griffith M. (1999) Purification, immunolocalization, cryoprotective, and antifreeze activity of PCA60: a dehydrin from peach (*Prunus persica*). *Physiologia Plantarum*, **105**, 600–608.
- Wormit A., Trentmann O., Feifer I., Lohr C., Tjaden J., Meyer S., Schmidt U., Martinoia E., Neuhaus H.E. (2006) Molecular identification and physiological characterization of a novel monosac-

- charide transporter from *Arabidopsis* involved in vacuolar sugar transport. *The Plant Cell*, **18**, 3476–3490.
- Xiao W., Sheen J., Jang J.C. (2000) The role of hexokinase in plant sugar signal transduction and growth and development. *Plant Molecular Biology*, **44**, 451–461.
- Xiong L., Zhu J.-K. (2001) Abiotic stress signal transduction in plants. Molecular and genetic perspectives. *Physiologia Plantarum*, **112**, 152–166.
- Xiong L., Schumaker K.S., Zhu J.-K. (2002) Cell signaling during cold, drought, and salt stress. *The Plant Cell*, **14**, S165–183.
- Yamaguchi-Shinozaki K., Shinozaki K. (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stress. *Annual Review of Plant Biology*, **57**, 781–803.
- Zarka D.G., Vogel J.T., Cook D., Thomashow M.F. (2003) Cold induction of *Arabidopsis* CBF genes involves multiple ICE (inducer of CBF expression) promoter elements and a cold-regulatory circuit that is desensitized by low temperature. *Plant Physiology*, **133**, 910–918.
- Zhang J.Z., Creelman R.A., Zhu J.-K. (2004) From laboratory to field. Using information from Arabidopsis to engineer salt, cold and drought tolerance in crops. *Plant Physiology*, **135**, 615–621.
- Zhu J., Jeong J.C., Zhu Y., Sokolchik I., Miyazaki S., Zhu J.-K., Hasegawa P.M., Bohnert H.J., Shi H., Yun D.-J., Bressan R.A. (2008) Involvement of *Arabidopsis* HOS15 in histone deacetylation and cold tolerance. *Proceedings of the National Academy of Sciences USA*, **105**, 4945–4950.
- Zuther E., Buchel K., Hundertmark M., Stitt M., Hincha D.K., Heyer A.G. (2004) The role of raffinose in the cold acclimation response of *Arabidopsis thaliana*. *FEBS Letters*, **576**, 169–173.