Chapter 27
Gene Expression Profiles Involved in Development of Freezing Tolerance in Common Wheat

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Wheat Cold Acclimation and Freezing Tolerance

Exposure of plants to low, nonfreezing temperatures leads to an increase in freezing tolerance, and this adaptive process, called cold acclimation, involves drastic physiological, biochemical and metabolic changes. Most of these alterations are regulated through changes in gene expression. One of the mechanisms behind development of freezing tolerance is induction of the *Cor* (cold-responsive)/*Lea* (late-embryogenesis-abundant) gene family (Thomashow 1999). In common wheat, major loci controlling freezing tolerance (*Fr-1* and *Fr-2*) have been assigned to the long arm of group five chromosomes (Galiba et al. 1995; Snape et al. 1997). *Fr-2* is coincident with a cluster of genes encoding C-repeat binding factors (CBFs) in wheat and barley (Miller et al. 2006; Francia et al. 2007), which directly induce the downstream *Cor*/*Lea* gene expression during cold acclimation (Takumi et al. 2008). In expression quantitative trait locus (eQTL) analysis of *Cor*/*Lea* and *CBF* genes, four eQTLs controlling cold-responsive genes were found, and the major eQTL with the greatest effect was located on the long arm of chromosome 5A (Motomura et al. 2013). The 5AL eQTL region, which plays important roles in development of freezing tolerance in common wheat (Motomura et al. 2013), coincides with a region homoeologous to a frost-tolerance locus (*Fr-A^{m}2*) reported as a *CBF* cluster region in einkorn wheat (Vágújfalvi et al. 2003; Miller et al. 2006). Allelic differences at *Fr-A2* might be a major cause of cultivar differences in extent of freezing tolerance in common wheat (Motomura et al. 2013). It was recently reported that

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large deletions in the *CBF* cluster at *Fr-B2* significantly reduced frost tolerance in tetraploid and hexaploid wheat (Pearce et al. 2013). In barley, two QTLs for low-temperature (LT) tolerance, *Fr-H1* and *Fr-H2*, are found on the long arm of chromosome 5H (Francia et al. 2004), and the *Vrn-H1*/*Fr-H1* genotype affects both the expression of *CBF* genes at *Fr-H2* and LT tolerance (Stockinger et al. 2007; Chen et al. 2009). Thus, the barley *Vrn-H1*/*Fr-H1* and *Fr-H2* regions function to develop freezing tolerance through *Cor*/*Lea* gene expression during cold acclimation. In contrast to barley, the functions of *Vrn-A1*/*Fr-A1* and *Vrn-D1*/*Fr-D1* in regulation of cold-responsive gene expression in common wheat remain unclear.

A lot of other genes, including *Wlip19* and *Wabi5* bZIP transcription factor genes (Kobayashi et al. 2008a, b), contribute to cold acclimation and freezing tolerance in common wheat. These transcription factors, which act in abscisic acid (ABA) signaling, bind to ABA-responsive elements in the promoters of *Cor*/*Lea* genes. Thus, ABA induces expression of a variety of genes that function in the regulation of gene expression, signal transduction and abiotic stress tolerance in common wheat. In fact, ABA sensitivity strongly affects the basal levels of freezing tolerance (Kobayashi et al. 2006, 2008c), and some QTLs on wheat chromosomes controlling ABA sensitivity at the seedling stage are also related to *Cor*/*Lea* gene expression and putatively associated with freezing tolerance (Kobayashi et al. 2010). Recent reports showed that QTLs for ABA sensitivity at the seedling stage could be also associated with dehydration tolerance, seed dormancy and preharvest sprouting tolerance (Iehisa et al. 2014a, b). The QTLs for ABA sensitivity do not correspond to *Fr-1* and *Fr-2*, and the two *Fr* loci act independently of ABA signal transduction pathways (Fig. 27.1).

Transcriptome Analysis During Cold Acclimation

Wheat *CBF* gene expression is temporal and upregulated at least two-fold by LT (Kume et al. 2005). The first upregulation occurs within 1–4 h, which might correspond to the rapid response to LT, while the second upregulation occurs between 2 and 3 weeks after the start of cold acclimation. Maintenance of a high *CBF* transcript level in freezing tolerant cultivars might represent a long-term effect of cold acclimation (Kume et al. 2005). Effects of long-term LT treatment on gene expression profiles could be distinct from rapid changes in response to cold stress. A comprehensive image of transcriptome alteration in cells and tissues of common wheat during cold acclimation and subsequent freezing stress conditions is not yet available. The above-ground tissues of wheat plants become wilted and wither under freezing conditions. However, cold-acclimated seedlings of freezing tolerant wheat cultivars rapidly recover from freezing stress and develop new shoots from surviving meristems of the crown tissues (Ohno et al. 2001). Therefore, biologically important events in the development of freezing tolerance should occur in the crown tissues.

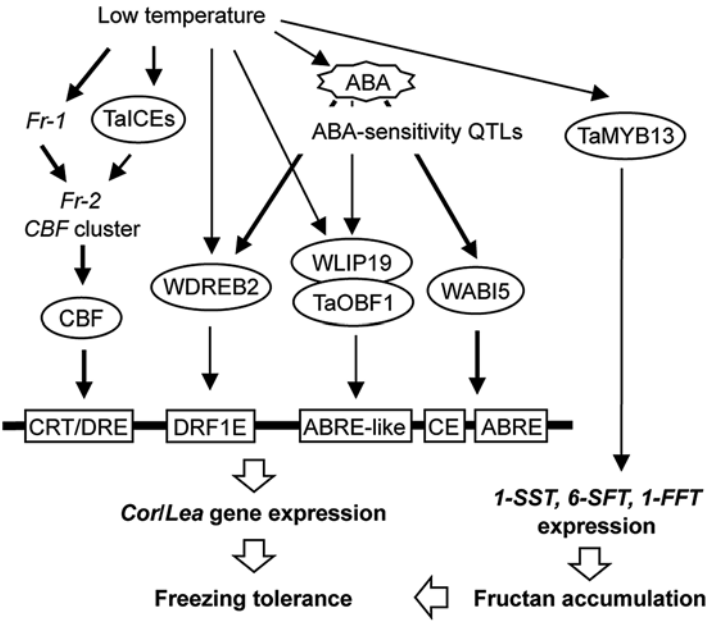

Fig. 27.1 Cold stress signaling pathways in common wheat. Low temperature leads to accumulation of transcription factors (indicated by *ovals*) through ABA-dependent and -independent pathways. Specific binding of each transcription factor to *cis*-acting elements (indicated by *boxes*) activates *Cor*/*Lea* gene expression. *TaMYB13* activates fructan biosynthesis-related genes

Freezing stress treatment significantly alters gene expression profiles of more than 400 genes in the crown tissues of cold-acclimated wheat plants (Skinner 2009). This transcriptome analysis revealed that 68 genes, including CBF, WRKY and zinc finger transcription factor genes, were more than fivefold upregulated by freezing stress. The upregulated genes also encoded kinases, phosphatases, calcium trafficking-related proteins and glycosyltransferases. This observation implied the presence of genetic variation among wheat cultivars in the ability to alleviate the damage to crowns exposed to freezing stress (Skinner 2009). Thus, many genes besides the *CBF* and *Cor*/*Lea* genes presumably participate in each step to develop freezing tolerance in the crown tissues of wheat.

To identify other LT-responsive genes related with cold acclimation in hexaploid wheat, we compared comprehensive gene expression patterns of a synthetic hexaploid line under normal and LT conditions using a wheat 38k DNA microarray (Yokota et al. 2015). For hybridization, total RNA samples were extracted from 3-week-old seedling leaves exposed to LT for 12 weeks, and from crown tissues exposed to LT for 6 weeks. The microarray analyses showed that *TaWRKY45*, *TaWRKY72*, and *TaMYB73* transcription factor genes and two fructan synthesis-related genes, *Ta1FFT* and *Ta6SFT*, were highly upregulated by long-term LT treatment, in addition to a number of *Cor*/*Lea* genes (Yokota et al. 2015). The transcript accumulation levels of these upregulated genes reflected the freezing tolerance

levels of two distinct lines of synthetic hexaploid wheat. Our observations suggest that, in addition to COR/LEA proteins, the WRKY and MYB transcription factors and fructan biosynthesis play important roles in development of freezing tolerance.

Fructan Biosynthesis Pathway and Freezing Tolerance

Severe abiotic stresses induce detrimental changes in cellular compounds, and sugars are regarded as one of the metabolites preventing detrimental changes (Valluru and Van den Ende 2008). In particular, long-term stress conditions lead to higher soluble sugar concentrations and lower amounts of starch (Silva and Arrabaca 2004). Fructans, soluble fructosyl polysaccharides, are storage carbohydrates in a large number of higher plants. Fructans accumulating in perennial grasses can be considered as longer-term reserve carbohydrates to survive the winter period (Yoshida et al. 1998). Transgenic perennial ryegrass plants with an increased amount of fructans showed significantly increased levels of freezing tolerance (Hisano et al. 2004). Genetic transformation of two wheat fructan-synthesizing enzymes conferred fructan accumulation and enhanced chilling tolerance in rice (Kawakami et al. 2008). Therefore, fructans play important roles as anti-stress agents in overwintering plants (Kawakami and Yoshida 2005), and are considered to function in membrane stabilization through formation of a fructan-lipid interaction under water stresses such as cold and drought (Valluru and Van den Ende 2008).

In wheat and barley, three enzyme families, sucrose:sucrose 1-fructosyltransferrase (1-SST), sucrose:fructan 6-fructosyltransferase (6-SFT) and fructan:fructan 1-fructosyltransferase (1-FFT), synthesize graminian-type fructans consisting of β-2,6 linked fructosyl units with β-2,1 branches (Ritsema and Smeekens 2003). The TaMYB13 transcription factor binds to the promoters of wheat *1-SST* and *6-SFT* genes and activates fructosyltransferase gene expression (Xue et al. 2011). Overexpression of *TaMYB13* results in upregulation of *1-SST*, *6-SFT* and *1-FFT* and enhances fructan accumulation and yield-related traits under water-limited conditions in transgenic wheat plants (Kooiker et al. 2013). Snow mold resistant cultivars accumulate and maintain higher fructan levels in the crown tissues from autumn to the end of winter (Yoshida et al. 1998). Yoshida et al. (1998) also reported that fructan may increase freezing tolerance, although its efficiency is lower than mono- and disaccharides in common wheat. Livingston (1996) suggested that fructan is indirectly involved in freezing tolerance of oat and barley. Therefore, fructans surely play important roles in development of water stress tolerance.

As mentioned above, our transcriptome analysis showed that fructan biosynthesis-related genes were significantly upregulated during long-term LT treatment in crown tissues of wheat synthetics (Yokota et al. 2015). In fact, fructan accumulation levels also reflected the distinct freezing tolerance levels of two synthetic wheat lines (Yokota et al. 2015). These observations support a significant association of fructan biosynthesis with development of freezing tolerance in common wheat

(Fig. 27.1). The relationship between carbohydrate accumulation in crown tissues and wheat freezing tolerance and winter hardiness should be elucidated in more detail in future studies.

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