Low-Temperature Tolerance Related *CBF* and *fructosyltransferase* Genes in Forage Grasses

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Introduction Many plants exhibit an increase in freezing tolerance in response to low , non-freezing temperatures , a phenomenon known as cold acclimation . A number of genes respond to cold and condition the plant cells against the effects of freezing temperature during cold acclimation . It has been suggested that the CBF (C-repeat binding factor) / DREB1 (dehydration-responsive element-binding protein 1) regulon is the most important transcription factor involved in cold acclimation in plants . Fructans , the major non-structural carbohydrate reserve , accumulate during cold acclimation . Fructans are synthesized by a combination of multiple fructosyltransferases (FTs) . We describe here genomic characterization of *CBF* genes and *FT* genes in perennial ryegrass (*Lolium perenne* L .) .

Materials and methods cDNAs encoding CBF and FTs have been isolated from cold-treated ryegrass plants . The positions of *CBF* and *FT* loci on the perennial ryegrass genetic map were determined . The mRNA levels of *CBF* and *FT* genes during cold treatment were analyzed . For *FT* genes , recombinant proteins were produced in *Pichia pastoris* and their enzymatic activity was characterized .

Results Ten novel putative *CBF* cDNAs have been isolated from cold-treated leaf tissue . Their primary structures contain some conserved motifs characteristic of the gene class . Phylogenetic analysis revealed that *LpCBF* genes were attributable to the HvCBF3- , and HvCBF4-subgroups following the previously proposed classification of barley *CBF* genes (Skinner et al . , 2005) . RT-PCR analysis revealed that the expression of the *LpCBF* genes was rapidly induced in response to low temperature , and that the expression pattern , under a long period of low temperature conditions differed between the various *LpCBF* genes . Five of the 10 *LpCBF* genes were assigned to the genetic linkage map . Four *LpCBF* genes were mapped on linkage group (LG) 5 forming a cluster within 2 .2cM , while one , the *LpCBF* gene mapped on LG 1 .

Six cDNAs encoding FTs (*prft1-prft6*) were also isolated from cold-treated perennial ryegrass plants . The *prft1* and *prft4* genes were both located near a gene for soluble invertase in the distal part of the LG 7 . The *prft3* gene was located in the distal part of LG 3 . Functional characterization using *Pichia pastoris* revealed that the *prft4* encodes sucrose-sucrose 1-fructosyltransferase (1-SST) , and *prft3* and *prft5* encode fructan-fructan 6G-fructosyltransferases (6G-FFT) . Protein sequences for the other genes (*prfts 1* ,*2* , and *6*) were similar to sucrose-fructan 6-fructosyltransferase (6-SFT) . The mRNA levels of *prft1* and *prft2* gradually increased during cold treatment while those of the *1-SST* and *6G-FFT* genes first increased but then decreased before increasing again during a longer period of cold treatment .

Conclusions Based on comparative genetic studies , conserved synteny for the *CBF* gene family was observed between the Triticeae cereals and perennial ryegrass (Tamura & Yamada , 2007) . A cluster of *CBF* genes is positioned at the frost resistance locus , *Fr-H2* in barley (Francia et al . , 2007) . Determination of the functional role of each different type of *LpCBF* gene will be necessary for the development of specific genetic markers associated with low-temperature tolerance . At least two different patterns of expression of *FT* genes appear to have developed during the evolution of the *FT* genes and this expression is coordinated with fructan synthesis in a cold environment (Hisano et al . , in press) .

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