Isolation and Characterization of a CBF Gene from Perennial Ryegrass (Lolium Perenne L.)

Y. Xiong
Iowa State University

S. Fei
Iowa State University

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Isolation and characterization of a *CBF* gene from perennial ryegrass (*Lolium perenne* L.)

Y. Xiong and S. Fei
Department of Horticulture, Iowa State University, Ames, IA, USA, Email: sfei@iastate.edu

Keywords: *Lolium perenne* L., CBF, cold acclimation, freezing tolerance

Introduction Maximum freezing tolerance of many temperate plant species is achieved after exposure to a period of non-freezing low temperatures, a phenomenon called "cold acclimation". Multiple mechanisms appear to operate in conferring freezing tolerance in plants. The discovery of a class of transcription factor genes, *CBF* genes (C-repeat binding factor), in *Arabidopsis* demonstrated that *CBF* genes can serve as 'Master switches' to activate downstream cold-related (*COR*) genes during cold-acclimation (Liu *et al*., 1998). They act by binding to the core sequence (CCGAC) which is present in *COR* genes and thus activate the expression of *COR* genes and enhance freezing tolerance. The components of this cold acclimation pathway are conserved across a number of plant species including both eudicot and monocot species (Jaglo *et al*., 2001). Perennial ryegrass (*L. perenne* L.) is an important turf and forage grass but it lacks adequate winter hardiness which is a limiting factor for its adaptation to the northern regions of the US. Development of improved germplasm with enhanced winter hardiness would be highly desirable. The objective of this research was to isolate and characterize *CBF* gene(s) in perennial ryegrass and to determine the genomic location and the function of the *CBF* gene(s).

Materials and methods Total RNA was extracted using TRIZOL Reagent from a perennial ryegrass plant (cv. Caddieshack) that was exposed to 4°C for 72 hours. The RT-PCR approach was used to obtain a partial sequence of *CBF* gene from perennial ryegrass with degenerate primers that were designed based on conserved sequences from known *CBF* genes. 5' and 3' RACE were used to obtain the full length sequence of the *CBF* gene. Sequence alignment was performed using BLAST. Northern blot was performed on leaf, root and stem tissues that received no cold treatment and on leaf tissues treated with 4°C cold for 15 min, 30 min, 1h, 4h, 6h, 9h, 24h and 48h. MEGA 3 software was used to construct a dendrogram for the *CBF* genes from both perennial ryegrass and other species.

Results We have identified a *CBF*-like (designated as *LpCBF*) gene in perennial ryegrass. *LpCBF* gene is 942bp long without introns. *LpCBF* gene has all the conserved domains of known *CBF* genes. Dendrogram showed that *LpCBF* is clustered with *CBF* orthologs from other grass species, particularly with the rice *CBF3* gene (*OsDREB1C*). Ninety one percent of the *LpCBF3* DNA sequence is identical to the rice *CBF3* gene and more than half of the amino acids in *LpCBF* are identical to those in rice *CBF3*. Our results indicated that the expression of *LpCBF* was induced between 15min and 30 min of cold treatment with the highest expression level at 30min of cold treatment. No *LpCBF* expression was detected after 2 hour of cold treatment which indicates that the *CBF* gene we isolated from perennial ryegrass functions in the early steps of cold acclimation. Similar to the expression patterns of other known *CBF* genes, no *LpCBF* expression was detected in non treated leaves, stems, crowns and roots.

Conclusions The *LpCBF* gene shares significant sequence similarity with other known *CBF* genes and has similar expression patterns as other *CBF* genes. Additional homologs of the *LpCBF* gene are being isolated and their map locations will be determined. The potential functions of the *CBF* genes are currently being determined with a heterologous transformation system using *Arabidopsis*.

References

Jaglo, K.R., Kleff, S., Amundsen, K.L., Zhang, X., Haake, V., Zhang, J.Z., Deits, T., and Thomashow, M.F. (2001). Components of the *Arabidopsis* C-repeat/dehydration-responsive element binding factor cold-response pathway are conserved in *Brassica napus* and other plant species. Plant Physiol. 127: 910–917.

Liu, Q., Kasuge, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., & Shinozaki, K. (1998). Two transcription factors, *DREB1* and *DREB2*, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10: 1391–1406