

# Identification and characterization of CBF gene in perennial ryegrass (*Lolium perenne* L)

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## Introduction

Perennial ryegrass (*Lolium perenne* L.) is an important turf and forage grass used extensively throughout the temperate regions in the U.S. The primary breeding goal of perennial ryegrass is to improve its tolerance to abiotic stress conditions, particularly winter hardiness. Winter hardiness is the ability for plants to tolerate a wide range of winter environmental stresses including low temperatures, rapidly fluctuating temperatures, low light intensity, desiccation, wind, snow, ice cover, and disease. Freezing tolerance is an important component of winter hardiness in plants. It has been reported to be highly correlated with field winter survival in many plants including perennial ryegrass (*Lolium perenne* L). Freezing tolerance of plants is controlled by multiple genes, each of which contributes a small effect. Identifying these quantitative genes is difficult because their effects on the plant phenotype are often influenced by the environment. Because of these shortcomings, using conventional breeding to improve abiotic stress tolerance has met with limited success.

In the model plant *Arabidopsis*, a class of regulatory genes, CBF / DREB (C-repeat Binding Factors / Dehydration Response Element Binding factor) activate stress responses. Such regulatory genes can serve as “master switches” that activate a signal transduction cascade that leads to enhanced abiotic stress tolerance. Similar regulatory genes have been isolated in a number of different species. Overexpression of some of these CBF genes enhanced tolerance to freezing and dehydration. Our goal is to use the molecular approach to improve winter hardiness in perennial ryegrass. The specific objective of this project is to use the Reverse Transcriptase – Polymerase Chain Reaction (RT-PCR) approach to isolate and characterize CBF genes from perennial ryegrass.

## Method and material

To isolate CBF genes in perennial ryegrass, we designed primers based on conserved regions of all known plant CBF genes. DNAs and RNAs from the perennial ryegrass cultivar of “Caddieshack” were extracted and used as a template in the following experiment. Because CBF genes are cold inducible in other plants, we prepared RNAs from both cold treated plants and non-treated plants.

RT-PCR was performed on the RNA samples. The amplified products were then cloned and sequenced. Specific primers were then designed for RACE (Rapid Amplification of cDNA End) to obtain the sequences on both ends of the conserved region. Genomic DNAs were then used as templates for PCR and the amplified products were sequenced to obtain the full gene sequence. Protein sequence was deduced from the full length cDNA sequence.

Northern hybridization was performed in roots, stems, leaves and flowers to determine the expression pattern of the isolated CBF gene after exposing the plants to low temperatures for various times.

## Results

(1) *Gene Isolation*: A CBF gene (designated as LpCBF) was isolated using the RT-PCR and RACE methods from the perennial ryegrass cultivar of Caddieshack. LpCBF gene has all of the conserved domains of known CBF genes from other plant species, and it has 60% similarity to the rice CBF gene. Sequence alignment showed that more than half of the amino acids are identical between LpCBF and CBF genes of rice.

(2): *Expression Pattern of LpCBF*: To characterize the spatial-temporal expression pattern of the LpCBF gene, Northern blot was performed using RNAs extracted from plant leaves treated by 4°C cold for 15 min, 30 min, 1hr, 4hr, 6hr, 9hr, 24hr and 48hr. RNAs extracted from untreated plants were used as a control. The expression of LpCBF RNA was detected only at the 30min and 1hr treatment, with the highest level detected at 30min. Our results showed that the expression of LpCBF was induced between 15 min and 30 min of cold treatment. The expression then reaches its highest level at 30 min of cold acclimation and lasts for at least another 30 min. No more LpCBF expression was detected after 2 hours of cold treatment, which indicated that the CBF gene isolated from perennial ryegrass functions in early steps of the cold acclimation process. No LpCBF expression was detected in non-cold treated leaves, stems, crowns and roots. This is similar to other known plant CBF genes. Whether LpCBF gene expression is tissue specific in cold-treated plants will be examined.

Further studies will focus on the functional analysis of LpCBF by transferring this gene to the model plant *Arabidopsis* to determine its function in freezing tolerance.