

Identification and Characterization of a CBF Gene in Perennial Ryegrass (*Lolium perenne* L.)

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Introduction

Perennial ryegrass is widely used as both turf and forage in temperate regions. Perennial ryegrass cannot survive severe winters, which is a major limitation for its distribution. Plants from temperate regions can increase freezing tolerance after an exposure to non-freezing low temperatures for a period time. This process is called cold acclimation. Cold acclimation is at least partly due to gene expression induced by low temperatures. Many cold inducible genes have been identified and characterized in Arabidopsis, barley, wheat and other crops. These cold inducible genes all have a C-repeat (CRT)/dehydration response element (DRE) in their promoter regions. In model plant Arabidopsis, a family of transcription factors that bind to CRT/DRE promoter region has been identified and designated as CBF (CRT/DRE Binding Factor) genes. CBF is a small family from which CBF1, CBF2, CBF3 have been isolated and identified in Arabidopsis. Overexpression of CBF1 and CBF3 led to constitutive expression of cold-inducible genes and enhanced freezing tolerance in non-cold acclimated Arabidopsis. This cold-stress signal transduction pathway has been reported to be conserved in flowering plants. CBF genes have also been identified and characterized in rice, maize, barley, and Brassica, in addition to Arabidopsis. The objective of this study was to identify and characterize CBF-like genes in perennial ryegrass with the hope that we can improve freezing tolerance of perennial ryegrass by transgenic technology in the future.

Materials and methods

Degenerate primers were designed based on the AP2 domain and C-terminal region conserved in all CBF genes known in monocot plant species. RNA was isolated from the perennial ryegrass cultivar 'Caddyshack' that was cold-treated by using Trizol reagent. RT-PCR was performed on the isolated RNA by using the degenerate primers to obtain partial length of a CBF-like gene and 5' and 3' RACE (Rapid amplification of cDNA end) were used to obtain the complete 5' and 3' end of the CBF-like gene. Southern and northern blots were used to determine the copy number and the expression pattern of the CBF-like genes in various stress treatments. Mega3 program was used to conduct phylogenetic analysis.

Results

A CBF-like gene was isolated by using RT-PCR and 5' and 3' RACE in perennial ryegrass cultivar 'Caddyshack'. Phylogenetic analysis indicated that perennial ryegrass CBF-like gene was clustered with a group of CBF3 genes from rice, maize, and barley. We, therefore, designated the perennial ryegrass CBF-like gene as LpCBF3 gene and deposited it to GeneBank with an accession number of AY970831. The expression pattern of LpCBF3 was similar to those of other reported CBF genes that function in ABA-independent cold stress signal transduction pathway. We are currently working on functional analysis of the LpCBF3 gene by transferring the gene into Arabidopsis. Expression pattern of downstream target genes and freezing tolerance testing of transgenic plants will be analyzed.