ISOLATION OF A GENE RESPONSIBLE FOR COLD TOLERANCE IN RYEGRASS (*Lolium Perenne*)

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Perennial ryegrass is the most common grass grown in the temperate regions throughout the world. It is used for turf and forage purpose of its high quality and yield, except it has a low winter hardiness (ability of a plant to survive winter), which limits its application in northern continental climates. Improving cold tolerance is one of the most important objectives for this species in our breeding program. Modern biotechnological methods can be used to improve freezing tolerance and winter hardiness by artificially driving some cold-related genes overexpression. *CBF* gene is considered to be responsible for cold tolerance in plant.

In this study, we isolated an *LpCBF3* gene from the most cold-tolerant perennial ryegrass accessions. *LpCBF3* encodes a protein of 237 amino acids with a molecular mass of 25.5kDa. The most closely related plant *CBF* gene (70% similarity) is from rice (Fig. 1). The *CBF* gene sequences in monocots are closely related to each other as compared with the dicots. Southern analysis indicated the presence of at least three homologous of *Lpcbf3* gene in the perennial ryegrass genome. Northern blotting and RT-PCR analysis found *LpCBF3* reached the highest expression after 3 hour cold-treatment (Fig. 2 and 3). A *COR* homologous gene in ryegrass, as a downstream gene of *CBF*, can be expressed in the plant stem of cold-tolerant ryegrass even without cold treatment.

In *Arabidopsis*, the over-expression of *CBF3* under 35S promoter was resulted in a plant with a striking dwarf-like appearance and a stunted phenotype that is late flowering, drought and cold tolerance (Fig. 4). These results lead us to propose potential implications and applications of *LpCBF3* gene in turfgrass cold-tolerance and other quality traits.

CBF3 gene full sequence

.....cggaccaagttcagggagacgcggcacccggtgtatc
gcggtgtgcgtcgtaggggcaatgccgggaggtgggtatgcgaggtgcgcgtcccagggaggcgc
gggagcaggctgtgggtcggcaccttcgacactgccgagatcgccgcgcgagcacacgacgccgcc
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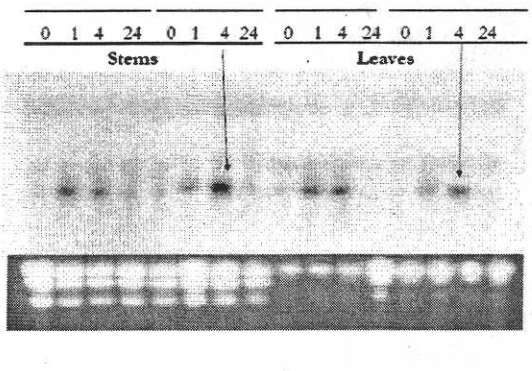

Figure 1. *LpCBF gene*

Figure 2. Northern expression

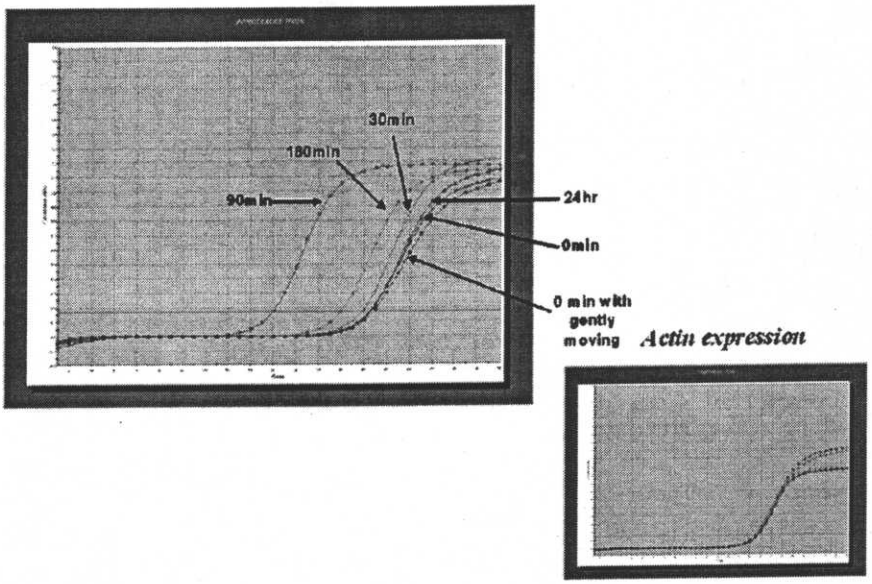

Figure 3. RT-PCR

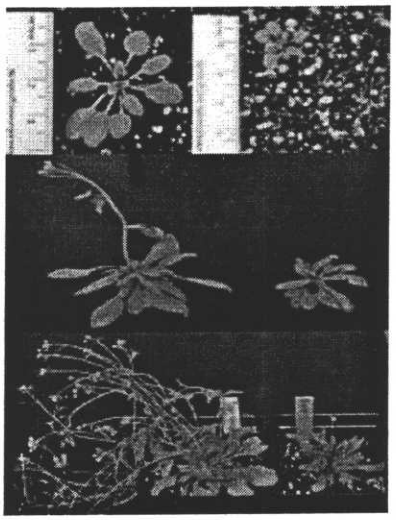

Figure 4. *Phenotypes of wild type and transgenic plants*