Transgenic barley lines prove the involvement of *TaCBF14* and *TaCBF15* in the cold acclimation process and in frost tolerance

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Running title: *TaCBF14* and *TaCBF15* improve frost tolerance

ABSTRACT

The enhancement of winter hardiness is one of the most important tasks facing breeders of winter cereals. For this reason, the examination of those regulatory genes involved in the cold-acclimation processes is of central importance. The aim of the present work was the functional analysis of two wheat CBF transcription factors, namely TaCBF14 and TaCBF15 shown by previous experiments to play a role in the development of frost tolerance. These genes were isolated from winter wheat and then transformed into spring barley, after which the effect of the transgenes on low temperature stress tolerance was examined. Two different types of frost tests were applied; plants were hardened at low temperature before freezing, or

plants were subjected to frost without a hardening period. Our analysis showed, that *TaCBF14* and *TaCBF15* transgenes improve the frost tolerance to such an extent that the transgenic lines were able to survive freezing temperatures several degrees lower than that which proved lethal for the wild type spring barley. After freezing, lower ion-leakage was measured in transgenic leaves showing that these plants were less damaged by the frost. Additionally, a higher F_v/F_m parameter was determined, indicating that PSII worked more efficiently in the transgenics. Gene expression studies showed that *HvCOR14b*, *HvDHN5* and *HvDHN8* genes were up-regulated by TaCBF14 and TaCBF15. Beyond that, transgenic lines exhibited moderate retarded development, slower growth and minor late flowering compared to the wild type, with enhanced transcript level of the GA catabolic *HvGA2ox5* gene.

Key words

Barley, CBFs, frost tolerance, gene expression, gibberellin metabolism, transformation, wheat