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ARTICLE

Are *Cbf* genes involved in copper tolerance?

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ABSTRACT A cluster of *Cbf* (*C-repeat binding factor)* genes have been shown to have a critical role in cold stress response in many plant species. Extensive cross-talk between abiotic stress-signalling pathways have also been described in recent publications. Since the expression of *Cbf* genes is known to be influenced by low-temperature, drought or salinity stress, it would be not surprising if their expression would be also influenced by other abiotic stresses such as heavy metal tolerance. In this work the expression pattern of 8 *Cbf* genes, located on chromosome 5A were investigated in wheat, subjected to different copper stress conditions (hydroponics and soil). Wheat cultivar 'Chinese Spring' (moderate copper tolerance) and two 5A chromosome substitution lines, especially 'Chinese Spring /*T. spelta* 5A' (copper sensitive) and 'Chinese Spring /Cheyenne 5A' (copper tolerant) showed different expression profiles in the course of copper stress. *Cbf3* and *Cbf10* were up-regulated by copper stress, especially in the tolerant genotype, however down-regulation of other *Cbf* genes was also found. These findings suggest that the *Cbf* genes might have roles in the enhanced copper tolerance.

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KEY WORDS

Cbf genes
copper tolerance
wheat
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Copper (Cu) is an essential micronutrient, but it is phytotoxic in higher concentration. Despite of the fact that the morphological and physiological aspects of copper toxicity are intensively investigated, the genetic background and the signalling pathways of copper tolerance is not well understood yet. It is known, that Cu tolerance is associated with linkeage group 5A in wheat and also known, that a cluster of C-repeat binding factor genes (*Cbf*) are located on this chromosome. *Cbf* genes are one of the most extensively studied stress-related transcription factors in the plant kingdom, having important role in low-temerature, drought and salt stress response. Considering the crosstalk between the abiotic stress signal transduction pathways, the role of *Cbf* genes in copper tolerance might be presumed.

Materials and Methods

Plant material and growing conditions

Hydroponics and soil pot experiment were made on a hexaploid wheat (*Triticum aestivum*) genotypes 'Chinese Spring' (CS) (moderate copper tolerance) and two CS/5A substitution lines 'CS/Cheyenne5A' (copper tolerant) and 'CS/*Triticum spelta* 5A' (copper sensitive). In the hydroponics experiment the germinated seedlings were grown in Hoagland nutrient solution (Cu concentration 10^{-7} M) in a plant growth chamber (PGR-15, Conviron, Manitoba, Canada) for 7 days on 18/13ºC. Copper stress was started on the 8th day by the plants into nutrient solution containing Cu^{2+} in a concentration of 10^{-4} M. Leaves for RNA isolation were harvested 10 min, 25 min, 1h, 3h and 5h after the beginning of the stress treatment. In the soil-system experiment the germinated seedling were planted in pots containing soil and they were grown under identical condition to the hydroponics. No copper was added to the control while 1500 mg/kg final Cu was used for the soil-treatment. Samples were taken at the third leaf stage.

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RT-PCR analysis

Total RNA was extracted from leaves by means of Trizol reagent (Invitrogen) following the manufacturer's instructions. The cDNA was synthesized using 200 U of M-MLV Reverse Transcriptase (Promega), oligo$(dT)_{15}$ primer (Promega) on 2 µg of total RNA according to the manufacturer's recommendations. First strand cDNA was used as template for PCR amplification with gene specific primer described by Vágújfalvi *et al*. (2005). PCRs were performed with GoTaq Flexi DNA Polymerase (Promega) under the following procedure: 95ºC for 2 min, then 30-35cycles of denaturation at 95ºC for 30 s, annealing at 55-63ºC for 30 s and extension at 72ºC for 30 s, followed by a final extension for 5 min. Normalization of the PCR reaction was performed with a primer pair designed on wheat GAPDH gene.

Results

Primer pairs specific for 9 *Cbf* genes were used in the RT-PCR analysis. Two *Cbf* genes (*Cbf3* and *Cbf10*) showed fast, transient expression during the hydroponics experiment. Copper

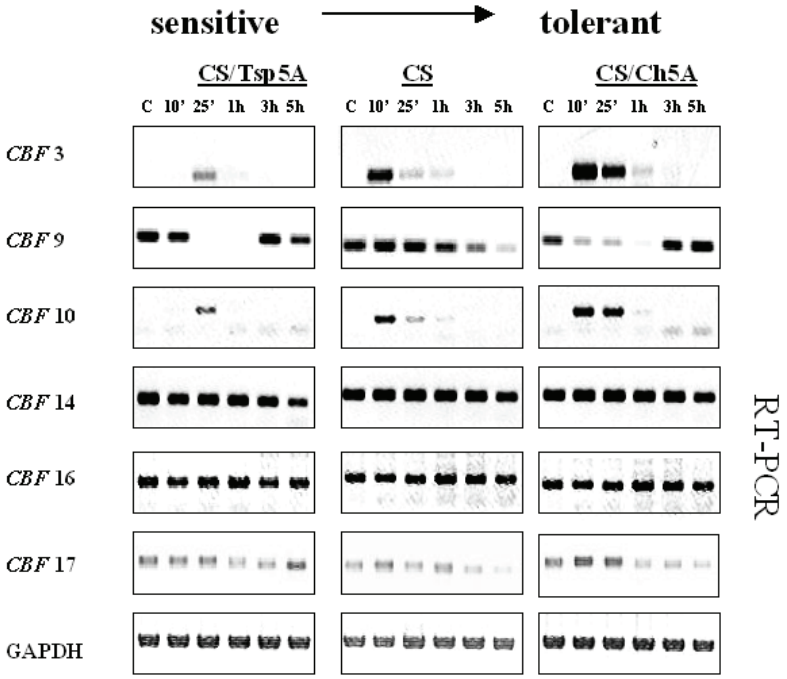

Figure 1. RT-PCR analysis of *Cbf* genes. Plants were subjected to copper stress in hydroponics. The figures of gel separation are presented in inverted colours.

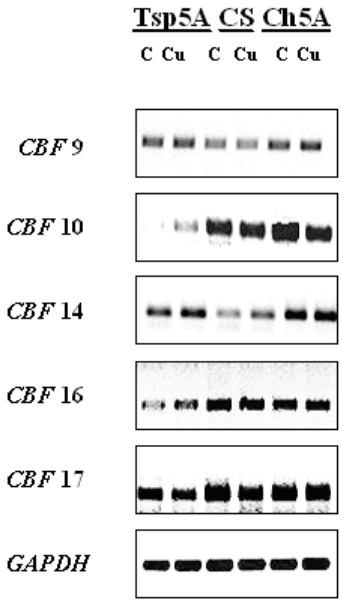

Figure 2. RT-PCR analysis of *Cbf* genes. Plants were subjected to copper stress in soil. The figures of gel separation are presented in inverted colours

stress dependent up-regulation of these two genes was found in all genotypes but the lowest expression was detected in the susceptible genotype (Fig. 1). In soil pot experiment the expression level of *Cbf10* was nearly unaffected by Cu treatment but differed among the genotype (Fig. 2). No expression was detected for *Cbf3* in the soil pot experiment. *Cbf9* was down regulated in hydroponics in genotype dependent manner, but remained unchanged in the soil pot experiment. The mRNAs corresponding to *Cbf14*, *Cbf16* and *Cbf17* were completely unaffected in both experiments in all sampling dates. The primer pairs of two genes (*Cbf12, Cbf13*) did not give any detectable amplification product.

Discussion

This work demonstrates that several *Cbf* genes regulated not only by cold or dehydratation but by copper stress as well. Changes in the transcript level of *Cbf* genes were analysed under two different stress conditions. In hydroponics the fast and in soil experiment the slow differences were examined. Cluster analysis of orthologue CBF proteins suggests the existence of three major subgroups (Skinner *et al.* 2005). In *Arabidopsis, Cbf3* gene, which belongs to the HvCbf3-subgroup, is not involved in the self-regulation of other *Cbf* genes, however it is positively regulated during cold acclimation and by activating a subset of *Cbf*- target genes (Novillo *et al.* 2007) finally it increases the cold tolerance. Wheat *Cbf3* and *Cbf10* genes are also belong to HvCbf3- subgroup, and here we found that their transcript levels are up-regulated in the tolerant genotypes (Fig. 1).

The expression profile presented here suggests a possible involvement of *Cbf* genes in the regulation of copper tolerance, but further experiments required to clarify their exact role in this process.

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