Abstract

Transcription factors of the HD-Zip I subfamily play a key role in regulating plant responses to abiotic stress and linking environmental signals to internal developmental processes, including circadian rhythms. In the genome of oilseed rape (*Brassica napus* L.), an amphidiploid species with a complex evolutionary history, many genes of this subfamily are present in multiple copies reflecting previous whole-genome duplications and genome reorganization. Although their coding sequences are highly conserved, the HD-Zip I homologs show significant differences in spatial and temporal expression patterns and in response to environmental stimuli, suggesting subfunctionalization or incipient neofunctionalization. The main objective of this PhD thesis was to investigate the molecular mechanisms underlying the potential functional divergence of the four *BnaHB6* homologs, with particular emphasis on their role in the plant response to water deficit. In addition, their potential target genes and associated regulatory pathways under dehydration conditions should be identified.

Bioinformatic (sequence and phylogenetic analyzes, identification of cis-regulatory elements), transcriptomic (RNA-Seq and RT-qPCR) and molecular approaches (promoter activity assays in transgenic plants, ChIP-qPCR and characterization of the TILLING mutant) were used for the research. All four *BnaHB6* homologs exhibited a conserved gene structure and functional domains (HD domain and LZ motif) and showed transcriptional activation potential. However, they differed in their regulatory sequences and expression profiles. Among them, *BnaC08HB6* and *BnaA09HB6* showed strong induction under dehydration, salinity and darkness. The presence of characteristic *cis*-regulatory elements associated with abiotic stress responses (ABRE, DRE, MYB) in their promoter regions correlated with the expression patterns and promoter activity in transgenic *Arabidopsis thaliana* plants. ChIP-qPCR analyzes confirmed that both genes directly regulate the expression of selected *BnaABF4* and *BnaDREB2A* homologs, which are key components of the ABA-dependent osmotic stress response pathway. Remarkably, *BnaC08HB6* and *BnaA09HB6* act as transcriptional repressors under control conditions and activators under stress conditions, highlighting their dynamic regulatory function. In contrast, *BnaA04HB6* and *BnaC04HB6* showed limited induction under abiotic stress, indicating their possible specialization in other physiological processes, such as light-dependent development. The functional diversification of *BnaHB6* homologs supports the hypothesis of subfunctionalization,

allowing fine-tuned adaptation to environmental conditions. Mutations in *BnaC08HB6* resulted in both morphological and molecular changes. Transcriptome profiling of the *bnac08hb6* mutant under dehydration stress revealed deregulation of signaling pathways related to water stress, redox homeostasis, and plastid metabolism, highlighting the key role of this gene in the integration of environmental signals with organelle functions and plant development.

The results of this study provide new insights into the organization and evolution of transcriptional regulatory networks in plants and identify potential targets for future biotechnological and breeding strategies to improve tolerance to abiotic stress factors.