Summary

Every year drought causes serious loss of crop yield due to the progressive climate changes. Therefore, there is an urgent demand for development of new cultivars with better tolerance to stress. The part of this process is identification and description of mechanisms which ensure plant adaptation to drought. Abscisic acid (ABA) is a main phytohormone regulating plant response to abiotic stresses, including drought. At the physiological level, ABA causes stomata closure and photosynthesis inhibition, whereas at the molecular level it regulates expression of stress-responsive genes. In Arabidopsis, ABA INSENSITIVE 5 (ABI5) and ABRE BINDING FACTORs/ABRE-BINDING PROTEINs (ABFs/AREBs) act as ABA-dependent transcription factors with BASIC LEUCINE ZIPPER (bZIP) domain. They regulate expression of genes associated with plant adaptation to unfavorable environmental conditions.

The aim of the presented PhD thesis was to describe the function of *HvABI5*, a barley (*Hordeum vulagre*) homolog of *AtABI5* and *AtABF/AREB*, in response to drought and to identify putative target genes of HvABI5. The application of barley TILLING population developed at the Department of Genetics, University of Silesia in Katowice, enabled identification of *hvabi5.d* mutant carrying a G1751A point mutation in *HvABI5* gene. The identified mutation caused arginine to lysine substitution at the 274 amino acid position which is close to the bZIP domain of HvABI5 protein. *hvabi5.d* showed a much lower sensitivity to ABA during seed germination than its parent variety 'Sebastian'. It also exhibited decreased values of photosynthetic parameters: the performance index for the photochemical activity (PI_{ABS}) and the maximum quantum yield of primary photochemistry (φP_0), together with increased level of osmolyte proline after ABA treatment at the early seedling stage. Based on *hvabi5.d* reaction to ABA, it was assumed that *HvABI5* may be involved in regulation of barley response to the drought stress. After 5 days of water withdrawal and 10-day drought treatment, *hvabi5.d* showed a 13% higher value of Relative Water Content (RWC) parameter than 'Sebastian'. Increased drought tolerance of *hvabi5.d* was related to the better membrane protection, higher flavonoid content (flavonols and anthocyanins) and faster stomatal closure than observed in the parent variety. Moreover, the known HvABI5 target genes: *HVA1* and *HVA22*, as well as *DEHYDRATION-RESPONSIVE FACTOR 1* (*HvDRF1*), encoding ABA-dependent transcription factor, showed the higher expression in mutant when compared to 'Sebastian' under drought. On the other side, *hvabi5.d* showed decreased chlorophyll content and lower values of photosynthetic parameters, PI_{ABS} and φP_0, under drought. To verify if *HvABI5* regulates response to drought in the ABA-dependent way, the expression of genes

related to the ABA metabolism and signaling was analyzed under drought in both genotypes. Expression of key ABA-pathway genes differed between mutant and 'Sebastian' under stress. In response to drought *hvabi5.d* showed 2-20 times higher expression of genes involved in ABA biosynthesis and metabolism, *HvNCED1* and *HvBG8*, and gene encoding main components of ABA signaling, *HvSnRK2.1* and *HvPP2C4*. Moreover, the mutant showed 2-times higher endogenous content of ABA than its parent variety after drought treatment. Furthermore, in the promoters of *HvNCED1*, *HvSnRK2.1* and *HvPP2C4* putative binding sites for ABI5 were identified. The increased expression of *HvNCED1* and *HvSnRK2.1* and the faster stomatal closure was also observed in *hvabi5.d* after ABA treatment which confirms the ABA-dependent HvABI5 activity in barley response to drought.

Global transcriptome analysis using Agilent microarrays revealed differentially expressed genes (DEGs) between *hvabi5.d* mutant and its parent variety after application of stress. More genes (2688) were specifically up- or down-regulated in the mutant after 5- day decrease of soil moisture (drought onset) than after 10-day drought treatment (1959 genes). Among them were genes which could be related to the mechanisms responsible for increased drought tolerance of *hvabi5.d*. In order to identify putative HvABI5 target genes, the promoters of DEGs were analyzed for the presence of *cis*-elements ABA RESPONSIVE ELEMENT (ABRE) recognized by ABI5. ABRE elements were found in the promoters of 49 genes showing differentiated expression at drought onset and in the promoters of 48 genes showing differentiated expression after drought. Twenty-two selected HvABI5 putative target genes were selected and their expression after drought and ABA treatments was analyzed. It showed a different transcription activity of 12 genes between *hvabi5.d* and its parent variety under both treatments, which indicates that they may be regulated by HvABI5 in response to drought in the ABA-dependent way. Function of these putative HvABI5 target genes is associated with response to stress, phytohormone biosynthesis, transcription regulation, phosphorylation, lipid function and cell function. Only 5 of 22 analyzed genes, which are related to stress response, gibberellin response, pathogen defense and translation regulation, showed a different expression in the mutant only under drought treatment. This indicates that HvABI5 can also act in the scope of other signaling pathways. It has to be underlined that the identified potential HvABI5 target genes were not described in literature, and function of these genes was assigned based on GO terms and functional annotation available in the databases for sequences corresponding to their HORVU ID.

Taken together, the presented results indicate that *HvABI5* regulates barley drought response in the ABA-dependent way. The role of *HvABI5* is to regulate stress-responsive genes

which are related to mechanisms ensuring plant adaptation to drought. Moreover, *HvABI5* can participate in the regulation of ABA biosynthesis and signaling *via* a feedback loop in response to drought. It should be underlined that the mode of ABA-dependent *HvABI5* action during regulation of seed germination and drought response is different.