Summary

Root hairs are cylindrical extensions of the root epidermal cells that play a role in anchoring the plant in the soil, taking in nutrients, and interacting with microorganisms within the rhizosphere. Their presence is crucial under drought and phosphorus deficiency. The development of root hairs is a complex process that requires the involvement of many genes. Their development can be divided into the following stages: 1) determination of the rhizodermis pattern, 2) initiation and formation of the root hair bulge, 3) tip growth of the root hair. In contrast to the model plant *Arabidopsis thaliana*, knowledge of the molecular basis of root hair morphogenesis in monocots, including barley (*Hordeum vulgare*), is limited.

The aim of this study was to identify new genes related to the processes of root hair development and growth and to characterize their molecular function in barley. To achieve this goal, two research approaches were used: the TILLING strategy and classical genetic analysis but enhanced with the exome sequencing technique. In the first part of the study, using bioinformatics tools, three candidate genes were selected for TILLING analysis: *HvRIC10 (ROP-INTERACTIVE CRIB MOTIF-CONTAINING PROTEIN 10)*, encoding a protein involved, for example, in cytoskeletal rearrangements and regulation of exocytosis, *HvOPR1 (12-OXOPHYTODIENOATE REDUCTASE 1)*, encoding 12-oxophytodienan reductase 1 and *HvEXPB5 (EXPANSIN B5)*, which product is β-expansin 5. These genes were selected on the basis of the results of a microarray experiment carried out previously in the Department of Genetics US, in which the root transcriptomes of the hairless mutant *rhl1.a* and its parent variety 'Karat' were analyzed (Kwasniewski et al., 2016). Genes, whose expression in the mutant roots was significantly reduced compared to the parental variety, their biological function have suggested a possible involvement in root hair morphogenesis, but their role in this process has not yet been experimentally confirmed in any model or cultivated species. The research material was the barley *Hor*TILLUS population, obtained after chemical mutagenesis of spring barley cv. 'Sebastian'. For each gene, 5376 - 6912 M_2 plants were screened for mutations. In the case of the *HvRIC10* gene, 12 nucleotide substitutions were identified, including three missense mutations, one splice-junction and one nonsense mutation. As a result of the analyzes conducted for the *HvOPR1* gene, 12 mutations were found, of which three were missense and one a splice-junction mutation. Furthermore, 15 mutations were identified in the *HvEXPB5* gene, including five missense mutations and one splice-junction. The other changes in each of the analyzed genes were silent or occurred in non-coding regions, unrelated to splicing. The phenotypic analysis of the root hair zone of mutants that were homozygous for the identified missense, splice-junction and nonsense mutations in the *HvRIC10* and *HvOPR1* genes has not revealed

any differences in the development and growth of root hairs compared to the parental variety. Among them, there were mutants carrying a premature STOP codon, causing shortening of HvRIC10 protein by 50% and 70%, and those in which the retention of the intron resulted in the insertion of new amino acids and, as a result, the synthesis of a longer HvOPR1 protein in compared to the wild type. This indicates that the activity of the *HvRIC10* and *HvOPR1* genes in barley roots is not necessary for the proper development of root hairs.

However, the analysis of the root hair zone of mutants with changes in the *HvEXPB5* gene showed one mutant (carrying the *hvexpb5.i* allele), which was characterized by the phenotype of strongly shortened root hairs, reaching only 3% of the length of the root hairs of the parental variety. The *hvexpb5.i* mutant carries the C759T transition, which results in the replacement of proline at position 144 with serine in the protein sequence. However the analysis of the F_2 population resulting from the crossing of the *hvexpb5.i* mutant with the parental variety, showed a lack of co-segregation of the *hvexpb5.i* allele with the changed root hair phenotype of the mutant. This indicates that the *HvEXPB5* gene does not play a role in maintaining the tip growth of root hairs in barley, and another, as yet unidentified, gene is responsible for the observed mutant phenotype.

Therefore, in the second part of the study, an attempt was made to identify the gene with the mutation responsible for growth inhibition at the early stage of root hair development of the *hvexpb5.i* mutant. For this purpose, the identified mutant was crossed with *rhp1.a - rhp1.d* (*root hair primordia 1*) mutants, with a similar root hair phenotype. The complementation test showed that these mutants were allelic in terms of the gene responsible for the strong shortening of root hairs, hence the name *hvexpb5.i/ rhp1.e* was proposed, after the next allele in the *Rhp1 locus*. In the previous studies, the *Rhp1 locus* was positioned in the long arm of chromosome 1H (Janiak et al., 2007), but the specific sequence and molecular function of the gene underlying the described phenotype have not been identified.

Further studies have been carried using the “exome capture” strategy, which involves a selective sequencing of coding regions captured from the genomic DNA by especially designed probes (Ng et al., 2009). For this purpose, the SeqCap EZ HyperCap protocol (Roche, Basel, Switzerland) was used. The exome sequencing of F_2 individual with shortened root hairs derived from the F_2 population and the wild type cv. 'Sebastian' were sequenced. Sequencing was performed for 2 × 75 paired-end sequencing according to standard protocols with an Illumina HiSeq 4000 (Illumina) at the Medical University of Bialystok. Based on the results of exome sequencing of the *rhp1.e* mutant, eight candidate genes were selected, identified within chromosome 1H, in which mutations were detected in the homozygous state. Based on data on

the physical location of the *Rhp1 locus*, information on gene ontology, as well as the potential impact of mutations on the protein activity, the most probable candidate gene selected for further analysis was *HORVU1Hr1G077230*, encoding a cellulose synthase C1 (HvCSLC1), involved in the synthesis of xyloglucan in barley.

The mutation identified in the *HORVU1Hr1G077230 (HvCSLC1)* gene in the *rhp1.e* mutant was a G1674A substitution in a donor splice-junction site, causing the retention of intron 2 and, consequently, the mature mRNA sequence longer by 117 nucleotides. This resulted in the creation of a premature STOP codon in the inserted intron, which caused shortening of the 699-amino-acid protein by a 344-amino-acid fragment containing a part of the functional CESA domain and transmembrane domains (TMD). As a result, the identified mutation may interfere with the activity of the HvCSLC1 protein. Interestingly, the mutant showed reduced expression levels of the *HORVU1Hr1G077230 (HvCSLC1)* gene in all zones of seminal roots, with the largest difference noted in the root hair zone. A complete co-segregation of the identified *hvcslc1.a* allele with the changed root hair phenotype was found in the F_2 population of the cross between the mutant and the parental variety. The obtained results indicate that the presence of the identified mutation in the *HORVU1Hr1G077230 (HvCSLC1)* gene is associated with disorders in root hair development. This conclusion was confirmed by the detection of various nucleotide substitutions within the *HORVU1Hr1G077230 (HvCSLC1)* gene sequence in all identified allelic mutants (*rhp1.a – rhp1.d* and *rhp1.f*). Moreover, in the *rhp1.b* mutant, no mutations were detected in the remaining candidate genes from chromosome 1H.

Taking into account the obtained results and available literature data, the role of the *HORVU1Hr1G077230 (HvCSLC1)* gene in the development of root hairs in barley, and more specifically in the biosynthesis of xyloglucan during tip growth, was discussed. Other genes, potentially interacting with the *HvCSLC1* gene were identified and their expression was analyzed, resulting in the proposed scheme of xyloglucan biosynthesis in the *H. vulgare* root hairs.