The goal of the presented project is to explore the molecular mechanisms of the response of monocotyledonous plants to environmental stress related to the presence of phytotoxic aluminum ions (Al^{3+}) in the soil. Aluminum is the most common metal in the Earth's crust. Hence, it is present in almost all types of soils worldwide. In near-neutral or alkaline soils, it is not toxic – it occurs in forms harmless to plants or is bound to various minerals. However, in low pH conditions, it is released from the minerals in the form of highly phytotoxic Al^{3+} ions that penetrate the roots and inhibit their growth, which translates into a reduction of the plant's above-ground parts and, as a result, reduced yield. It is estimated that about 50% of cultivated lands in the world are acidic (with a pH below 5.5), and in such conditions, Al is the main factor limiting the yield. **Understanding the molecular mechanisms of crop plant responses to stress related to the toxicity of aluminum ions is crucial and may enable the development of new varieties tolerant to this stress.**

The research material will be barley (*Hordeum vulgare* L.), one of the model species for monocotyledonous plants, the fourth cereal in terms of cultivation area in the world, and one of the most sensitive to Al^{3+} ions crop plant species. As part of the work, **it is planned to develop barley mutants characterized by mutations in the *HvSTOP1* gene**, which is a homolog of the *AtSTOP1* gene encoding **a key transcription factor controlling the response to aluminum ions** in Arabidopsis. For this purpose, we will use the *Hor*TILLUS platform - the TILLING population of barley derived at the University of Silesia. This population will be analyzed using the TILLING strategy to identify plants carrying new mutated *HvSTOP1* alleles. In parallel, knock-out mutants for this gene will be derived using the CRISPR/Cas9 strategy (in collaboration with dr. Ales Pecinka from the Czech Academy of Sciences). All generated mutants will be tested under hydroponic conditions for Al response. The influence of aluminum on various parameters of the root system (such as the number of seminal and lateral roots, their length, diameter, etc.) will be assessed using the specialized WinRHIZO program. Then, mutants with an altered, when compared to wild type (WT), Al response will be subjected to more detailed phenotyping, including morphological, physiological, and cytological analyses, such as the assessment of the frequency of cell divisions in the root meristem, cell cycle analysis using a flow cytometer, or evaluation of the level of DNA damage. **The planned research will allow the functional analysis of the *HvSTOP1* gene**. In addition, **for the selected *stop1* mutants** (one TILLING and one CRISPR/Cas9 mutant) **and their parent varieties, RNA-seq analysis will be carried out,** allowing the recognition of genes regulated (directly or indirectly) by the STOP1 transcription factor, and thus the **STOP1-dependent and STOP1-independent transcriptional regulatory pathways of the response to Al. The results will be compared with publicly available RNA-seq results obtained for Arabidopsis to assess the universality of these pathways in monocotyledonous and dicotyledonous plants and identify factors specific to particular groups.**

In the presented project, **we also plan to perform functional analysis for barley *ALS1* and *ALS3* genes encoding transporters potentially involved in Al transport. We will develop TILLING and CRISPR/Cas9 mutants for the *HvALS1.1* and *HvALS1.2* genes**, which we found in our earlier studies of the barley transcriptome to be induced by Al treatment. These two paralogs are homologs of the *AtALS1* gene, which encodes an ABC transporter **responsible for transporting Al^{3+} ions in root cells from the cytoplasm to the vacuole, where they are sequestered (deactivated).** Double mutants will also be created for functional analysis of *ALS1* paralogs. Both single and double mutants will be tested in the same manner as described above for *stop1* mutants. We expect that the mutants in these genes will be Al-hypersensitive due to the accumulation of Al ions in the cytoplasm of root cells (lack of sequestration in the vacuole). The next gene to be studied, ***HvALS3***, encodes another ABC transporter that is known, at least in Arabidopsis, to be **responsible for transporting Al^{3+} ions away from the root tissue to the upper, less Al-sensitive parts of the plant.** In our previous studies, we have identified a series of *als3* barley TILLING mutants. We assumed that they should be more sensitive to Al than WT because of accumulating Al ions in roots - however, surprisingly, none of them were more Al-sensitive. We have created a hypothesis that barley is in general so sensitive to Al because the mechanism of moving Al away from root tissue is not working efficiently in this species. Within the frame of this project, we would like to create CRISPR/Cas9 (knock-out) mutants in *ALS3* and test them under Al conditions. If knock-out mutants are not more sensitive to Al than WT it will indirectly enhance our hypothesis. We will also check the Al content in roots and leaves of analyzed genotypes, grown under Al stress, to finally prove it.

In the last part of the project, one *als1* mutant, showing Al-hypersensitivity, will be selected for **suppressor mutagenesis, which will be performed to identify mutants with the restored phenotype (not Al-hypersensitive). Obtaining suppressor mutants will enable the subsequent identification of genes responsible for the abolition of Al-hypersensitivity and the discovery of new elements of the Al response pathway.**