1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)

Project title:

The role of plant metallothioneins in response to environmental stresses: the *Arabidopsis thaliana* model

1.1. Project goals

The aim of this PhD project is to gain deeper insight into the role of plant metallothioneins in the response to environmental stresses. We aim to verify the hypothesis that plant metallothioneins are general stress proteins via CRISPR-Cas9 genome editing. Further, an in-depth analysis of mutant plans will allow us to determine the position of MTs in plant stress response pathways.

1.2. Outline

Genome editing, on the one hand, is a potent tool for analysing the role of various proteins and other genetic elements. On the other hand, genome editing allows to boost the yield of crop plants quickly in contrast to traditional breeding techniques. Among other methods, the most recent CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) technology offers great promise to develop a new era of plant breeding. Some obstacles to this technology's ability to overcome include potential off-targets and the development of multiplexed CRISPR. Moreover, the development of base modification in precisely specified genome positions is needed. Metallothioneins (MTs) are ubiquitous, low-molecular-weight proteins rich in cysteine residues. Due to the presence of multiple thiol groups, MTs bind various heavy metal ions, especially those with d^{10} electron configuration. The primary role of MTs is maintaining copper and zinc homeostasis and detoxification of xenobiotics, mostly cadmium. In addition, MTs are involved in reactive oxygen species scavenging and maintaining cell redox balance. Plant metallothioneins (pMTs) are much more diversified than MTs from other organisms. Based on the number and arrangement of cysteine residues pMTs were divided into 4 types. Structural diversification is related to functional diversification, there is no single unifying role for all pMTs, and each type of pMT may fulfil more than one function. Despite several years of research, the specific functions of each type of pMT are poorly understood. We hypothesise that plant metallothioneins are general stress proteins involved in the response to various environmental stresses.

1.3. Work plan

<u>1. Preparation and validation of DNA constructs.</u>

In this PhD project, the following vectors will be prepared:
- vectors for *A. thaliana* genome editing via CRISPR/Cas9 – deletion in single *MT* genes,
- vectors for *A. thaliana* genome editing via CRISPR/Cas9 – point mutations in *MT* genes,
- vectors for *A. thaliana* genome editing via CRISPR/Cas9 – deletion in multiple *MT* genes.

<u>2. Testing of the efficiency of prepared vectors in protoplasts.</u>

<u>3. Plant transformation and selection of transformants.</u>

Agrobacterium-mediated transformation will be used to transform *A. thaliana.* After selecting positive transformants, plants will be grown to homozygous F2 generation.

4. Analysis of modified plants for possible off-targets.
The potential off-targets will be predicted via online tools. Further analysis via PCR and sequencing of PCR products will be performed.

5. Analysis of the phenotype of modified plants.
The obtained lines of modified plants will be analysed for their stress resistance, e.g. drought, high temperature, and oxidative stress. To compare modified and wild-type plants, germination parameters, morphological parameters of seedlings and mature plants, and the number of seeds will be analysed. Moreover, biochemical parameters will be determined, including chlorophyll content, the activity of antioxidant enzymes, and the phytohormones level.

6. Transcriptome analysis.
Based on the results obtained in the previous experiment, wild-type and mutant plants will be grown in the conditions where the phenotype differences are observed. The whole transcriptome will be analysed via RNA sequencing to verify molecular mechanisms underlying the observed differences. Further selected genes will be verified by qPCR.

1.4. **Literature** *(max. 10 listed, as a suggestion for a PhD candidate)*

Mierek-Adamska A, Dąbrowska GB, Blindauer CA (2018) The type 4 metallothionein from Brassica napus seeds folds in a metal-dependent fashion and favours zinc over other metals. Metallomics 10: 1430-1443.
Mierek-Adamska A, Kotowicz K, Goc A, Boniecka J, Berdychowska J, Dąbrowska G (2019) Potential involvement of rapeseed (*Brassica napus* L.) metallothioneins in the hydrogen peroxide-induced regulation of seed vigour. Journal of Agronomy and Crop Science 205: 598-607.
Antoszewski M, Mierek-Adamska A, Dąbrowska GB (2022) The importance of microorganisms for sustainable agriculture – a review. Metabolites 12: 1100.
Konieczna W, Mierek-Adamska A, Warchoł M, Skrzypek E, Dąbrowska GB (2023) The involvement of metallothioneins and stress markers in response to osmotic stress in *Avena sativa* L. Journal of Agronomy and Crop Science 209: 371-389.
Konieczna W, Warchoł M, Mierek-Adamska A, Skrzypek E, Waligórski P, Piernika A, Dąbrowska GB (2023) Changes in physio-biochemical parameters and expression of metallothioneins in *Avena sativa* L. in response to drought. Scientific Reports 13: 2486.
Turkan S, Mierek-Adamska A, Głowacka K, Szydłowska-Czerniak A, Rewers M, Jędrzejczyk I, Dąbrowska GB (2023) Localization and expression of *CRSH* transcript, level of calcium ions, and cell cycle activity during *Brassica napus* L. seed development. Industrial Crops & Products 195: 116439.
Konieczna W, Mierek-Adamska A, Chojnacka N, Antoszewski M, Szydłowska-Czerniak A, Dąbrowska GB (2023) Characterization of metallothionein gene family in *Avena sativa* L. and the gene expression during seed germination and heavy metal stress. Antioxidants 12: 1865.
Garstecka Z, Antoszewski M, Mierek-Adamska A, Krauklis D, Niedojadło K, Kaliska B, Hrynkiewicz K, Dąbrowska GB (2023) *Trichoderma viride* colonizes the roots of *Brassica napus* L., alters the expression of stress-responsive genes, and increases the yield of canola under field conditions during drought. International Journal of Molecular Sciences 24: 15349.

Turkan S, Kulasek M, Zienkiewicz A, Mierek-Adamska A, Skrzypek E, Warchoł M, Szydłowska-Cerniak A, Bartoli J, Field B, Dąbrowska GB (2024) Guanosine tetraphosphate (ppGpp) is a new player in *Brassica napus* L. seed development. Food Chemistry 436: 137648.
Ikram M, Rauf A, Rao MJ, Maqsood MFK, Bakhsh MZM, Ullah M, Batool M, Mehran M, Tahira M (2024) CRISPR-Cas9 based molecular breeding in crop plants: a review. Molecular Biology Reports 51: article number 227.

1.5. Required initial knowledge and skills of the PhD candidate

- very good knowledge of molecular biology, plant genetics, and biochemistry
- practical knowledge of basic molecular biology techniques (i.e. nucleic acids isolation, PCR, RT-PCR, qPCR, DNA recombination)
- very good English language skills
- the ability to work in a team
- analytical thinking ability
- willingness to travel (domestic and foreign trips)

1.6. Expected development of the PhD candidate's knowledge and skills

- broadening the knowledge of plant mechanisms of stress adaptation
- broadening the knowledge of molecular biology techniques
- great extend the scope of known molecular biology techniques (i.e. plant transformation, protoplasts transformation, CRSPR/Cas9, analysis of physicochemical and biochemical parameters of plants, plant transcriptomics)
- increasing professional English language skills
- developing the ability to write scientific papers, and present research results in the form of posters and oral presentations
- gaining the ability to write grant proposals
- gaining the ability to plan research and organise own work
- gaining the ability to organise the work of others by supervising the master students