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

**Project title:**

Zn-metallothionein as a potential mediator of the antioxidant mechanism in response to environmental stresses in plants

### 1.1. Project goals

The aim of the project is to gain deeper insight into response of plants to environmental stress. We aim to verify the hypothesis that metallothioneins (MTs) and zinc ions bound by MTs play a crucial role in the regulation of stringent response in model plants. To determine the role of different types of RSH and MT proteins in response to various stresses ectopic expression of heterologous genes and gene editing technologies will be utilised. The analysis of transcriptoms of modified plants grants determination of the position of RSH and MT proteins in stress response pathways.

### 1.2. Outline

Plants as sessile organisms have evolved several mechanisms that allow them to adapt to unfavourable environmental conditions including abiotic (e.g. drought, insufficient amount of nutrients, the presence of xenobiotics) and biotic (e.g. viruses, parasite) factors. There are numerous proteins involved in response to particular types of environmental stressors. Other proteins are more universal and are involved in adaptation to various stresses. Those proteins are called stress proteins and among several other metallothioneins (MTs) and proteins homologous to bacterial RelA/SpoT proteins (RHS) are especially appealing.

MTs are a large protein superfamily present both in Procaryota and Eucaryota. All MTs are 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 in the maintenance of copper and zinc homeostasis and detoxication of xenobiotics mostly cadmium. In addition, MTs are involved in reactive oxygen species scavenging and maintenance of cell redox balance. Plant metallothioneins (pMTs) are much more diversified than MTs form other organisms. Based on the number and arrangement of cysteine residues pMTs were divided into 4 types (type 1-4). Based on the literature data it may be concluded that structural diversification is related to functional diversification. There is no single unifying role of all pMTs, and each type of pMT may fulfil more than one function.

Plant RSH proteins are homologous to bacterial stringent response proteins (RelA/SpoT). Stringent response is a bacterial mechanism to adapt to unfavourable environmental conditions. Plant RSH proteins are synthases and/or hydrolases of guanosine pentaphosphate and tetraphosphate i.e. atypical regulatory nucleotides called alarmones. Plant RSH proteins are encoded in nuclear genome and then proteins are directed to chloroplasts. In plants there are three groups of RSH proteins: RSH1 that

possess alarmone hydrolase activity, RSH2/3 that possess alarmone synthase and hydrolase activity, CRSH that possess alarmone synthase activity. CRSH proteins are the only group among RSH proteins that needs calcium ions for their activity. Plant stringent response in involved in multiple developmental processes e.g. flowering and seed development. Moreover, RSH proteins are involved in plant stress response to various stress conditions.

## 1.3. Work plan

1. Preparation and validation of gene constructs.

In this PhD project three types of vectors will be prepared:

- vectors for ectopic expression of *RSH* and *MT* genes from *Sorghum bicolor* in *Arabidopsis thaliana* under the control of constitutive promotor,
- vectors for *A. thaliana* genome editing via CRISPR/Cas9 – deletion and single point mutation in *CRSH*, *MT4a* and *MT4b* genes.

In our previous research in collaboration with Professor Gutierrez-Marcos *A. thaliana* plants with deletion in *MT4a* and *MT4b* genes have been created (Mierek-Adamska et al. manuscript in preparation). The efficiency of prepared vectors will be tested in protoplasts.

2. Plant transformation and selection of transformants.

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

3. Analysis of transgenic plants.

The obtained lines of modified plants will be analysed for their stress resistance i.e. drought, high temperature, and oxidative stress. To compare modified and wild type plants morphological parameters (the length of shoots and roots, the number of seeds) will be analysed. Moreover, biochemical parameters including the content of chlorophyl, the activity of antioxidant enzymes, and the level of abscisic acid will be determined. The level of alarmones will be measured via HPLC.

4. Gene expression analysis.

The level of expression of other types of *MT* and *RSH* genes will be verified via qPCR. Moreover, the expression of other stress-related genes will be analysed in response to plant conditions. In selected lines the analysis of whole transcriptome with total RNA sequencing will be performed.

## 1.4. Literature

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.

Dąbrowska GB, Turkan S, Tylman-Mojżeszek W, Mierek-Adamska A (2021) In silico study of the

RSH (*RelA/SpoT* Homologs) gene family and expression analysis in response to PGPR bacteria and salinity in *Brassica napus*. International Journal of Molecular Sciences 22: 10666.
Konieczna W, Mierek-Adamska A, Warchoł M, Skrzypek E, Dąbrowska GB (2022) Are metallothioneins involved in the reaction of oat (*Avena sativa* L.) to the osmotic stress? Journal of Agronomy and Crop Science, manuscript in review
Turkan S, Mierek-Adamska A, Głowacka K, Szydłowska-Czerniak A, Rewers M, Jędrzejczyk I, Dąbrowska GB (2022) Localization and expression of *CRSH* transcript, level of calcium ions, and cell cycle activity during *Brassica napus* L. seed development. Industrial Crops and Products, manuscript in review

### 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 physico-chemical 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 the 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