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

Metallothioneins as a potential markers of drought tolerance in oat (*Avena sativa* L.)

1.1. Project goals

The aim of the proposed experiments will be to determine the relationship between water-plant relations, selected physiological, biochemical and genetic parameters, and oat (*Avena sativa* L.) metallothionien (MT) genes under abiotic stress (drought and high temperature stress). The research will be aimed at understanding and characterization of *MT* genes in order to develop molecular markers that will be used to select seed for oat varieties with high drought tolerance. Identification of mechanisms underlying drought and high temperature tolerance in plants as well as the development of reliable tests for selection of plants with high level of tolerance to this stress may contribute to biological progress in oat farming by reducing yield losses and increasing the cultivation area of this species.

1.2. Outline

Metallothioneins are ubiquitous proteins, that are able to bind heavy metal ions in metal-thiolate clusters [1]. MTs play a role in many physiological functions in living organisms, such as maintenance of microelement homeostasis (Zn, Cu), detoxification of toxic ions (Cd), and maintaining redox balance [2,3]. Based on the pattern and number of the metal-binding cysteine residues, pMTs have been classified into 4 types. There are some clear indicators that pMTS may have a role in drought stress tolerance. Increased expression of MTs was observed in response to water-limited conditions in rice [4], cotton [5] and others. Transcriptome analysis of *A. thaliana* [6] and rice [7] showed that the expression of p*MT* genes increases in response to drought stress. In addition, the expression of many MTs is regulated by abscisic acid [8], which plays an important role in the activation of plant mechanisms for adaptation to drought conditions [9]. Thus, MTs may serve as molecular marker that allows the selection of plant varieties having high drought-tolerance and possibly other abiotic stresses.

In Polish agriculture, oat (*Avena sativa* L.) not only provides valuable feed grain, but also has high nutritional value for humans and animals. Currently, with a large share of cereals in plant production, oats should be grown as a phytosanitary plant, because it is not infected by fungal pathogens that cause cereal foot and root rot disease. Oat is a species belonging to plants with low input cultivation. However, compared to other cereals, oat has very highwater requirements, which results in reduced yield during drought.

1.3. Work plan

1. Oat plants growth in a field experiment and plant sample collection at different phenological growth stages listed in the BBCH classification for cereal.
2. Analysis of mRNA levels of *A. sativa MT* type 1-4 genes using qRT-PCR in all mentioned developmental stages.

3. Analysis (in cooperation with The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences) of 90 oat varietes and families in order to identify varieties/families with high and low tolerance to drought stress and high temperature, by analyzing the rate of water loss from leaves, by determining the content of phenolic compounds, and low-molecular and enzymatic antioxidants, by determining the content of fat and protein in seeds.
4. Analysis, in selected oat varieties/families differing in the degree of drought stress tolerance/high temperature, of mRNA levels of *AsMT1-4* genes, in order to determine the suitability of MT genes as molecular markers for selection of seed with a high degree of drought tolerance and/or high temperature.
5. Preparation of transgenic *Arabidopis thaliana* overexpressing oat MT genes, selected in previous stages, by transformation using *Agrobacterium tumefaciens*. Analysis of transgenic plants tolerance to soil drought stress and high temperature by analyzing the rate of water loss from leaves. Analysis of the level of reactive oxygen species as well as enzymatic and low-molecular weight antioxidants in plants grown under control, drought stress and high temperature conditions.
6. Analysis of the biochemical and biophysical properties of oat MTs (in cooperation with the Department of Chemistry of the University of Warwick, Coventry, UK, Dr Claudia A. Blindauer; in accordance with the procedures developed for sorghum MTs, Mierek-Adamska et al., manuscript in preparation) by recombinant protein analysis produced in bacterial cells using mass spectrometry (ESI-MS), UV-Vis spectroscopy and nuclear magnetic resonance (NMR) spectroscopy.

1.4. Literature

1. Koszucka AM, Dąbrowska G. 2006. Plant metallothionein. Advances in Cell Biology 33(2): 285-302.
2. Mierek-Adamska A., Dąbrowska G.B., Blindauer C.A. 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.
3. Mierek-Adamska A., Kotowicz K., Goc A., Boniecka J., Berdychowska J., Dąbrowska G.B. 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.
4. Yang Z, Wu Y, Li Y, Ling HQ, Chu C. 2009. OsMT1a, a type 1 metallothionein, plays a pivotal role in zinc homeostasis and drought tolerance in rice. Plant Molecular Biology 70: 219-229.
5. Xue T, Li X, Zhu W, Wu C, Yang G, Zheng C. 2004. Cotton metallothionein GhMT3a, a reactive oxygen species scavenger, increased tolerance against abiotic stress in transgenic tobacco and yeast. Journal of Experimental Botany 60: 339-349.
6. Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Taji T, Yamaguchi-Shinozaki K, Carninci P, Kawai J, Hayashizaki Y, Shinozaki K. 2002. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. The Plant Journal 31: 279-292.
7. Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. 2003. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. Plant Physiology 133: 1755-1767.
8. Ren Y, Liu Y, Chen H, Li G, Zhang X, Zhao J. 2012. Type 4 metallothionein genes are involved in regulating Zn ion accumulation in late embryo and controlling early seedling growth in Arabidopsis. Plant, Cell & Environment 35: 770-789.

9. Wang W, Vincur B, Altman A. 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218: 1-14.

1.5. Required initial knowledge and skills of the PhD candidate

- ➔ Analytical thinking
- ➔ Eager to learn
- ➔ Open for challenging tasks
- ➔ Understanding of molecular biology techniques
- ➔ Understanding of basic chemistry, physics and biology/biotechnology
- ➔ Ready to go abroad for traineeship or study (Erasmus+ or other program)
- ➔ Eager to work hard

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

- ➔ gain a knowledge about heavy metal metabolism, abiotic plant stress and molecular mechanisms underlying plant stress tolerance
- ➔ acquire novel skills and techniques with a range of biophysical techniques for studying (metallo-)proteins
- ➔ significantly widen present molecular biology and plant science skills
- ➔ develop transferable skills including scientific writing, oral communication, research data management, planning and budgeting
- ➔ improve the creativity thinking