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

1.1 Project title:

The involvement of plant cyclic nucleotide phosphodiesterases in hormonal signaling involving brassinosteroids in *Arabidopsis thaliana*

1.2 Project goals: The proposed project's goal is to thoroughly screen proteins implicated in the brassinosteroid pathway for the presence of phosphodiesterase domains in them, and to analyze, biochemically and functionally, these domains in detail. The next goal will be to create plant mutants lacking/ overexpressing the PDE domain, in order to clarify and place the role of cyclic nucleotides and their cross-talk in the brassinosteroid signaling pathway, involved in plant growth, development and immunity

1.3 Outline: Cyclic nucleotide monophosphates (cNMPs) are essential signaling molecules that initiate a variety of physiological and developmental reactions as well as molecular processes. In particular, 3',5'-cyclic adenosine monophosphate (cAMP) and 3',5'-cyclic guanosine monophosphate (cGMP) have become especially important. The balanced activity of cyclases, which are in charge of synthesis, and cyclic nucleotide phosphodiesterases (PDE), which are enzymes that can break down 3',5'-cNMPs into inactive 5'- and 3'-NMPs, results in the homeostasis of cNMPs.

For a long time, cNMPs in plants have been quite a controversial topic due to their negligible concentrations. Researchers were skeptical about their presence in plant tissues. However, with the development of techniques enabling more and more sensitive determination of compounds at the pico or femto molar level, it was possible to clearly determine the presence of these compounds in plants and determine the processes in which they may participate. Unfortunately, another problem emerged during the research - the lack of evidence for the presence of enzymes responsible for cNMP metabolism in plants. Classical homology approaches excluded the existence of such enzymes in plants. Only in 2003, an approach based on motifs, short functional amino acid sequences, allowed the discovery of the first plant cyclases, which differ significantly from their animal counterparts, because unlike animal enzymes that constitute large, separate units, plant cyclases are components of larger proteins and constitute only small catalytic domains. This approach allowed the discovery of several dozen cyclases and their position in plant signaling pathways, like in auxin. However, research on the discovery and determination of roles for phosphodiesterases, the only enzymes that hydrolyze cNMP, has come to a standstill. It was only in 2021 that the first such enzyme was discovered in higher plants, using a methodological approach analogous to that used for cyclases. A motif was established to identify potential areas of PDE activity in multi-domain proteins. Since then, more and more attention has been paid to discovering these enzymes and determining the specific processes on which their activity may depend.

Our preliminary bioinformatic results indicate the presence of a PDE domain in the BAS1

protein, which is a brassinosteroid catabolic protein and a positive modulator of photomorphogenesis in Arabidopsis. We also speculate that other proteins related to this phytohormone may also have this function. Brassinosteroids play crucial roles in diverse aspects of plant biology, including cell elongation, cell division, root growth, photo-morphogenesis, stomatal and vascular differentiation, seed germination, immunity and reproduction. In previous years, it was shown that the brassinosteroid receptor BRI1 can generate cNMPs. Due to the presence of cyclases in this signaling pathway, we hypothesize that there must be a checkpoint that will turn off cNMP signaling. Therefore, our goal is to find proteins with PDE activity in the brassinosteroid pathway, and then determine where in the pathway their activity is needed, as well as determine the role of cNMPs themselves in this pathway, as they have not been identified at all to date.

1.4 Work plan:

- Motif-based search of *A. thaliana* proteomes for putative PDE domains in proteins associated with brassinosteroid signalling (comprehensive candidates *in silico* analysis; 3D modeling of the structure of selected proteins)
- Obtaining RNA and transcribing it into cDNA encoding fragments of PDE domains. Multiplication of fragments by PCR and cloning them into appropriate expression vectors in the bacterial system
- Isolation and purification of the PDE domain of protein candidates. Conducting full biochemical characterization of recombinant PDE domains.
- Performing point mutagenesis to disable the activity of PDE domains
- Checking the relationship between brassinosteroids and PDE domain - changes in PDE activity and cNMP level determined by using LCMS
- Creation of a plant mutants with the PDE domain knocked/overexpressed and their role in the brassinosteroid pathway especially impact on photomorphogenesis

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

https://doi.org/10.3389/fpls.2017.01704
https://doi.org/10.1038/s41586-022-05369-7;
https://doi.org/10.1016/j.csbj.2021.01.036;
https://doi.org/10.1016/j.plantsci.2022.111493
https://doi.org/10.3389/fpls.2017.01704;
https://doi.org/10.1038/s41586-022-05369-7;
https://doi.org/10.1016/j.csbj.2021.01.036;
https://doi.org/10.1016/j.plantsci.2022.111493;
https://doi.org/10.1073/pnas.96.26.1531;
https://doi.org/10.1186/s12915-016-0340-82-2

1.6 Required initial knowledge and skills of the PhD candidate:

Knowledge of techniques such as molecular cloning (including PCR, DNA electrophoresis, plasmid isolation, restriction enzymes), bacterial transformation (using heat shock, electroporation) and the basics of protein isolation and purification (AKTA system, affinity chromatography) is necessary. An additional advantage will be the ability to operate HPLC and LCMS systems and molecular docking.

1.7 Expected development of the PhD candidate's knowledge and skills:

- The student will improve knowledge and skills in basic molecular biology and genetic engineering techniques and the preparation, purification and analysis of recombinant proteins.
- The student will improve skills in enzyme detection and biochemistry.
- The student will refine skills in analytical chemistry - various chromatography techniques (affinity, ion exchange, high pressure liquid chromatography with tandem mass spectrometry (LC-MS-MS)) for the separation, detection and identification of substances.
- Enhance knowledge and skills in the creation and analysis of genetically modified plants.