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

1.1 Project title: Searching for proteins with hidden adenylate and guanylate cyclase activity domains among the strigolactones (SLs) signalling pathway in *Arabidopsis thaliana*.

1.2 Project goals: The aim of proposed project is a detailed bioinformatical, biochemical and functional analysis of proteins with hidden adenylate (AC) and guanylate cyclase (GC) domains involved in new class of plant hormones – strigolactones (SLs) signalling pathway. The role of cyclases and cyclic nucleotides (cAMP and cGMP) as a products of those proteins activity will be determined in the context of various developmental processes and responses to abiotic stress factors in *Arabidopsis thaliana*.

1.3 Outline: Strigolactones (SLs) have been recently defined as new phytohormones that regulate plant metabolism and, in turn, plant growth and development. This group of phytohormones is derived from carotenoids. It has been implicated in a wide range of physiological functions including regulation of plant architecture (inhibition of bud outgrowth and shoot branching), photomorphogenesis, seed germination, nodulation, and many reactions to abiotic factors (especially drought and salinity).

Like most plant growth regulators (auxins, gibberellins, jasmonates), SL signaling mechanisms are executed by proteosomal degradation. The SL signaling machinery comprises at present: (1) the α/β-fold hydrolase named (At)D14/DAD2/RMS3, (2) the F-box leucine-rich protein MAX2/RMS4/D3, and (3) the SMXL6/D53 a repressor protein, which holds some similarity to class I

Clp ATPase enzymes and belongs to a small family of proteins [SMAX1-like (SMXL)]. MAX2, leucine rich F-box proteins is part of the SKP1-CUL1-F-box-protein (SCF)-type ubiquitin ligase complex, which ubiquitinates target proteins tagging them for proteosomal degradation. Interestingly, components in the auxin, gibberellin, jasmonic acid and salicylic acid perception and signal transduction machinery share similarities with either D14, MAX2 or both. Basing on those similarities and the breaktrough discovery concerning the involvement of cyclic AMP and adenylate cyclases (AC) in auxin signalling pathway, we suppose that cyclic nucleotides (cAMP and cGMP) can also acts as signalling molecules in SLs pathway.

Two years ago, when we thought that the major components in the auxin signaling pathway had been identified, an adenylate cyclase, embedded in the TIR1/AFB receptors, has emerged as an essential component of auxin signaling in gravitropism and root growth inhibition (Qi et al., 2022). The product of the AC - cAMP could also mediate effects of auxin, especially in the fast-growing cells at the apical meristems and in the polarized tip growth of pollen tubes. That paper, together with an increasing number of plant adenylyl and guanylyl cyclases discovered in recent years, and detailed descriptions of AC and GC motifs in complex molecules such as ion channels are now dispelling some persisting doubts about cyclic nucleotide signaling in plants. Taken together, these findings guide the search for intra- and inter-molecular mechanisms enabled by moonlighting ACs and GCs in other complex multi-domain proteins.

Our preliminary bioinformatics data indicate that some of the SLS pathway elements (D14, MAX2, SMXL6) could be good candidates for adenylate or guanylate cyclases, because they contain the AC/GC motif within the molecules. It is important to find out the role of hidden AC/GC cyclases and cyclic nucleotides in strigolactones signalling and explain whether it is universal or uniqe mechanism of cNMP activity in different plant hormones pathways.

1.4 Work plan: The following steps will be taken to implement the above project:

1. bioinformatics analysis of SLs signalling pathway elements (e.g. D14, MAX2, SMXL6) to identification of AC/GC motif within the amino acid sequences;
2. the design of specific primers for studied genes containing putative AC/GC domain, preparing a gene constructs with expression vector, transformation into *E.coli* cells;
3. isolation, purification and biochemical characterization of AC/GC motif containing proteins; the measurement of kinetic parameters of obtained recombinant proteins;
4. obtaining AC or GC knockout proteins by directed point mutagenesis (switch off the key functional amino acids responsible for substrate specificity/binding or co-factor binding), production and purification of mutated recombinant proteins, analysis of involvement of point mutation on biochemical paramenters of all produced proteins;
5. creation of transgenic plants with impaired function of AC and GC motif within studied genes;
6. analysis of the physiological and morphological parameters (e.g. root and shoot growth, germination, bud formation) *of A. thaliana* transgenic plants (compared to wild type plants) growing under optimal and stress conditions (salinity, drought);
7. analysis of the effect of exogenous strigolactones treatment on endogenous level of cyclic nucleotides in *A.thaliana tissues.*

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

1. Saeed W, Naseem S, Ali Z. **Strigolactones Biosynthesis and Their Role in Abiotic Stress Resilience in Plants: A Critical Review.** Front Plant Sci. 2017, 8:1487. doi: 10.3389/fpls.2017.01487.
2. Świeżawska-Boniecka B., Szmidt-Jaworska A. Phytohormones and cyclic nucleotides - Long-awaited couples?. J. Plant Physiol. 2023, 286:1-7, DOI:10.1016/j.jplph.2023.154005
3. Marzec M. **Perception and Signaling of Strigolactones**. Front Plant Sci. 2016, 7:1260. doi: 10.3389/fpls.2016.01260.
4. Wu F, Gao Y, Yang W, Sui N, Zhu J. **Biological Functions of Strigolactones and Their Crosstalk With Other Phytohormones.** Front Plant Sci. 2022, 13:821563. doi: 10.3389/fpls.2022.821563.
5. Qi L, Kwiatkowski M, Chen H, Hoermayer L, Sinclair S, Zou M, Del Genio CI, Kubeš MF, Napier R, Jaworski K, Friml J. **Adenylate cyclase activity of TIR1/AFB auxin receptors in plants**. Nature. 2022 Nov;611(7934):133-138. doi: 10.1038/s41586-022-05369-7.
6. Chen H., Qi L., Minxia Zou M., Lu M., Kwiatkowski M., Jaworski K. & Friml J. **TIR1-produced cAMP as second messenger in transcriptional auxin signalling**, Nature, 2025, 640, 1011-1016, https://doi.org/10.1038/s41586-025-08669-w
7. Al-Younis I, Moosa B, Kwiatkowski M, Jaworski K, Wong A, Gehring C. **Functional Crypto-Adenylate Cyclases Operate in Complex Plant Proteins**. Front Plant Sci. 2021, 12:711749. doi: 10.3389/fpls.2021.711749.
8. Świeżawska B, Duszyn M, Jaworski K, Szmidt-Jaworska A. **Downstream Targets of Cyclic Nucleotides in Plants.** Front Plant Sci. 2018, 9:1428. doi: 10.3389/fpls.2018.01428.
9. Wong, A., Chi, W., Yu, J. *et al.* **Plant adenylate cyclases have come full circle.** Nat. Plants 2023, 9:1389–1397, https://doi.org/10.1038/s41477-023-01486-x
10. Turek I, Irving H. **Moonlighting Proteins Shine New Light on Molecular Signaling Niches.** Int J Mol Sci. 2021;22(3):1367. doi: 10.3390/ijms22031367.

1.6 PhD candidate's knowledge and skills

1. Basic knowledge of molecular biology, biochemistry, plant physiology and bioinformatics.
2. Good knowledge of the biochemistry of proteins (especially receptor proteins and enzymes - structure and function).
3. Knowledge of basic techniques including: molecular biology, recombinant proteins, protein purification and analysis, and statistical methods.
4. Basic knowledge about plant hormone signalling pathways and other signalling molecules (e.g. cyclic nucleotides, reactive oxygen species, calcium ions) in plant cells.

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

1. Improved knowledge and skills in preparing, purifying, and analysing recombinant proteins.
2. Improved analytical chemistry skills- handling and using Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) to detect and identify residual chemical compounds.
3. Improved knowledge and skills in the field of creation and analysis of transgenic plants.