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

Project title: The effect of microorganisms on the functioning of flower abscission zone of yellow lupine under abiotic stress conditions

1.1 Project goals

Examination of the biological activity of microorganisms (selected previously in my scientific team), including bacteria (*Bacillus subtilis*, *B. licheniformis*) and mycorrhizal fungi (*Glommus*) and assessment their usefulness in increasing the resistance of yellow lupine growing under unfavorable conditions of water deficit in the soil.

Checking whether microorganisms can activate defense mechanisms lupine in response to drought stress in flowers' abscission zone (AZ).

Selection of proteins appearing in AZ of lupine flowers under drought conditions and after application of microorganisms that may increase the resistance of lupine to drought stress.

Identification of molecular components of the defense mechanism stimulated by microorganisms in flower AZ lupines cultivated under drought stress.

1.2. Outline

Yellow lupine (*Lupinus luteus* L.), an agriculturally important species, is characterized by a high correlation between drought stress and yielding. We previously shown that water deficit in the soil leads to premature and excessive flower abscission of lupine, which prevents the development of pods with high-protein seeds [1,2,3,4]. The process of flower abscission occurs in specialized layers of cells located at the base of pedicels, which forms the abscission zone (AZ) [4,5]. Our previous investigations showed that drought activates the AZ and causes molecular and biochemical transformations in this tiny piece of tissue, leading to a loosening of the cell wall structure, a break in tissue continuity, and, consequently, the flower separation from maternal plant [2,3,4]. Among the elements coordinating the changes taking place in the AZ are hormones, which, by influencing specific genes expression and hydrolytic enzymes activity, determine the rearrangement of cell wall components [4]. The main site of perception of drought stress is the root. Rhizosphere microorganisms composition changes in root environment in response to drought. These microorganisms can produce in metabolic processes many protective substances that improve plant growth. In agriculture, selected strains of microorganisms appearing in response to drought can be used to increase plant resistance to this type of stress and thus improve yield. Therefore, the aim of the proposed project is examination of the biological activity of microorganism strains (selected previously in my scientific team), bacteria (*Bacillus subtilis* and *B. licheniformis*) and mycorrhizal fungi (*Glomus*) and assessment their usefulness in increasing the resistance of lupine growing under water deficit in the soil. The implementation of the assumed goal will be possible given the use of various research techniques, including analyzes carried out at many levels of organization, both local - changes occurring in the AZ of flowers, the activity of which determines their abscission, and systemic - modifications of lupine physiological processes (biometric

measurements). The results obtained in this multifaceted way will make it possible to determine whether the tested microorganisms inhibit the AZ-activating transformations of lupine flowers cultivated in conditions of soil drought and will enable their detachment on the plant, and thus positively affect its yield.

1.3. Work plan

1. Plant cultivation, microorganisms application, biometric measurements. Cultivation of the *L. luteus* in conditions described by [3]. Determination of the effectiveness of microorganims in the counteracting the effects of drought will be assessed by biometric parameters (plant morphology, water content, chlorophyll level, photosynthetic activity, flowers and pods abortion rate), and histological analysis of the flower AZ.

2. Estimation of the effectiveness of microorganisms in the AZ flowers of lupine based on changes in the redox homeostasis, lipid and hormonal changes in plants subjected to soil drought stress. The control flower AZ and flower AZ from stressed plants simultaneously treated with the microorganisms will be subjected to detailed analyzes: determination of proline, ROS, malondialdehyde, the activity of enzymatic antioxidant system (cooperation with EEZaidin, CSIC, Granada, Spain and SGGW, Warsaw); total fatty acid content and composition, (GC analysis, cooperation with IFB of University of Gdańsk); level (GC-MS analysis, cooperation with ICNT Toruń) and localization of stress phytohormones (immunocytological methods).

3. Analysis of proteins isolated from lupine flowers AZs grown under drought conditions and simultaneously treated with the analyzed microorganisms and from control plants, determination of differences in the pattern of obtained protein maps (electrophoresis, MALDI-TOF, cooperation with ICNT Toruń).

4. qPCR analysis of genes involved in redox, hormonal and lipid metabolism in control flower AZ and AZ stressed plants simultaneously treated with the microorganisms.

1.4. Literature:

[1] Florkiewicz et al. 2020, IJMS, 21(18), 6848
[2] Kućko et al. 2022, IJMS, 21(11), 3815
[3] Wilmowicz et al. 2016, JPP, 206, 49-58.
[4] Wilmowicz et al. 2022, IJMS 23(3), 1680
[5] Tranbarger et al. 2017, Front. Plant. Sci., 8, 196.

1.5. Required initial knowledge and skills of the PhD candidate

- Willing to learn;
- Analytical thinking, the ability to solve scientific questions;
- General knowledge in plant physiology;
- Open for challenging tasks ;
- Knowledge of biochemistry techniques;
- Understanding of molecular techniques;
- Research interests and experience related to the subject of the project are desirable;
- Prior experience in working with plant material is highly desirable;

- Ready to go abroad for traineeship or study (Erasmus+ or other program).

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

- Gained skills: qualitative and quantitative analysis of lipids and plant hormones (isolation, extraction, GC, GC-MS), microscopy techniques (preparation and embedding of material for analysis, staining, immunocytochemical reactions), molecular biology techniques (nucleic acid isolation, PCR, qPCR), biochemical techniques (protein isolation, SDS-PAGE, in-gel assay, Western Blot), interpretation of MALDI-TOF analysis results;
- Acquire the ability to analyze research results, statistical analysis, presenting research results at scientific conferences, participating in the data preparation and writing of the manuscripts, the ability of cooperate with other scientific institutes, especially abroad.