1. PHD PROJECT DESCRIPTION

Project title: Assessment of the usefulness of poly-γ-glutamic acid in increasing the resistance of yellow lupine to drought stress and improving the yield of this species

1.1. Project goals:

- Investigation of the influence of γ-PGA on defense mechanisms triggered by yellow lupine in response to drought stress
- Selection of microorganisms, which appear in the lupine rhizosphere under drought conditions and can increase resistance lupine against the stress
- Identification of protein components of the defense mechanism stimulated by drought stress
- Determination of the ability of microorganisms producing γ-PGA to improve the resistance of yellow lupine to drought

1.2. Outline

Drought is the main abiotic factor that negatively affects not only the growth and development of plants but also limits the yield of economically important species, e.g. yellow lupine. This plant is a high-protein material used in the production of animal feed. Moreover, lupine interacts with bacteria binding atmospheric N_2, which reduces the use of chemical fertilizers [1]. The lupine cultivation area in Poland does not exceed 1% due to its high sensitivity to drought [2]. This stressor is received by root. Drought disturbs the development and functioning of a root, reduces the number of nodules, results in symbiosome degradation, and reduces the amount of fixed N_2, leading to yield decrease [3]. This process is correlated with the changing endogenous level of stress phytohormones [4] and the upregulation of their biosynthesis genes. Furthermore, it is accompanied by modifications of the transcriptional activity of genes responsible for the proper functioning of the nodules [1]. Interestingly, the results of analyzes carried out on *Z. mays, Amaranthus, S. italica, S. bicolour* indicate that soil drought affects the number and composition of rhizosphere microorganisms, which are capable to produce substances that increase plant resistance to unfavorable conditions [7, 8]. One of such molecules - poly-γ-glutamic acid (γ-PGA), is a natural product of the metabolic activity of soil bacteria, mainly from the genus *Bacillus* [9, 10]. It has been pointed that γ-PGA counteracts the negative effects of drought in *B. napus* [11, 12]. The effectiveness of γ-PGA in the inhibition of the harmful effects of water deficiency in any legumes has not been investigated up to date. Therefore, the proposed project aims to find an answer to the question: does γ-PGA can act through the mobilization of the plant defense system and increase the resistance of lupine to drought? Obtained results and the acquired knowledge could be crucial in the future for the production of bioproducts, which improves the tolerance of plants to drought. This project will be carried out in tight cooperation with foreign institutes (Spain, France).

1.3. Work plan

1. Plant cultivation, γ-PGA application, biometric measurements

Cultivation of the *L. luteus* in conditions described by [1].

Optimization of concentration, time and number of applications of synthetic γ-PGA.

Determination of the effectiveness of γ-PGA in counteracting the effects of drought given biometric parameters (plant morphology, water content, chlorophyll level, photosynthetic activity, flowers and pods abortion rate), and histological analysis of the root and nodules.

2. Estimation of the effectiveness of γ-PGA in reducing the sensitivity of lupine to drought by molecular,

biochemical, and chromatography methods
The roots and nodules of control and stressed plants simultaneously treated with the most effective γ-PGA solution (results of task 1) will be subjected to detailed analyzes:
- genetical: expression of soil drought markers;
- biochemical: determination of proline, ROS, malondialdehyde, the activity of enzymatic antioxidant system, level of stress phytohormones;
- proteomic: identification of proteins appearing in the root and root nodules in response to the drought and simultaneous application of γ-PGA, determination of differences in the pattern of obtained protein maps;
- immunocytochemical localization of stress markers in roots and nodules subjected to drought and γ-PGA.

3. Metagenomic analysis
- Isolation of the DNA from the rhizosphere of control and drought + γ-PGA treated plants, sequencing of the variable regions of microorganisms (16S rDNA);
- evaluation of the biodiversity of microorganisms and selection of strains capable to produce γ-PGA;
- Determination of the level of γ-PGA produced by selected microorganisms.

4. Assessment of the influence of rhizosphere bacteria on the sensitivity of lupine to drought
- Checking whether the application of strains of microorganisms (selected based on the results of task 3) will affect the resistance of lupine to drought;
- further determination of parameters described in 2.

1.4. Literature
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1.5. Required initial knowledge and skills of the PhD candidate
- General knowledge in plant physiology;
- Research interests and experience related to the subject of the project are desirable. In particular, experience in the molecular biology methods, knowledge of biochemistry techniques;
- Prior experience in working with plant material is highly desirable;
- Being open to developing bioinformatic skills;

- Analytical thinking, the ability to solve scientific questions;
- Ready to go abroad for 1-6 months traineeship/study;
- Good command of spoken and written English.

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

- Gained skills: qualitative and quantitative analysis of plant hormones (isolation, extraction, 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.