Framework description of research topic

Institute: The Franciszek Górski Institute of Plant Physiology Polish Academy of Sciences

Title: Regulation of secretory trichome metabolism using new genomic techniques (NGT)

The research topic will be implemented as part of the program „Doktorat Wdrożeniowy" organized by the Ministry of Science and Higher Education (Republic of Poland).

Scientific discipline: Biological Sciences

Name of potential supervisor: Dr hab. Ewa Dubas (IPP PAN); e.dubas@ifr-pan.edu.pl

Background information: Plant secretory trichomes are epidermal structures specialized in the production, secretion and storage of a wide range of secondary metabolites. Many of these compounds perform key functions for the plant's vegetative and generative development processes, and are also involved in defence and protective mechanisms. Their diversity and level of biosynthesis are important for the proper development and yield in crop plants. Plant secondary metabolites are also a valuable source of biologically active substances, and are widely used as fragrances, dyes, plant growth agents, pesticides and, also, pharmaceuticals.

The rich and autonomous nature of secretory trichome metabolism makes them an interesting model system useful in biochemical research. These structures are also perceived as "metabolic biofactories" with great biotechnological and agronomic potential. The scientific goal of the project is to modify the secondary metabolism of secretory trichomes in crop plants using the latest genetic engineering tools, molecular and biotechnological techniques. The model plant in the project will be *Cannabis sativa* L. Targeted regulation of the activity of specific biosynthetic pathways in glandular trichomes resulting in secondary metabolite profile changes may have a beneficial effect on the processes related to the growth and development of the plant. Such modifications could increase the resistance to biotic and abiotic stresses, which as a result, could reduce the amount of plant pesticides used during cultivation, increase yield, and increase the content of substances produced (especially those produced in small quantities), and increase the attractiveness of producer plant as a raw material for various industries, e.g. for medicinal purposes. By using new genetic techniques such as CRISPR/Cas9 system for directed mutagenesis, it will be possible to obtain more precise results.

The doctoral scholarship will be financed as part of the research scholarship provided for in the project.

The main questions to be addressed in the project:

- Can changes in the profile of secondary metabolites have a positive impact on the processes related to the growth and development of the plant as well as defence mechanisms? And, as a result, translate into a reduction of pesticides used during cultivation, increased yield, and increase the attractiveness of the producer plant as a raw material for processing in various industries, e.g. for medicinal purposes?

Information on the methods/description of work: Project is interdisciplinary in nature and combines research in the field of plant sciences, analytics, omics technologies (proteomics, metabolomics, mass spectrometry), bioinformatics, genetic engineering and biotechnology. The project focuses on research on Cannabis sativa L., which is a source of many valuable phytochemical compounds with interesting therapeutic potential. The CRISPR/Cas9 system will be used to modify genes involved in specific metabolic pathways. One of the goals of the project is to silence genes involved in cannabinoid pathway, which could result in cannabinoid profile changes. A complementary biotechnological tool which will be used in the project will be microspore embryogenesis (EM). This process allows to generate homozygous lines from F1 hybrid offspring in one vegetation cycle. Isolated microspores also constitute a unique object for genetic transformation. To perform metabolomic and proteomic identification, techniques including chromatographic and mass spectrometry systems will be used: GC/MS/MS, LC/MS/MS and MALDI-TOF/TOF. We also plan to incorporate mRNA sequencing data, RT-qPCR data and analyse gene expression. The results obtained from genetic and „omics" studies conducted on F1-F2 populations will be integrated. In the project several implementations are planned, such as: development of procedure for cannabis glandular trichome modification using CRISPR/Cas9 system, development of procedure for cannabis microspore embryogenesis and double haploids, development of plants with changed metabolic profile, development of procedures for genetic, metabolomic, and proteomic studies for cannabis, etc.

Requirements from the candidate:

- MSc in natural sciences, e.g. biotechnology, biology, chemistry or related fields,
- Interest in science and R&D,

- Willingness to acquire knowledge and develop skills in the field of plant biochemistry, molecular biology, analytics, genetic engineering, biotechnology,
- Good knowledge of English in speech and writing,
- Ability to organize one's own work,
- Creativity, communication skills, conscientiousness.

Place/name of potential collaborator: Project will be realized in consortium with the team of experts from the field of plant science, omic science (IPP PAS, Cracow), and in cooperation with company Małopolska Hodowla Roślin (Cracow).

References:

- Ma, Xingliang et al. (2016) CRISPR/Cas9 Platforms for Genome Editing in Plants: Developments and Applications, Molecular Plant, 9:7, 961 - 974
- Wada, N., Ueta, R., Osakabe, Y. et al. (2020) Precision genome editing in plants: state-of-the-art in CRISPR/Cas9-based genome engineering. BMC Plant Biol 20, 234
- Żur I., Kopeć P.; Surówka E., Dubas, E., et. al. (2021) Impact of Ascorbate—Glutathione Cycle Components on the Effectiveness of Embryogenesis Induction in Isolated Microspore Cultures of Barley and Triticale. Antioxidants, 10, 1254.
- Krzewska, M., Dubas, E., et. al. (2021) Comparative proteomic analysis provides new insights into regulation of microspore embryogenesis induction in winter triticale (× Triticosecale Wittm.) after 5-azacytidine treatment. Scientific Reports, 11, 22215.

Application documents:

- CV
- Motivation letter
- Other documents required by Doctoral School*

The complete set of documents should be sent electronically to Dr. Ewa Dubas to the following address: **e.dubas @ifr-pan.edu.pl** by September 1, 2024.

The interview with the candidates will take place in early September 2024 - details will be provided at a later date.

*) Information and documents for recruitment:
www.botany.pl/index.php/pl/teaching-pl/doctoral-school-pl/admissions-pl