INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS
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Review of doctoral dissertation thesis by Serhii Kozin, “Engineering of tryptophan specialized metabolism in *Arabidopsis thaliana* for plant immunity”.

Tryptophane-derived metabolites are abundant plant secondary metabolites with diverse chemical structures and metabolic activities. Glucosinolates, Camalexins and Brassinins are all tryptophane-derived secondary metabolites produced in Brassicaceae with functions in plant defence. They do, however, have a negative value for plant taste and therefore use it as food. Development of plant varieties with a low level of glucosinolate, like *Brassica napus* (rapeseed), Bronowski variety was indeed a game changer in plant breeding programs designed to produce rapeseed for oil production for human consumption. This, however, comes at the cost of reduced plant defence, which translates into the use of more pesticides. The applicant Serhii Kozin has taken upon himself the challenging task of engineering tryptophan specialised metabolism in the epidermis. The thesis can therefore be considered a step towards plants that produce tryptophan-derived defence metabolites in the epidermis, contributing to plant defence while restricting the presence of Glucosinolates, Camalexins and Brassinins in other tissues.

The thesis follows a standard structure for a PhD thesis and, in my opinion, contains all the necessary parts required from a PhD thesis.

The Introduction is a well-written part that summarises the broad subjects that the thesis touches upon, including plant defence, tryptophan metabolism, as well as spatial aspects of the above-mentioned processes. I am grateful to the author for his comprehensive description of this aspects as for a non-specialist they are really needed to understand the following experimental parts.

The method section gives an in-depth description of the methods used, is well structured and I am sure can serve for the future generation of scientist willing to continue this work.

The result section details the experimental strategy and provides a description of the obtained results. The results are presented with sufficient description for the reader to follow the logic of the experiments. My first concern is the nature of the method used for the engineering of the pathway.

1. The candidate used two different promoters to drive expression of the CYP71A13 and PAD3 enzymes. Why? Would it not be better to use one of the system that allow for use of one promoter for two proteins? The strategy employed by the author risk that the two genes show disconnected expression domains and strength.
2. Also, I am lacking the experimental validation of CYP71A13 and PAD3 transgenes expression pattern. Assuming that the promoters will ensure the desired tissue specificity could be misleading.
3. Why was CYP71A13 selected and not CYP71A12? Given that CYP71A12 is at least partially redundant with CYP71A13 it would be interesting to test CYP71A13 side by side with CYP71A12.
4. If I understand the logical flow of the pathway would a localized PAD3 overexpression in the epidermis of WT plants not be sufficient to result in localised high camalexins levels?

The use of transgenic plants comes with inherent risks related to the methodology of Agro mediated plant transformation. I have several questions related to the way the candidate has analysed the obtained transgenic lines:

1. In terms of the single transgenic the author screened the T1 for expression of the transgene, selected promising lines followed by metabolic analysis at T2 level. For the double transgenic lines, the authors used metabolic profiling both for T1 and T2 analysis. I think that development of stable transgenic lines characterized both for the transgene/s expression level, segregation and metabolic profiles would help the interpretation of the results.

Figure 4.1.7 shows a comparison of camalexins upon infection in single and double transformants. When compared to internal Col-0 infected control one can see that in panel 4.1.3 and 4.1.5 the reduction in the mutants is maintained but the Col-0 is much more

variable. This suggest that the infection assay maybe be irreproducible or that the number of bio repeat sis not sufficient. Can you comment on this?

Why Flagg22 dose not induce camalexins but induce HCX-Hex in the double transformants? Is this not contradictory with the HCX-Hex being the degradation/downstream processing products of camalexins?

The level of intermediates IAOx and IAN are elevated more in double transgene but not in Col-0, however, camalexin level is lower or similar to Col-0 level, any explanation on this? Can this be tested/corroborated by checking expression level of the enzymes involved?

Finally, the aim of the thesis was to engineer a plant with localized tryptophane derived metabolites accumulation in epidermis. I missed the analysis of accumulation of the engineered compounds in the epidermis. Are there methods available for spatial metabolomic analysis of metabolites in question?

Despite my multiple questions I regard this thesis as a clear advancement in our understanding of the tryptophan derived metabolites engineering. The quality of the results, the scope of methods employed, clarity of the result presentation, are of very good standard.

Discussion is well written and summarise the results placing them in context of tryptophane metabolism in plants. I however missed somehow the future outlook, and would therefore like the author to discuss during the defence the potential application, advantages and disadvantages of tryptophane derived metabolites engineering in agriculture.

The dissertation fulfils all requirements indicated in the currently binding regulations (art. 187 Ustawy z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce, tekst jednolity: Dz.U. z 2021 r., poz. 478). In particular, it provides an original solution of a scientific problem, it confirms the general theoretical knowledge of Mr. Serhii Kozin in the discipline of biological sciences and his ability to conduct research. Thus, I put forward a motion to the Scientific Board of the Institute of Bioorganic Chemistry, Polish Academy of Sciences, to admit the PhD candidate, Mr. Serhii Kozin, to further stages of the procedure.

Dr. hab Szymon Swiezewski

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