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Review of the doctoral dissertation by

Ms. PREETI SHAKYA

entitled "A Proteomic Approach to Understanding the Mechanisms of Underlying Interactions between Hypericum perforatum L. and Agrobacterium tumefaciens,"

supervisor Dr. hab. Franklin Gregory, Prof. IGR PAN,

auxiliary supervisor Prof. dr. hab. Robert Malinowski.

Doctoral dissertation carried out at the Institute of Plant Genetics, Polish Academy of Sciences in Poznań

A.tumefaciens is often used as a gene carrier in functional genomics of model plants and some crop species. In the case of a large group of plant species, transformation using this bacterium is difficult due to "plant resistance," a phenomenon whose nature has not yet been fully explained. Achieving desired transformation results in treatment-resistant plants such as *Hypericum perforatum* L. remains difficult due to incompatible interactions between plants and bacteria.

In her doctoral thesis, the doctoral student attempted to determine the factors responsible for the resistance mechanism of Agrobacterium-mediated transformation, using the *H. perforatum-Agrobacterium* interaction as a model.

Assessment of the doctoral thesis layout and formal aspects

Ms. Shakya P.'s doctoral dissertation was prepared as a series of papers, published in 2016, 2019, and 2025. Articles included in the dissertation:

1. Hou W, **Shakya P.**, and Franklin G. (2016). A Perspective on Hypericum perforatum Genetic transformation. Front. Plant Sci. 7:879. doi: 10.3389/fpls.2016.00879

2. Shakya, P., Marslin, G., Siram, K., Beerhues, L., and Franklin, G. (2019). Elicitation as a tool to improve the profiles of high-value secondary metabolites and pharmacological properties of *Hypericum perforatum*. J Pharm Pharmacol, 71:70-82. doi:10.1111/jphp.12743

3. Preeti Shakya, Rajendran K. Selvakesavan, Dawid Perlikowski, Vimala Antonydhason, Leonard Kiirika, Gregory Franklin, (2025). *Agrobacterium tumefaciens*-induced proteomics remodeling and physiological adaptations in *Hypericum perforatum* L,. Plant Stress, Volume 18, doi.org/10.1016/j.stress.2025.100986.

The doctoral dissertation includes one experimental work and two review works. The papers were published in reputable journals from the JCR list with a high impact factor. The total IF value for these papers was approximately 15. the publications included in the doctoral dissertation are co-authored, in two of them the doctoral student is the first author and, in addition, according to the declarations provided, she had a significant contribution to the creation of these publications. The publications included in the Dissertation are very well and carefully prepared, they contain clear graphic documentation, appropriately selected to present a specific type of results.

Individual elements of the dissertation were assessed substantively by competent reviewers cooperating with publishing houses and made available for broad verification by the scientific community.

Ms. Preeti Shakya's doctoral dissertation addresses an important and current topic in the field of plant molecular biology and plant-pathogen interactions. The analysis of proteomic and physiological changes induced by *Agrobacterium tumefaciens* in *Hypericum perforatum* is a valuable contribution to understanding the mechanisms of plant response to genetic transformation and biotic stress.

This topic is particularly relevant in the context of plant biotechnology, where *A. tumefaciens* is commonly used as a transformation vector. Understanding proteomic and adaptive changes may have both practical (optimization of transformation) and cognitive (mechanisms of immunity and stress) importance.

Furthermore, research on plant proteomics in response to *A. tumefaciens* infection is part of the rapidly developing field of systems biology and functional proteomics.

Particularly noteworthy is the holistic approach to the problem studied by combining proteomic analyses, physiological parameters (e.g., oxidative stress, antioxidant enzyme activity), and functional interpretation of proteins.

Methodology Assessment

The research approach is highly commendable. The author utilized:

- antibacterial assays to assess the survival of A. tumefaciens,
- LC–MS/MS proteomic analysis,
- functional analysis of identified proteins,
- measurements of physiological parameters related to the stress response.

The selection of methods is appropriate for the research objectives and demonstrates the doctoral student's high research competence. Proteomic analysis was conducted to identify both proteins associated with the immune response (e.g., PR-10) and enzymes associated with oxidative stress (peroxiredoxin, L-ascorbate peroxidase) and secondary metabolism (PAL, benzophenone synthase, 1,3,7-trihydroxyxanthone synthase).

The correlation of proteomic changes with the observed physiological changes is particularly valuable.

Analysis and Interpretation of Results

The interpretation of the results is logical and consistent. The author demonstrated that contact between *Hypericum perforatum* and *A. tumefaciens* triggers a coordinated defense response, which includes:

- activation of pathogen-related proteins,
- increased activity of enzymes neutralizing oxidative stress,
- modification of cell wall structure,
- activation of secondary metabolic pathways,
- increased energy demand (increased levels of malate dehydrogenase and sucrose synthase).

The conclusions are well grounded in the obtained data and do not exceed the scope of the presented results. The author convincingly demonstrates that a strong plant defense response may be a key factor limiting the effectiveness of genetic transformation.

In addition to the experimental work, the doctoral dissertation also includes two review articles. Although they do not provide new experimental results, they serve an important synthetic and conceptual purpose. They are valuable not only for systematizing knowledge but also for guiding further research directions regarding *Hypericum perforatum*.

Comments and Questions for Discussion

The work is of a high standard, but during the defense, it is worth addressing the following issues:

- Variation in bacterial viability depending on cell density – did the author consider the influence of specific secondary metabolites (e.g., hypericin) secreted into the medium in addition to the identified proteins?

- It would also be useful to relate the results to other plant species to place the results in a broader comparative context.

Final Conclusion

The doctoral dissertation by Ms. **Preeti Shakya**, submitted for review, entitled "**Proteomic approach to understanding the mechanisms underlying interactions between *Hypericum perforatum* L. and *Agrobacterium tumefaciens*,**" meets the requirements of the Act of March 14, 2003, on degrees and titles in the field of art (Journal of Laws No. 65, item 595), as amended on March 18, 2011 (Journal of Laws No. 84, item 455), in conjunction with Article 179, section 1 of the Act of July 3, 2018 (Journal of Laws item 1669). The author has demonstrated scientific maturity, and the presented results regarding the proteomic basis of *H. perforatum* resistance represent significant progress in the fields of plant physiology and biotechnology.
Therefore, I request the Disciplinary Council for Agriculture and Horticulture, IGR in Poznań, to admit Ms. **Preeti Shakya** to the next stages of the doctoral process.

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