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REVIEW

of a dissertation for the acquisition of the educational and scientific degree "Doctor" in the field of higher education: 4. Natural Sciences, Mathematics and Informatics, professional field: 4.3. Biological Sciences, Scientific specialty: Botany, Scientific supervisor: Prof. Dr. Strahil Hristov Berkov

Author of the dissertation: GABRIELA IVANOVA HAIST, full-time PhD student in the research group "Chemoecology and Natural Products", Applied Botany Section, Department "Plant and Fungal Diversity and Resources" at the Institute of Biodiversity and Ecosystem Research – BAS

Title of the dissertation: "STUDY OF THE BIOSYNTHESIS OF GALANTHAMINE IN HIPPEASTRUM PAPILIO (RAVENNA) VAN SCHEEPEN"

Reviewer: Assoc. Prof. Dr. Ivayla Nedyalkova Dincheva, AgroBioInstitute – Sofia, Agricultural Academy; Field of Higher Education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, scientific specialty "Biologically Active Substances", appointed as a member of the scientific jury by Order No. 42/02.05.2025 of the Director of IBER – BAS

1. Brief presentation of the candidate.

PhD student Gabriela Ivanova Haist was born on 25.08.1987. In the period 10/2006–07/2010, she was a student at the Faculty of Biology, Sofia University "St. Kliment Ohridski", where she obtained a Bachelor's degree in Molecular Biology in 2010. In the period 10/2011–06/2013, she studied at the same university and received a Master's in Molecular Biology – Biochemistry in 2013. She has conducted full-time PhD studies in the scientific specialty

01.06.03 “Botany” during the period 10/2019–present at the Department “Plant and Fungal Diversity and Resources” of IBER–BAS. She has been granted the right to defend her dissertation, which was approved for submission by the Collegium of the Department, according to the Protocol No. 26/14.04.2025.

Since 10/2019, she has also held a position as an assistant at the Institute of Biodiversity and Ecosystem Research – BAS, including during her doctoral studies. She has participated in two scientific forums in Bulgaria and abroad. She is the author of four scientific publications in peer-reviewed journals indexed in global scientific databases (Web of Science and Scopus), three of which are in a quartile Q1 and one in Q3. A total of six citations have been noted. The dissertation was developed within the framework of project КП-06 ПН76-24, funded by the National Science Fund.

2. Submitted documents for the procedure

The materials submitted by PhD student Gabriela Haist comply with the regulations for the conditions and procedures for acquiring academic degrees and holding academic positions at the Institute of Biodiversity and Ecosystem Research – BAS. The following mandatory documents are included: Inventory of documents; Dissertation; Abstract; Statement of the contributions of the dissertation; List of publications on the dissertation topic and copies of the publications themselves; List of participations in scientific forums with presentations and posters reflecting the research in the dissertation, with copies of printed abstracts; List of citations of the works on the dissertation topic; Scientific CV with a complete list of publications attached; Master’s diploma – copy certified by the Scientific Secretary of IBER–BAS; Bachelor’s diploma – copy certified by the Scientific Secretary of IBER–

BAS; Protocol from the meeting that directed the dissertation for defense; Transcript of the completed coursework and obtained credits, certified by the Scientific Secretary of IBER–BAS; English summary of the dissertation, containing the title and main points of the work.

3. General characteristics of the dissertation – volume and structure

The structure of the dissertation is standard and includes all the required components as specified in the Regulations for acquiring academic degrees and holding academic positions at IBER–BAS. The dissertation is presented in a total volume of 93 pages and includes the following sections: Title page, which contains the name of the institution, the candidate's name, the title of the dissertation, the academic degree, scientific specialty, and the supervisor's name, as well as the year of completion of the dissertation; Acknowledgements (1 page); List of abbreviations (1 page); Table of contents (2 pages); Introduction (1 page); Literature review (16 pages); Aims and objectives (1 page); Materials, methods, results, and discussion – presented in the form of 4 publications (43 pages); Conclusion (1 page); Main findings (2 pages); Declaration of originality and authenticity (1 page);
Contributions (1 page); References (20 pages); List of scientific publications with full bibliographic details and citations (2 pages). The literature review includes 2 figures, while the four attached publications contain a total of 24 figures and 5 tables.

4. Relevance of the topic

The topic of the dissertation is extremely relevant in light of the growing need for effective treatments for Alzheimer's disease, which affects over 55 million people worldwide. Galantamine is one of the few approved drugs for symptomatic therapy of this disease, but its natural sources are limited and often endangered. This creates a significant need for sustainable and economically viable alternatives for the industrial production of the active substance.

Hippeastrum papilio emerges as a promising new source of galantamine, as it contains high amounts of the alkaloid and is characterized by rapid growth, lack of dormancy, and substantial biomass. However, there is a lack of in-depth data on the distribution of galantamine in different plant organs, as well as on the dynamics of its accumulation. The factors influencing its biosynthesis, such as ploidy, plant age, nutrient medium composition, and application of elicitors, are not sufficiently studied. Understanding these aspects is key to developing an effective agrotechnological approach. This would allow for the sustainable cultivation of H. papilio and a reliable yield of galantamine.

The research in the present dissertation is fully aligned with the global trend toward the use of environmentally friendly, renewable resources for the production of pharmaceutical substances. For these reasons, the topic is of high scientific, medical, and economic significance.

5. Literature awareness and theoretical preparation of the candidate

Gabriela Haist demonstrates a high level of literature awareness and excellent theoretical preparation, which is reflected in the content and argumentation of the dissertation. A thorough and up-to-date literature review has been carried out on the family Amaryllidaceae and the subfamily Amaryllidoideae, focusing on the genus Hippeastrum and the species Hippeastrum papilio. The groups of Amaryllidaceae alkaloids are presented in detail, with an emphasis on galantamine- its biosynthesis, biological activity, and significance as a medication for Alzheimer’s disease.

The PhD student demonstrates a deep understanding of the mechanisms of secondary metabolism in plants, tracing the role of key enzymes such as PAL, TYDC, NBS, NR, CYP96T, their expression, and localization in various plant

organs. Moreover, good knowledge is shown of the molecular-genetic, physiological, and biochemical aspects of the studied processes.

Modern concepts and techniques are presented, such as polyploidization, elicitation, hydroponic cultivation, and agronomic optimization of conditions for the accumulation of bioactive compounds. The candidate successfully applies an interdisciplinary approach, connecting botany, phytochemistry, and biotechnology, and takes an analytical approach to solving applied scientific problems – for example, identifying sustainable plant sources of galantamine.

The cited literature is current and covers leading research up to the year 2024, which indicates excellent orientation in the scientific field. A total of 173 sources of information are cited in the dissertation, all written in Latin script.

All of this testifies not only to theoretical and methodological maturity but also to the candidate's potential for independent scientific work at a high level.

6. Consistency of the chosen research methodology with the stated aim and objectives of the dissertation

The methodology used in Gabriela Haist's dissertation is logically structured and fully corresponds to the stated aim and the formulated research tasks. In the investigation of the influence of ploidy, karyological and morphological analyses were applied, supplemented by gas chromatography-mass–mass spectrometry (GC-MS) to assess the chemical composition. This combined approach provides a comprehensive characterization of diploid and autotetraploid plants.

To analyze the organ- and tissue-specific biosynthesis of galantamine, methods such as metabolite profiling, histochemical staining, and microscopy

were used, allowing precise localization and quantitative measurement of the alkaloids in various plant organs.

The influence of macroelements and plant age was studied through hydroponic cultivation under controlled conditions. Both galantamine accumulation and plant growth and biomass over time were measured, allowing for monitoring of developmental dynamics.

In the fourth task, the effect of salicylic acid as an elicitor was assessed by applying different concentrations and treatment durations, followed by GC-MS analysis to determine quantitative changes in galantamine content.

The techniques used are scientifically grounded, modern, and suitable for achieving the objectives of the study. Validated methods were applied, including statistical analyses (ANOVA, PCA), which contribute to the reliability and reproducibility of the results. The methodology demonstrates a high level of professionalism and excellent alignment between theoretical foundations and experimental design.

7. Significance and Convincingness of the Obtained Results, Interpretations, and Conclusions

Gabriela Haist has submitted four scientific publications that present the research work carried out about the goals and objectives of the dissertation. Based on Article 9 of the “Regulations on the Conditions and Procedures for Obtaining Scientific Degrees and for Occupying Academic Positions at the Institute of Biodiversity and Ecosystem Research at the Bulgarian Academy of Sciences,” the chapters “Materials and Methods,” “Results,” and “Discussion” are replaced by copies of the publications related to the dissertation topic.

The results obtained in the dissertation are significant, clearly presented, and scientifically convincing. They cover a wide range of factors influencing the

biosynthesis of galantamine in Hippeastrum papilio and address specific gaps in the existing scientific literature. The interpretations are logically consistent, supported by quantitative data, and current scientific reasoning.

Regarding ploidy, it has been convincingly demonstrated that autotetraploid plants have significantly higher galantamine content compared to diploids, despite reduced biomass. This is an important conclusion with practical significance for the selection of high-yielding lines.

The organ- and tissue-specific localization of alkaloids has been demonstrated through histochemical and metabolic analyses, providing a clear understanding of the sites of biosynthesis and accumulation. This provides a basis for more precise targeting of plant biomass for extraction.

The dynamics of galantamine accumulation and the influence of macroelements and plant age have been systematically tracked. It was established that younger plants, under specific fertilization conditions, have better productivity, which has direct applications in agro-technological practice.

8. Evaluation of the Scientific Contributions

The main scientific contributions of Gabriela Haist's dissertation are based on clearly formulated and well-founded conclusions that reflect the significance and originality of the research conducted. The work reveals new aspects in the study of Hippeastrum papilio as a potential source of galantamine, a bioactive compound with proven application in the symptomatic treatment of Alzheimer's disease.

It has been demonstrated that autotetraploid plants of H. papilio contain significantly higher levels of galantamine compared to diploids, despite reduced biomass. This represents an important contribution to the selection of high-yielding forms and justifies the benefits of polyploidization as a method for improving the metabolic profile of medicinal plants.

It was established that galantamine synthesis occurs predominantly in the leaves, while its accumulation is strongest in the bulbs. This knowledge, acquired through histochemical and metabolic analyses, is of practical importance in processing plant raw material and directs attention to the use of specific organs for alkaloid extraction.

Research under hydroponic conditions showed that plant age, as well as the concentrations of macroelements (nitrogen, potassium, calcium), directly influence galantamine accumulation and biomass. The data indicate that the highest galantamine content is observed in 2-year-old plants under certain nutritional conditions, allowing for optimization of cultivation practices.

An important contribution is the demonstrated stimulatory effect of salicylic acid as an elicitor of galantamine biosynthesis — under appropriate concentration and duration, an increase of up to 19% in the active substance content was recorded. This is the first study of its kind on H. papilio and opens

the way for using elicitors as a means of controlled enhancement of secondary metabolite production.

Based on these results, the dissertation proposes an integrated model for optimizing the cultivation and industrial use of Hippeastrum papilio as an alternative, sustainable, and economically viable source of galantamine. Existing knowledge on the biosynthetic pathways of Amaryllidaceae alkaloids and their regulation by internal and external factors has been expanded, enriching theory and supporting practice in plant biotechnology and pharmaceutical science.

9. Quality of the Scientific Works Reflecting the Dissertation Research

The scientific works included in Gabriela Haist's dissertation stand out for their high quality. They cover various aspects of the research, from genetic and morphological to biochemical and agro-technological. Together, they build a consistent and in-depth scientific concept. The doctoral candidate is the first author of all four publications. All are published in reputable international scientific journals, including Planta (Springer Nature), Agronomy (MDPI), and Journal of Plant Physiology (Elsevier), all ranked in the top Q1 quartile, which speaks to the high level of the presented results. One publication was issued in the Proceedings of the Bulgarian Academy of Sciences, a recognized national scientific forum.

Content-wise, the works are well-structured, with clear experimental design, application of modern methods (GC-MS, histochemistry, cytology, PCA analysis), and well-justified results and conclusions. The statistical processing is correct and reliable, and the discussions demonstrate a profound understanding of the subject matter and skillful comparison with global literature.

The publications not only complement but constitute the core structure of the dissertation, with each addressing a specific research task. This gives the work coherence and proves the candidate’s full capability to conduct independent scientific activity at a high level.

The dissertation results have also been presented at two international scientific forums.

10. Critical Remarks on the Dissertation

Gabriela Haist's dissertation is written at a high scientific and linguistic level, characterized by precise terminology, clearly structured arguments, and an academic style of presentation. Nevertheless, a comprehensive review reveals some minor grammatical, stylistic, and linguistic inaccuracies that do not affect the content but could be corrected to improve readability and linguistic precision.

Some sections contain overly long sentences with multiple subordinate clauses, which complicates comprehension and leads to stylistic heaviness. Minor inaccuracies were noted — for example, inconsistencies in the spelling of Latin names (e.g., "Ravena" instead of the correct "Ravenna").

It should be noted that, although Hippeastrum papilio is proposed as an alternative cultivated source of galantamine, a more in-depth economic evaluation of profitability compared to already established plants, such as Narcissus pseudonarcissus, is lacking. In this context, it would be valuable in the future to add a comparative analysis of production costs and yields.

Although tetraploidization leads to increased galantamine content, it is recommended to examine more deeply the effect of reduced biomass in auto tetraploids and the potential agronomic challenges associated with their cultivation.

On the other hand, experiments were mainly conducted under controlled conditions — in vitro and hydroponic systems. The lack of data from real field conditions leaves open the question of the applicability of the results in a broader agroecological context.

11. Conclusion

Having thoroughly reviewed the submitted materials for defense, I am firmly convinced that the obtained results, formulated scientific contributions, and drawn conclusions represent an independent and personal contribution of the doctoral candidate. Gabriela Haist is the first author of all four publications related to the dissertation topic, which further confirms her leading role in the scientific work.

MOTIVATED CONCLUSION:

Based on the variety of methods successfully used by the candidate, the well-planned and executed experiments, the significant scientific results achieved, and the well-argued summaries and conclusions, I consider the presented dissertation fully meets the requirements outlined in the Law for Development of Academic Staff in the Republic of Bulgaria (ZRASRB), its implementing regulations, as well as the Regulations on the Conditions and Procedures for Obtaining Scientific Degrees and Academic Positions at IBER–BAS.

On this basis, I give a positive evaluation of the dissertation and allow myself to recommend that the esteemed Scientific Jury also vote positively to award Gabriela Ivanova Haist the educational and scientific degree of “Doctor” in the scientific specialty “Botany.”

Date: 16.06.2025 REVIEWER: .....

Sofia (Assoc. Prof. Dr. Ivayla Dincheva)