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STATEMENT

by Prof. Dr. Marina Ivanova Stanilova,

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences (IBER-BAS), member of the Scientific Jury, according to Order No. 42/02.05.2025 of the Director of IBER

on the dissertation thesis for the acquisition of the educational and scientific degree "Doctor" of Gabriela Ivanova Heist, a full-time doctoral student in the field of higher education: 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, scientific specialty "Botany", at the research group "Chemoecology and Natural Products", section "Applied Botany", department "Plant and Fungal Diversity and Resources", IBER-BAS, with scientific supervisor: Prof. Dr. Strahil Berkov and topic of the dissertation thesis: "Study of the biosynthesis of galanthamine in *Hippeastrum papilio* (Ravenna) van Scheepen"

The topic of Gabriela Heist's dissertation is very relevant given the importance of the alkaloid galanthamine in the symptomatic treatment of dementia, including Alzheimer's syndrome, which affects tens of millions of people worldwide. *Hippeastrum papilio* is characterized by a number of advantages over the plant species currently used in the production of galanthamine: lack of a dormant period, larger plant size, i.e. more biomass, as well as a higher content of the alkaloid. However, in order to be established as a high-quality raw material, it is necessary to clarify some important characteristics: the distribution of galanthamine in plant organs and tissues, the dynamics of its accumulation in them, and also the influence of various factors such as genotype, ploidy, age, macroelements and elicitors on the process of biosynthesis and localization. The aim of the dissertation is to clarify the influence of these factors on the biosynthesis and accumulation of biomass and galanthamine in various organs and tissues. It is clearly formulated and 4 tasks have been defined to achieve it, each of which is discussed in detail in the publications on the topic.

The doctoral student has chosen to present her dissertation in the form of bound scientific publications, adhering to the structure according to Art. 7 of the Regulations on the conditions and procedure for acquiring scientific degrees and for occupying academic positions at IBER. The literature review is well structured, meets the set goals and objectives and shows a very good knowledge of the literature on the topic. The results of the research, their interpretation and the conclusions drawn are presented in 4 publications. It is noteworthy that Gabriela Heist is the first author of all publications on the topic of the dissertation, and in one of them she is also the corresponding author. All publications are in prestigious international scientific journals with impact factor and impact rank according to Web of Science and Scopus and with quartiles, with 3 of the publications being in journals with Q1 (Planta, J. Plant Physiol.

and Agronomy) and one in a journal with Q3 (Compt. Rend. Acad. Bulg. Sci.), one published in 2023, two in 2024 and one in 2025. The abstract also meets the requirements for volume and structure. The lack of plagiarism is evident from the similarity report.

The methods used are suitable for the tasks set. The metabolic analysis was performed with GC/MS, and for the quantitative determination of the main alkaloids galanthamine and hemanthamine, calibration curves were prepared using several concentrations and codeine as an internal standard. The results of the study are convincing, obtained after preliminary planning of large-scale experiments with simultaneous monitoring of the impact of several factors. The data were statistically processed using Two-sample Student's t test, Minitab Statistical Software v. 19, one-way ANOVA and the software program multiple linear regression respectively to study the impact of one and more factors, followed by Tukey test, at a significance level of $p < 0.05$. The results are presented in well understandable tables and very well illustrated with figures (photographs, including very good quality microscopic ones, graphs, schemes and diagrams). The interpretation of the results is adequate, and the conclusions are clearly formulated.

The significance of the results obtained is related both to the establishment of new scientific facts and to the possibility of their application in the future use of *H. papilio* as a raw material source of valuable alkaloids. For example, of practical interest is the established significant decrease in biomass, atypical for autotetraploid plants, compared to diploids (both in terms of leaf mass and number of leaves, and bulb diameter), which cannot be compensated by the increased biosynthesis of galanthamine and hemanthamine. Of essential importance is the comparative analysis of growth and accumulation of alkaloids in plant organs and tissues of plants of different ages, as well as the establishment of the most favorable concentration of nutrient solutions for the biosynthesis of alkaloids. It was noted that the increased concentration of nitrogen, potassium and calcium did not positively affect either the accumulation of biomass or the biosynthesis of galanthamine and hemanthamine in hydroponic cultivation of *H. papilio*. Based on histological and metabolic studies of plant organs, it was assumed that alkaloids are synthesized in the aboveground parts of plants, and their presence in the phloem indicates that they are transported to the bulb, with their content in the central part of the bulbs being several times higher than in the other plant parts. The dynamics of galanthamine and biomass accumulation in the roots, bulbs and leaves during the vegetative period was also revealed. A scientific result with a perspective for practice is the established stimulation of alkaloid biosynthesis upon elicitation with salicylic acid, with

galanthamine statistically showing a 19% increase in content compared to control plants, and a similar trend was noted for hemanthamine. It is worth checking whether higher concentrations of salicylic acid would lead to a further increase in alkaloid biosynthesis, since the maximum content was noted at the highest concentration of the elicitor tested.

Seven contributions have been formulated, clearly distinguishable from each other, which I fully accept. It is striking that all contributions are of an original nature and meet the set goals and objectives of the dissertation work. For the first time: the chromosomal morphology of diploid plants *H. papilio* has been studied, and the formula of its karyotype has been established; comparative analyses of the primary and secondary metabolites in diploid and autotetraploid plants have been made; the tissue localization of the alkaloids has been clarified through histological studies; the content of galanthamine and hemanthamine in the all plant organs of *H. papilio* has been determined, as well as their metabolic profile; the influence of different concentrations of the nutrient solution on the accumulation of galanthamine and hemanthamine in roots, bulbs and leaves of plants of three ages has been studied; an analysis of the productivity (expressed in g/unit area) of hydroponically cultivated *H. papilio* as a source of galanthamine has been made; elicitation has been tested in hydroponic cultures of amaryllis plants to stimulate alkaloid biosynthesis.

In conclusion, in my opinion, the dissertation meets all the requirements. The research was conducted according to a well-thought-out preliminary plan, and its volume is significant. The results are correctly presented in tables, graphs and photographs and adequately interpreted. All contributions are of an original nature and have both scientific and scientific-applied value. Given all this, I strongly recommend that the members of the esteemed Scientific Jury evaluate and award Gabriela Ivanova Heist the ESD "Doctor" in scientific field 4.3. "Biological Sciences", scientific specialty "Botany".

Sofia, 18.06.2025 Signature: .....

(Prof. M. Stanilova)