STATEMENT

by **Assoc. Prof. Galina Dinkova Stoyancheva, PhD**
from The Stefan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences
regarding dissertation work for the acquisition of the educational and scientific degree
"Doctor"
professional field: **5.11 Biotechnology,**
scientific specialty: **Technology of biologically active substances**

Author of the dissertation: **Gergana Spasova Krasteva**, full-time doctoral student at The Stefan Angeloff Institute of Microbiology – BAS
Dissertation topic: **Plant bioengineered systems from *Gardenia jasmine* Ellis for obtaining active ingredients**
Scientific supervisor: **Assoc. Prof. Vasil Georgiev Georgiev**, **PhD,** Stefan Angeloff Institute of Microbiology – BAS

ABOUT THE PROCEDURE:
Full-time doctoral student Gergana Spasova Krasteva is directed to an official defense by Decision of the Scientific Council of the Institute of Microbiology - BAS (Protocol No. 10/30.09.2025). According to the Order of the Director of the Institute of Microbiology - BAS (No. I-143/1.10.2025) I am included in the scientific jury for the defense as an internal member, in accordance with The Act on the development of academic staff in the Republic of Bulgaria and art. 29, para. 3 of the Regulations on the conditions and procedure for acquiring scientific degrees in The Institute of Microbiology – BAS.

EVALUATION OF THE DISSERTATION:
Relevance and significance of the scientific topic. The dissertation examines an extremely topical scientific issue related to the development of sustainable bioengineering systems for obtaining biologically active substances of plant origin. In the context of modern environmental challenges and limited natural resources, the creation of effective *in vitro* technologies for the production of metabolites with high added value is a priority direction in biotechnology. Research on the species *Gardenia jasmine* Ellis are of high scientific and applied value, as the plant is rich in iridoid and phenolic compounds with proven antioxidant and pharmacological activity. The development of model plant systems in which the biosynthesis of target metabolites can be controlled has the potential for implementation in the pharmaceutical, cosmetic and food industries. The work is aimed at an in-depth study of the factors influencing the genetic stability and metabolic profile of various *in vitro* systems, which represents a significant contribution to modern trends in plant biotechnology.

The candidate's literary awareness and theoretical preparation. The literature review is targeted and well-structured, addressing the following aspects: botanical features of the species, geographical distribution, phytochemical composition, antioxidant activity, types of plant *in vitro* systems and molecular aspects of genetic stability. The author has presented current sources from the international scientific literature, including publications from the last decade. The analysis of existing research clearly outlines the unresolved problems and argues the need for conducting the present study.

The overview is concise, specific and clear, illustrated with 6 tables and 1 figure and, together with the methods and discussion of the results, is based on over 200 literary sources, approximately 25% of which have been published in the last 5 years. The excellent awareness and theoretical preparation of the doctoral student is impressive.

The main goal of the dissertation is the development and optimization of plant *in vitro* systems from *Gardenia jasmine* Ellis for obtaining biologically active substances while maintaining genetic stability and high antioxidant activity of the biomass.

The tasks set are clearly formulated and logically related to the purpose of the study. They include: creating different types of in vitro cultures; optimizing the nutrient medium and cultivation conditions; studying the dynamics of growth and accumulation of secondary metabolites; determining antioxidant activity by various methods; and assessing genetic stability by molecular markers.

Analysis of methodological approaches to achieve the goal of the dissertation . The applied methods are well selected and suitable for solving the experimental tasks set. Classical and modern biotechnological approaches were used - callus, suspension and germination culture, combined with biochemical and analytical methods, such as High Performance Liquid Chromatography and Gas Chromatography to determine metabolic profiles. Various spectrophotometric tests (DPPH, FRAP, CUPRAC, TEAC) were also applied to determine antioxidant activity. Genetic stability was studied using SSR markers, which gives high reliability to the results. The experimental design used, including two-factor optimization and statistical data processing, guarantees objectivity and reproducibility of the results. The rich set of classical and modern methods mastered by the dissertation candidate during her doctoral studies will also find application in her future scientific research.

Evaluation of the achieved results and contributions of the dissertation work. All the tasks set have been consistently and successfully solved. In the dissertation work, several types of in vitro systems have been obtained and compared - callus, suspension and germination culture. A thorough analysis of their growth dynamics, biomass accumulation and synthesis of secondary metabolites was carried out. The results show that the composition of the nutrient medium, the concentration of growth regulators and the aeration conditions have a significant impact on the productivity of the cultures. Conditions leading to maximum accumulation of phenolic compounds and high antioxidant activity were established. By High-performance liquid chromatography and gas chromatography-mass spectrometry main groups of compounds have been identified – phenolic acids, flavonoids, iridoids glycosides and triterpenoids, and a relationship was made between their concentration and biological activity. Parameters for media optimization were determined, leading to increased synthesis of target metabolites and better stability of the cultures.

A particularly significant contribution is the study of somaclonal variability during prolonged cultivation and when changing the composition of the nutrient medium. SSR analysis shows a high degree of genetic stability in certain combinations of phytohormones and medium composition. This is of great importance for the practical application of the created systems in the industrial production of biologically active substances. The dissertation presents a complex approach for combining biochemical, physiological and molecular analysis, which distinguishes it with an interdisciplinary character. The results obtained contribute to a better understanding of the factors determining the metabolic activity of plant crops *in vitro* , and can be used as a model for other economically important species. Publications on the topic confirm the scientific maturity and consistency of the author in the research work.

The results are illustrated with 26 tables and 34 figures. The most significant results are included in four publications, two of which have an impact factor, and have been reported at three scientific forums. **The abstract** fully corresponds to what is stated in the dissertation.

The conclusions are correctly formulated, and the contributions reflect the most significant results of the dissertation, namely:

1. Differentiated (germination) and dedifferentiated (callus and cell suspension) *in vitro* cultures of *Gardenia jasminoides* Ellis were obtained.
2. The differences in the metabolic profile and antioxidant activity of the obtained *in vitro* systems and *Gardenia jasminoides* Ellis were studied for the first time.
3. The genetic stability of a differentiated and dedifferentiated culture was studied for the first time during the study of the dynamics of development as well as when changing the main components of the nutrient medium.
4. The genetic stability of differentiated and dedifferentiated *in vitro* systems compared to that of the parent plant was studied for the first time.
5. For the first time, the relationships between changes in the metabolic profile and genetic variability during the cultivation of differentiated and dedifferentiated *in vitro* cultures of gardenia have been proven.

Questions and recommendations. The dissertation is well structured and methodologically correct. However, some recommendations can be made for future research. In some parts, clearer graphical representations of the relationships between growth parameters and antioxidant activity are missing. It is recommended that the results be linked in more detail to the potential applications of the obtained extracts in specific industries. Technical notes: there are several repeated sources in the list of cited literature**,** it would be good if all citations were in the same format and font. Nevertheless, the presentation is scientifically sound, well-reasoned and demonstrates in-depth knowledge in the field of plant biotechnology.

I have a question for the doctoral student: How can the fact that the differentiated seedling culture produces more phenolic compounds than the parent plant be explained?

CONCLUSION

The dissertation represents a comprehensive, original and in-depth scientific study with high theoretical and practical value. The results achieved are convincing, well-argued and supported by experimental data. The work meets the requirements for a dissertation for the award of the educational and scientific degree "Doctor". This gives me grounds for an overall high assessment of the dissertation, on the basis of which I confidently propose to the esteemed scientific jury to award **Gergana Spasova Krasteva the educational and scientific degree "DOCTOR"** in the professional field: **5.11 Biotechnology,** scientific specialty: **Technology of biologically active substances**.

November 13, 2025 (Assoc. Prof. Galina Stoyancheva, PhD)