R E V I E W

Of the Ph.D. Thesis of Eng. Gergana Spasova Krasteva

for the acquisition of the educational and scientific degree "Doctor"

In the Field of Higher Education 5. Technical Sciences, Professional Direction 5.11. Biotechnologies, Scientific Specialty "Technology of Biologically Active Substances"

Topic: "Plant Bioengineering Systems from Gardenia jasminoides Ellis for Obtaining Active Ingredients"

Reviewer: Prof. Dr. Kaloyan Petrov

Head of the Laboratory "Biochemical Engineering", Institute of Chemical Engineering - BAS

Member of the Scientific Jury, determined by order I-143/01.10.2025

1. Biographical data of the PhD student

Gergana Krasteva was born in 1995. She earned both a bachelor's and a master's degree in 'Analysis and Control of Food Products' from the University of Food Technologies in Plovdiv. Her professional journey began at the Stefan Angelov Institute of Microbiology, Bulgarian Academy of Sciences, Cellular Biosystems Laboratory, where she has worked as a technologist since 2018 and has also been a doctoral student since then. In this role, she actively participated in research focused on isolating biologically active substances from plant in vitro systems and exploring their applications in medicine, the cosmetic industry, and the food sector. She also has experience working in analytical laboratories, including Coca-Cola Bulgaria, where she gained expertise in quality management systems and analytical procedures. She is fluent in both English and German.

2. Relevance of the topic of the dissertation

The dissertation topic is important because plant cell cultures have emerged as the most promising platform for the sustainable production of valuable biologically active substances over the past decade. This is due to their ability to be synthesized in vitro, regardless of climate or location. Gardenia jasminoides is a highly valuable species for the pharmaceutical, cosmetic, and food industries, as it contains numerous secondary metabolites with distinct antioxidant, anti-inflammatory, and neuroprotective properties, including crocin, crocetin, genipin, and geniposide. Interest in these compounds is increasing because they are used in therapeutic products for human medicine (such as neuroprotective, antidepressant, antihypertensive, and anti-inflammatory agents), as well as natural dyes and bioactive additives. Meanwhile, there is a lack of detailed studies on large-scale in vitro production of these metabolites and how cultivation factors affect their genetic stability, making this research even more significant. Developing bioengineered systems that allow controlled production of bioactive compounds aligns with the global movement toward green technologies, sustainable production, and reducing pressure on natural resources.

3. Data on the PhD Thesis

The dissertation is 170 pages long and follows a traditional structure, comprising a comprehensive literature review (45 pages), an introduction to the aim and objectives, a section on "Materials and Methods" (12 pages), and a section on "Results and Discussion" (74 pages).

The final section includes a conclusion, summaries, and a section on contributions. The sources cited number around 257, with most from the last ten years, and over 25% from the past five years. The layout features a substantial number of tables and figures (35 and 33, respectively), which illustrate the experimental data, optimization models, biochemical analyses, and the results of molecular studies.

The literature review is one of the most extensive parts of the dissertation. The doctoral student is well informed about the current knowledge regarding Gardenia jasminoides and its biotechnological potential. The candidate describes the botanical features of the species, its geographical distribution, and its rich history of use across different cultures, including traditional Chinese and Japanese medicine. The review considers the economic, pharmacological, and biotechnological significance of the plant. It discusses the main groups of natural substances found in G. jasminoides: flavonoids, iridoid glycosides, phenolic acids, saffron glycosides, and triterpenoids. Among these, crocin, crocetin, genipin, and geniposide are examined in detail, as they are considered key to the plant's biological activity. The section on antioxidant properties is particularly thorough. The author clearly explains the connection between phenolic components and antioxidant biological activity, citing experimental data that support this relationship. Various methods used to assess antioxidant capacity are purposefully reviewed, including their mechanisms and practical applications. An important part of the review also focuses on plant in vitro systems as promising models for producing biologically active metabolites of value. The influence of cultivation conditions—such as light, nutrient medium, and growth regulators—on the quantity and types of secondary metabolites is emphasized. This analysis highlights the growing scientific interest in somaclonal variability, with the author providing detailed descriptions of various groups of DNA markers (SSR, RAPD, AFLP, and others) and their roles in monitoring genetic stability in in vitro cultures.

The volume, systematicity, and precision of the literature review are clear evidence of the doctoral student's scientific maturity and knowledge of the topic.

The methodological section is well-organized and convincingly argued. The author employs widely accepted methodologies for obtaining and analyzing plant in vitro systems from G. jasminoides. The procedures for extracting sprout cultures, callus tissues, and cell suspensions are detailed thoroughly. This highlights efforts to develop a biotechnological platform capable of producing high-yield plant growth and metabolic accumulation models. GC-MS and HPLC were used for analyzing primary and secondary metabolites, providing high accuracy in identifying and quantifying bioactive components. Four different methods—DPPH, TEAC, FRAP, and CUPRAC—were employed to assess

antioxidant activity, ensuring objectivity in evaluating the biological properties of the extracts.

To evaluate genetic stability, SSR marker analysis was employed, a modern and reliable molecular method for detecting somaclonal variation. Additionally, the doctoral student employed statistical data processing using ANOVA and optimization models, demonstrating an approach focused on the quantitative verification of the results.

The most voluminous part of the dissertation presents the results obtained in the various experimental directions, as they are considered in their scientific and applied context. The author has successfully created three types of in vitro systems – sprouting, callus, and cell suspensions – and has followed their development, biomass, and metabolic profile. It is essential to note that specific cultivation conditions result in a significant accumulation of biologically active components, including phenolic compounds, and an increased antioxidant capacity.

Comparative analyses between the donor plant and in vitro cultures reveal significant differences in both the phytochemical profile and antioxidant activity. These findings are crucial for understanding how cultivation conditions influence plant metabolism. A key contribution is the established link between cultivation conditions, metabolic composition, and genetic stability of the bioengineered systems. This was accomplished through precise analyses with SSR markers, which demonstrate the dynamics of genetic changes under different nutrient medium regimes and cultivation durations.

The results are supported by a detailed discussion that compares findings with existing literature data. This part of the work showcases not only an analytical approach but also a comprehensive scientific interpretation, indicating strong research skills. Additionally, systematic optimization of cultivation conditions, including the selection of nutrient medium, aeration, and the use of two-factor models, highlights the potential for future applications of these systems in producing valuable plant metabolites of industrial significance.

The dissertation concludes with seven key findings and five significant contributions, based on extensive experimental data. Notable contributions include: (1) the successful preparation and characterization of three bioengineered in vitro systems from G. jasminoides, with differentiated sprout cultures accumulating metabolites at comparable or higher concentrations than the plant itself, whereas dedifferentiated in vitro cultures (callus and cell suspension) show lower biosynthetic potential; (2) a systematic comparison of the phytochemical profile and antioxidant activity between the donor plant and in vitro cultures; (3) an analysis of somaclonal variability using SSR markers; (4) the development of optimization models for targeted accumulation of biologically active substances; (5) establishing a relationship between cultivation conditions, genetic stability, and metabolic profile.

4. Publications on the topic of the PhD Thesis

The total number of publications by Eng. Gergana Krasteva on her dissertation topic is four, two of which are in peer-reviewed journals in the first and second quartiles

(Molecules, MDPI and Engineering of Life Sciences, Wiley), and two are published in collections. The articles have been cited 136 times. In all four articles, Eng. Krasteva is the first author, demonstrating that the results are the product of her independent work. Additionally, her participation in reports at scientific events in Bulgaria and abroad further highlights her strong performance as a doctoral student.

5. Critical remarks

The dissertation would benefit from including a more comprehensive economic evaluation of the potential for industrializing in vitro systems. Additionally, providing a diagram of the main biosynthetic pathways could enhance understanding of metabolic processes. These suggestions are intended as recommendations and do not diminish the scientific value of the work.

6. Conclusion

The presented dissertation demonstrates a mature scientific concept, skilful use of modern methods, and an analytical approach. Its results represent a significant contribution to the development of plant biotechnology and reveal potential for practical application. In conclusion, I strongly recommend that Eng. Gergana Spasova Krasteva be awarded the educational and scientific degree "Doctor" in Professional Field 5.11. Biotechnology.

11.11.2025 Signature:

/Prof. Kaloyan Petrov, DSc/