REVIEW

of a dissertation on the topic:

"Plant bioengineering systems from *Gardenia jasminoides* Ellis for the production of active ingredients" for the acquisition of the ESD "Doctor" in the field of higher education: 5.11 Biotechnology, scientific specialty: Technology of biologically active substances

Author: Gergana Spasova Krasteva, full-time doctoral student, dismissed with the right to defend on 28.09.2023

Scientific supervisor: Assoc. Prof. Dr. Vasil Georgiev

Reviewer: Prof. Dr. Lyudmila Kabaivanova

Gergana Spasova Krasteva was born on July 18, 1995. She completed her higher education at the University of Food Technology - Plovdiv in June 2019, where she graduated as a Master of Engineering with a degree in "Analysis and Control of Food Products". In October 2020, she was enrolled as a full-time doctoral student at the "Cellular Biosystems" laboratory at the Department of "Applied Microbiology" of the "Stephan Angeloff" Institute of Microbiology - BAS - Plovdiv branch.

The present dissertation work of Gergana Krasteva is submitted for assessment for the educational and scientific degree "Doctor" in the field of higher education: 5.11 Biotechnology, scientific specialty: Technology of biologically active substances.

The topic of the dissertation is relevant, both in scientific and applied aspects. Plant cell cultures are considered a promising technology for sustainable production of plant biomass, as well as for obtaining valuable metabolites for the needs of the pharmaceutical, food and cosmetic industries. In this context, research on the chemical composition of different parts of plants, in vitro cultivation and production of secondary metabolites with the lowest environmental impact are timely and economically promising.

The dissertation is 176 pages long, structured as is customary, in the following sections: introduction, literature review (46 pages), aim and objectives, materials and methods (11

pages), results and discussion (70 pages), conclusions and contributions. The work contains 32 tables and 35 figures.

The introduction presents in a synthesized form the importance of plant cell cultures for the extraction of valuable metabolites. The relevance and significance of the research to which the presented scientific work is dedicated is clearly justified. Special attention is paid to the emergence of somaclonal variability during *in vitro* cultivation with a role in the production of metabolites.

The literature review provides an in-depth description of the selected object of research - *Gardenia jasminoides* Ellis, with emphasis on the historical significance of the flowers (essential oil) and fruits, which have been used in the folk medicine of Asian countries, as well as in the food industry and pharmacy. Based on the existing scientific literature, the role of jasmine gardenia (Gardenia jasminoides Ellis) as a promising source of active ingredients is highlighted. The phytochemical composition is examined with attention focused on the content of phenolic components. Literature data on the strong antioxidant activity of extracts from *Gardenia jasminoides* Ellis are presented. The list of cited literature contains 257 sources covering the period 1971 - 2025. This highlights the long-term efforts of scientists worldwide to clarify the importance of plant natural products, plant cell cultures, the advantages of micropropagation, the role of somaclonal variation and *in vitro* selection for plant improvement in various aspects. All this indicates the high degree of relevance of the topic being developed**.**

The purpose of the dissertation is clearly stated.

The five tasks form a specific plan for conducting research in the correct sequence and logical connection to each other.

The **Materials and Methods** section is presented in 8 parts, which describe classical and mainly modern analysis approaches used in the course of the development. Molecular genetic methods are described in detail in connection with the determination of the genetic changes occurring in in vitro gardenia cultures.

In the chapter **Results and Discussion**, significant data are presented, following the set goal of the dissertation work. Differentiated and dedifferentiated in vitro cultures of *Gardenia jasminoides* Ellis were obtained. A comparison was made between the phytochemical profile of gardenia with the in vitro systems obtained from it, with a total of 67 metabolites identified in the plant and the obtained cultures, 41 and 34 metabolites in callus and cell suspension cultures, respectively. When analyzing the phenolic content, clear differences were reflected in

the leaves of the *G. jasminoides* Ellis plant and *in vitro* cultivated sprout cultures, callus and cell suspension cultures. In this dissertation, new data are provided on the antioxidant activity of phenolic extracts from gardenia leaves and the obtained *in vitro* cultures. It was shown that the antioxidant potential of extracts from undifferentiated *in vitro* cultures (callus and cell suspension) was significantly lower compared to plant leaves and differentiated shoots. Somaclonal variability was detected in gardenia and the resulting *in vitro* cultures by selecting suitable effective markers for assessing genetic diversity. By cluster analysis, it was shown that the significant differences observed in the metabolic profile of the differentiated sprout culture correlate with the observed large genetic distance compared to the plant *G. jasminoides* Ellis and undifferentiated *in vitro* cultures (callus and cell suspensions).

A central part of the results is the monitoring of the genetic stability of *in vitro* systems derived from jasmine gardenia and analyzing the causes of the changes that occurred during prolonged cultivation and with changes in the concentration of major components of the culture medium.

Conclusions and contributions have been drawn that are clearly stated and correspond to the results obtained in the course of the scientific work. I cannot miss to note the enrichment with new data on the differences in the metabolic profile of the obtained *in vitro* systems and *Gardenia jasminoides* Ellis, determination of the genetic stability of differentiated and dedifferentiated culture in the dynamics of development and in the change of basic components of the nutrient medium, as well as the proof that the differentiated sprout culture shows high potential for developing a biotechnological method for the production of phenolic compounds from *G. jasminoides* Ellis with high antioxidant potential.

I accept author contributions of a scientific-fundamental and scientific-applied nature.

The Results and Discussion section ends with a general **Conclusion**, which emphasizes the most important achievements, which logically leads to the formulated conclusions and contributions.

The abstract in Bulgarian has been prepared according to the requirements. It is presented on 80 pages and is illustrated with 29 figures and 26 tables and corresponds to the data presented in the dissertation.

Conclusion

The dissertation submitted to me for review on the topic: "PLANT BIOENGINEERING SYSTEMS FROM *Gardenia jasminoides* ELLIS FOR OBTAINING ACTIVE INGREDIENTS" contains scientific and scientific-practical results, representing significant scientific contributions. The author has managed to publish the obtained results in 2 scientific publications with impact factor and open access, and 2 more with impact rank, which speaks of their significance. This is also an important reason for me to confidently recommend to the members of the Scientific Council of the Institute of Microbiology "Stephan Angeloff" - BAS to vote positively for the acquisition of the educational and scientific degree "Doctor" in the field of higher education: 5.11 Biotechnologies, scientific specialty: Technology of biologically active substances by the doctoral student Gergana Spasova Krasteva.

08.10.2025 **Reviewed by:**

(Prof. Dr. Lyudmila Kabaivanova)