OPINION

by Assoc. Prof. Dr. Dasha Spasova Mihaylova, University of Food Technologies – Plovdiv
elected as a member of the Scientific Jury with Order No. I- 143/01.10.2025 by the Director of the Institute of Microbiology "Stefan Angelov"
of a dissertation for the award of the educational and scientific degree "Doctor of Philosophy" (PhD)
in the field of higher education 5.Technical Sciences
professional field 5.11 Biotechnologies
scientific specialty "Technology of Biologically Active Substances"

Author: **Eng. Gergana Spasova Krasteva**
Topic: Plant bioengineering systems from *Gardenia jasminoides* Ellis for obtaining active ingredients
Scientific supervisor: Assoc. Prof. Vasil Georgiev, PhD – IMiKB – BAS

1. General presentation of the procedure and the PhD candidate

The author of the dissertation is Eng. Gergana Spasova Krasteva – a doctoral student in full-time education at Department of Biotechnology, Laboratory – Cellular Biosystems - Plovdiv with scientific supervisor Assoc. Prof. Vasil Georgiev, PhD – IMIkB – BAS.

The set of materials presented for consideration on paper and in electronic form is in accordance with the requirements of the Act on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the Regulations of the BAS for the Implementation of ZRASRB and meets the criteria of the Regulations on the Terms and Procedure for Acquiring Scientific Degrees and Holding Academic Positions at the Institute of Microbiology "Stefan Angelov", BAS for Acquiring the Educational and Scientific Degree "Doctor".

The doctoral student has attached 4 publications, of which 2 in journals with IF, and all four publications are in refereed and indexed journals. More than 130 noted citations on publications attached to the dissertation without self-citations and a list of 3 participations in scientific conferences in the country and abroad..

2. Relevance of the topic

The topic of the dissertation is undoubtedly relevant, since for years there has been a persistent trend to study plant species in terms of their potential as a source of valuable phytocomponents. In recent years, there has been increased interest in plant cell cultures as a

sustainable approach to obtaining plant biomass, which is of interest for isolating important metabolites with potential application not only in pharmacy, but also in the food and cosmetic industry.

In this regard, *Gardenia jasminoides* Ellis as a plant species, in addition to its decorative effect, is also a valuable source of metabolites with potential for application in medicine and the food industry. The lack of data in the literature for a detailed study of the chemical composition of the various parts of the plant determines not only the relevance of the topic, but also its scientific validity and contribution.

Therefore, I believe that the goal set to study the effects of the duration of cultivation and the content of basic nutritional components on the biosynthesis of phenolic compounds and somaclonal variability of differentiated and dedifferentiated *in vitro* systems of the jasmine gardenia plant (*Gardenia jasminoides* Ellis) is not only relevant, but also of particular scientific interest. It is believed that somaclonal variability during in vitro cultivation of the plant has aroused great interest in recent years. this has a direct impact on the production of secondary metabolites. There are no detailed studies on the influence of the composition of the nutrient medium, the duration of cultivation, light and the growth regulators used, and their influence on the genetic stability of *in vitro* cultures. So far, there is no published information in the world database on the causes that induce somaclonal variability and the factors that influence it in differentiated and dedifferentiated *in vitro* cultures of jasmine gardenia.

3. Degree of knowledge of the state of the problem and the literature

The dissertation work covers 170 pages, 35 figures and 33 tables. The bibliography covers more than 220 titles after removing duplicates. I believe that the dissertation work provided to me for consideration meets the requirements for sufficient volume and is properly structured.

The “Introduction” section outlines the main supporting points that will be discussed in the dissertation work (1 page). The “Literature Review” is properly structured and built in accordance with the topic being developed and covers 45 pages. A clear and systematic presentation of a lot of information in the form of tables is included. The depth of knowledge of the problem by the doctoral student is impressive. The literature review comprehensively presents the data known to date regarding the phytochemical composition and antioxidant activity of *Gardenia jasminoides* Ellis., as well as information on plant cell cultures as a source of biologically active substances and emphasizes genetic variability and somoclonal variability of plant *in vitro* systems.

4.Compliance of the selected research methodology with the set goal and tasks in the dissertation work

The goal of the dissertation work is precisely and clearly defined, and five well-formulated and clear tasks have been derived to achieve it:

1. Obtaining differentiated and dedifferentiated *in vitro* cultures

2. Comparing the phytochemical profile of the jasmine gardenia plant with with its studied *in vitro* systems

3. Tracking the dynamics of development of cell suspension culture and seedling culture of jasmine gardenia

4. Optimizing the conditions of growth and development of cell suspension culture and seedling culture of jasmine gardenia

5. Tracking the genetic stability of *in vitro* systems obtained from jasmine gardenia. Analyzing the causes of the changes that occurred during prolonged cultivation and when changing the concentration of main components of the nutrient medium.

Based on this, in the "Materials and Methods" section, the doctoral student includes a detailed description of the selected and applied methodologies. They have been selected and mastered skillfully and are in accordance with the set goals and objectives.

5. Contributions of the dissertation

In the section "Results and Discussion" on 74 pages, the scientific results are presented - differentiated (germination) and dedifferentiated (callus and cell suspension) *in vitro* cultures of *Gardenia jasminoides* Ellis were obtained, for which various studies were conducted for the first time. Studies were carried out regarding the metabolic profiles of the obtained *in vitro* systems and *Gardenia jasminoides* Ellis. and a comparative analysis was conducted. The results regarding their antioxidant activity were also compared.

In addition, the doctoral student studies the genetic stability of differentiated and dedifferentiated culture during the study of the dynamics of development and the change of the main components of the nutrient medium. In parallel, the genetic stability of differentiated and dedifferentiated *in vitro* systems was also studied compared to that of the source plant *G. jasminoides* Ellis.

Based on the conducted studies and the obtained results, 5 contributions have been formulated in the dissertation, which are original and are the personal work of the doctoral student. The contributions thus formed are not divided into those of fundamental importance and those of a scientific-applied nature. Such an approach would be useful in highlighting the benefits of the research conducted and my recommendation is to make such a differentiation.
For the first time, a detailed study has been carried out on the relationships between observed changes in the metabolic profile and genetic variability during the cultivation of differentiated

and dedifferentiated *in vitro* cultures of *Gardenia jasminoides* Ellis. For this reason, I believe that among the contributions there are those of both a scientific and a scientific-applied nature.

6. Assessment of publications on the dissertation

The doctoral student has presented 4 publications in connection with the dissertation. Two of them are in journals with IF The doctoral student has submitted 4 publications, of which 2 in journals with IF, and all four publications are in refereed and indexed journals, with a total IF for the year of publication - 8. Two of the publications are in journals with Q2 (*Engineering in Life Sciences* and *Molecules*) according to Clarivate Journal Citation Reports. Two of the publications are co-authored, with the doctoral student being the first author, and in two she is an independent author. More than 130 citations of the PhD candidate's publications (excluding self-citations) were also noted. The documents also include a list of participations in scientific forums for presenting results from the dissertation - a total of 3. All these data testify to the relevance of the topic, the interest it has aroused in scientific circles and the good scientific activity of Eng. Gergana Krasteva.

7. Evaluation of the abstract

The abstract of the dissertation reflects the main results achieved and meets all generally accepted requirements in the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria (PPZRASRB).

8. Opinions, recommendations and notes

I have no comments on the work performed. I have several comments and recommendations that in no way belittle the results achieved and the contribution of the presented dissertation and can rather be considered technical omissions.

The use of a decimal point instead of the correct one - a decimal comma when writing fractional numerical values is noticeable; a discrepancy between the actual arrangement of the points and that indicated in the content of the dissertation work on pages; the attention in the literature review is not equally distributed in the description of the methods for assessing AOA, which the doctoral student focused on in her research. I recommend using citation of literary sources in the text by author in the language of the publication itself, without transliteration, as well as a unified spelling of all terms used, including "*in vitro*".

CONCLUSION

The dissertation contains scientific and applied scientific results that represent an original contribution to science and meet the requirements of the Law on the Development of the

Academic Staff in the Republic of Bulgaria (ZRASRB), as well as the specific requirements of the Regulations of the Institute of Biotechnology - Bulgarian Academy of Sciences for the implementation of the ZRASRB.

The dissertation shows that the doctoral student possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Technology of Biologically Active Substances", which allow her to conduct independent scientific research.

Due to the above, I confidently give my positive assessment of the conducted research, presented by a dissertation, abstract, achieved results and contributions, and I propose to the esteemed scientific jury to award the educational and scientific degree "doctor" to Eng. Gergana Spasova Krasteva in the professional field 5.11. "Biotechnologies", doctoral program "Technology of biologically active substances".

14.11.2025 Member of the scientific jury: .....

Assoc. Prof. Eng. Dasha Mihaylova, PhD