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### SCIENTIFIC OPINION

on a dissertation for obtaining the educational and scientific degree "Doctor" in: a field of higher education 6. Agricultural sciences and veterinary medicine professional field 6.1. "Plant Growing", scientific specialty "Selection and seed production of cultivated plants" ("Plant biotechnology").

### Author of the dissertation: Pervin Shengyun Halkoglu-Hristova

Full-time PhD student at the Department of Genetics and Breeding at the Agricultural University, Plovdiv

<u>Topic of the dissertation:</u> "In vitro culturess of Fabiana imbricata Ruiz. et Pav. as technological matrices for obtaining biologically active substances"

<u>Reviewer:</u> Assoc. Prof. Maria Todorova Georgieva from RIMSA Troyan, in the field of higher education 6. Agricultural Sciences and Veterinary Medicine, Professional field 6.1. "Plant Growing", determined according to order No RD-16-744 on 29.06.2021 of the Rector of the Agricultural University - Plovdiv as a member of the scientific jury,

The PhD student has presented all the necessary documents and materials in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its application.

Pervin Shengyun Halkoglu-Hristova graduated with a bachelor's degree in "Agronomy-Plant Biotechnology" in 2014, and in 2015 obtained a Master's degree in "Agronomy-Plant Protection" at the Agricultural University in Plovdiv. The candidate begins her scientific career as a full-time PhD student in "Plant Biotechnology", professional field 6.1. "Plant Growing" in 2016 in the Department of Genetics and Breeding on the above topic. The PhD student conducted a short-term specialization (October 27-December 24, 2016) at the Technical University, Dresden, Germany under a bilateral contract with University of Food Technologies in Plovdid, with a supervisor Atanas Pavlov, Corresponding Member. In addition, she participated in training in the program ENTER Expert Workshop 2018. She speaks English and has the necessary computer skills.

#### Relevance of the scientific topic.

Currently, the application of model plant *in vitro* systems as technological matrices for the production of biological substances is increasingly used in the pharmaceutical industry for the production of phytopreparations. *Fabiana imbricata* Ruiz et Pav. is an objective of the dissertation, which is a valuable medicinal plant, little known in Europe. Due to the lack of similar scientific developments for this species in order to obtain phytopreparations based on secondary metabolites, the topic, purpose and objectives of this dissertation are significant and relevant.

#### Purpose, tasks, hypotheses and research methods.

The dissertation is developed according to the requirements and in a traditional

style: on 118 pages illustrated with 16 tables and 31 figures. A rich reference review is presented in depth, including 191 reference sources, 188 of which are in English. The reference used is much more than what is needed for a doctoral dissertation. A good ratio between the reference review and the methodological part to the effective one is observed.

The relevance and significance of the topic are successfully presented in the introduction to the dissertation.

The aims and objectives of the dissertation are properly constructed and justified. Traditional and innovative approaches are used, which fully correspond to the author's research work.

The section "Material and methods" is methodically correctly structured. The conditions for conducting the experiments are described in detail: the classical system for *in vitro* propagation, the impact of different light sources, the bioreactor cultivation, the cultivation of callus and suspension cultures. Particularly impressive are the modern methods for analyzing the antioxidant activity of various plant extracts: from micropropagated plants, callus cultures, obtained from them plant cell suspensions and *in vivo* plants from *Fabiana imbricata* Ruiz et Pay.

## Discussion of the results and reference used.

In the section "Results and discussion", the results of the conducted experiments are interpreted with great precision and consistency. Optimization of the classical system for *in vitro* propagation of *Fabiana imbricata* Ruiz et Pav. allows its further rationalization and automation through the bioreactor method, providing faster increased rates of reproduction under controlled conditions.

The change of the parameters of the light regime, spectrum, photoperiod in combination with a certain nutrient medium helps to model the synthesis of target biologically active substances.

The spectrophotometric analysis of *in vivo* and *in vitro* cultures of *Fabiana imbricata* Ruiz et Pav. is impressive, which demonstrated high antioxidant activity of the analyzed extracts, with the highest value reported in callus culture RA, grown in red light.

Of particular interest is the use of plant cell, tissue and organ cultures as biotechnological tools for the production of biologically active substances, widely applicable in the pharmaceutical and cosmetic industries.

The HPLS analysis revealed a rich polyphenolic profile of plants *in vitro*, *in vivo*, calluses, plant cell suspensions obtained from *Fabiana imbricata* Ruiz et Pav.

The final part presents conclusions, which summarize the results of the main tasks. They based on the results obtained by the PhD student in the process of developing the dissertation.

The assessment of the reliability of the data presented in the dissertation is positive, as they are based on modern methodologies and analyzes applied by the author.

The presented abstract objectively reflects the structure and content of the dissertation.

#### Critical notes and questions.

I have no critical remarks on the presented dissertation and abstract. The topic of the scientific development is interesting, brought to a high scientific level and it is desirable for the PhD student to continue to learn new methods and analyzes in the future to upgrade and improve.

# Published articles and citations.

The scientific production of the PhD student consists of 6 articles, and 3 scientific papers are subject to analysis for the preparation of the current opinion. I am pleased to note that she is the first author of all three publications. It is noteworthy that all the scientific production of the candidate is in English, which is proof of the value of the results. The published articles are sufficient in number for the dissertation and fully cover the requirements of the law for scientific and educational degree "Doctor".

The PhD student participated in two national and one international forums.

Pervin Hristova has conducted practical exercises in the Department of Genetics and Selection at the Agricultural University - Plovdiv. Her teaching activity includes a total of 127 hours of full-time and part-time students in the disciplines "Plant Biotechnology" (112 hours) and "Biotechnological methods in plant selection" (112 hours).

### CONCLUSION:

Based on the learned and applied by the PhD student, different research methods, correctly performed experiments, summaries and conclusions, I believe that the presented dissertation meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Rules of the Agricultural University for its application, which gives me reason to evaluate it as **POSITIVE**.

The PhD student showed, through the development of the dissertation, that a specialist has been built, who has acquired the necessary experience, knowledge and skills for the application of modern research methods. The accumulated experimental experience will be a good basis for future scientific developments at a high scientific level.

I take the liberty of proposing to the esteemed Scientific Jury also to vote in favour and to award Pervin Shengyun Halkoglu-Hristova the educational and scientific degree "Doctor" in the scientific specialty "Breeding and seed production of cultivated plants" ("Plant biotechnology").

Date: 27.08.2021r.

The Scientific Opinion was prepared by: Assoc. Prof. Maria Georgieva