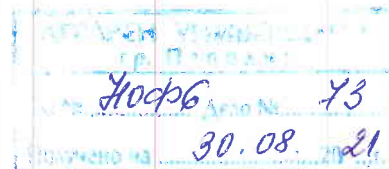


REVIEW



On a dissertation for obtaining an educational and scientific degree "Doctor" in :
Field of higher education – 6. Agricultural sciences and veterinary medicine,
Professional field – 6. 1. Crop science
Scientific specialty – Plant breeding and seed production (Plant Biotechnology)

Author of the dissertation: **Pervin Shengun Halkoglu-Hristova** – full time PhD student at the department of "Genetics and plant breeding" at the Agricultural University Plovdiv.

Thesis: "**IN VITRO CULTURES OF *FABIANA IMBRICATA* RUIZ. ET PAV., AS TECHNOLOGICAL MATRICES FOR THE PRODUCTION OF BIOLOGICALLY ACTIVE SUBSTANCES**"

Reviewer: **assoc.prof. PhD Marina Petrova Marcheva**,
Department of Crop science, Agricultural university Plovdiv,
Field of higher education – 6. Agricultural sciences and veterinary medicine,
Professional field – 6. 1. Crop science
Scientific specialty – Plant breeding and seed production
Appointed s member of the scientific jury by order № РД-16-744./ 29. 06. 2021 by the Rector of the Agricultural University, Plovdiv.
Supervisors: Associate Professor Dr. Svetla Yancheva. Corresponding Member Prof. D.T.S. Atanas Pavlov

1. Brief introduction of the candidate.

Pervin Shengun Halkoglu-Hristova was born on August 26th 1991. She obtained her higher education in 2014 at the Agricultural university Plovdiv, with bachelor's degree in Agronomy (Plant biotechnology). At the same university she successfully obtained master's degree in Plant protection in 2015. From 2016 she is full time PhD student in department of "Genetics and plant breeding" with scientific specialty Plant breeding and seed production (Plant biotechnology). During her study she completed a long term specialization at the Technical University Dresden, Germany. As PhD student, she participated in DAAD project in aim of education in the frame of two-sited project of UFT with scientific leader corr. Member prof. At. Pavlov.

In 2018 she improves her qualification with supplementary education in program ENTER Expert Workshop and acquire certificate "Cooperative learning for sustainable development". Pervin Halkoglu has good communicative skills and adaptability in teamwork. Her teaching activity refers to practical training of Plant biotechnology to bachelor students from different majors in the Agricultural University Plovdiv. She took part in several scientific forums on National level and with international participants. She has practical experience as

senior specialist in the Laboratory of Plant biotechnology in the AU Plovdiv.

2. Relevance of the problem.

The plant kingdom is rich in species used in various fields of human consumption. The food, chemical, technological and pharmaceutical industries rely heavily on resources obtained directly or after processing from various cultivated or under estimated species. Identification of their biological active substances and methods of use are constantly evolving. *Fabiana imbricata* Ruiz et Pav. is a valuable medicinal plant that remains unknown in Europe. There are a number of problems for its conventional reproduction and cultivation on farm. The development of appropriate techniques for cell and tissues culture opens up the possibility for propagation and research in order to obtain biologically active substances under controlled conditions.

3. Purpose, tasks, hypothesis and research methods.

After a detailed and well-structured review of the existing scientific information, gaps in the knowledge in specific areas have been identified. The conclusions made at the end of the section determine the need for this study. The goal is formulated clearly and precisely. The tasks correspond to the stages of research of the investigated problem. The chosen methodology is appropriate, wide accepted and sufficient. Software for “shaping and presenting” the results is indicated - Word, Excel, should focus on one for their analysis, such as SPSS and Breeze TM.

4. Visualization and presentation of the obtained results.

The thesis occupies 118 pages. The material is divided into 9 main sections with multiple sub-section. The introduction is brief – 1 page, but sufficiently informative and directing to the chosen topic. The literature review is 23 pages, divided into 5 chapters with up to 5 sub-chapters. There are 191 authors who have published in the last 40 years, of which only 3 are in Cyrillic. The conclusions of the review of the scientific information are indicated on a separate page. The goal and tasks are compact, specific and clear – on one page. The material and methods are described in detail on 13 pages in six chapters and fully reflect the experiment. They present the successive steps – from setting a seed germination test, through introduction into culture and experiments with different nutrient media and lights, obtaining callus and suspension cultures, bioreactor study system with temporary immersion, to extraction and analysis of biological active substances and statistical analysis of the results. The results and the discussion follow the course of the conducted and described experiments and are presented most extensively – on 46 pages, with 31 figures and photos and 16 tables. Two more tables for formulas and literature for primary and secondary metabolites are given in the appendix. The conclusions are presented on three pages. Contributions are not listed separately, but are found in the summary conclusion.

5. Discussion of the results and used literature.

The results, obtained during the investigation, are presented in four chapters, illustrated

with tables, photos and figures.

The inability of the purchased commercial seeds to germinate in laboratory conditions is described in detail. The successful sterilization of the initial explants and their primary development in a hormone free media were monitored. The effect of adding different growth regulators (BAP, IBA) was analyzed and the most suitable nutrition media for shoot proliferation was determined. Vitrification at specific auxin concentrations has been reported as a side effect. To prevent this, an attempt was made to add activated carbon to the media. This optimization leads to almost twice as high proliferation and no symptoms of hyperhydration.

The morphological characteristics of the plants grown on different nutrition media were traced. Shoots are rooting in all variants without the need for an additional stage and conditions for it. The best adaptation and the highest survival rate are marked for regenerants with the last subculture on a hormone free media.

The study of the influence of the light source - LED with different light spectrum and fluorescent lamps as a control, shows different efficiency compared to the characteristics of the plants *Fabiana imbricata* Ruiz. et Pav. It was found that the light source of white fluorescent light is most suitable for multiplication, compared to LED light sources.

The conducted experiment with an automated temporary immersion system (type RITA®) shows greater efficiency due to the reported higher values of the indicators characterizing the growth in comparison with the conventional *in vitro* system. The main advantage of the bioreactor system is that it provides process automation, minimal space and production of large amounts of biomass, despite the high cost of investment in equipment.

An experiment with digital holographic microscopy (DHM) was performed to measure the size of cell clusters in suspension cultures of *Fabiana imbricata* Ruiz. et Pav., which proves that it can be successfully used for: cell counting, measurement of cell size and cell clusters, analysis of cell culture viability, etc.

The polyphenolic profile of *Fabiana imbricata* Ruiz et Pav. was analyzed by HPLC analyzes of plants *in vitro*, *in vivo*, calluses, derived from them plant cell suspensions. The main metabolites - gallic, protocatechinic, chlorogenic, vanilla, syringic, caffeic, salicylic and rosmarinic acid have been identified and *in vitro* cultures with different degrees of differentiation have been identified, which show a higher antioxidant capacity close to that of a plant sample *in vivo*.

The experiments were conducted in the Laboratory of Plant Biotechnology at Plovdiv University, Physical Laboratory with Digital Holographic Microscope (DIHM), developed at Plovdiv University, Laboratory for Analysis of the Department of Analytical Chemistry at the University of Food Technology - Plovdiv, HPLC analyzes were performed in the Laboratory in Applied Biotechnology, BAS - Plovdiv. A large number of replicates were tested and statistical processing of the results was performed where applicable. In the course of laboratory experiments, full-time doctoral student Pervin Chalkoglu has mastered the necessary methods for *in vitro* cultivation and chemical analysis of biological active substances. The presented dissertation is entirely the work of her efforts and consistency..

6. Contributions.

The thesis ends with the formulation of 15 conclusions. They correctly reflect the results obtained. The analysis is methodical and thorough. The presentation is clear, concise and concrete.

The following can be indicated as scientific and scientific-applied contributions from the presented dissertation:

Scientific contributions

- ✓ *In vitro* plant material obtained from initial plants *Fabiana imbricata* Ruiz. et Pav., has been proved as suitable for use as a producer of biologically active substances.
- ✓ The influence of various factors (additives, growth regulators and light) on the regeneration of this culture has been studied:
 - the effect of the addition of activated carbon on the proliferation of shoots and overcoming of negative physiological conditions such as vitrification and malformations of the regenerants;
 - white fluorescent light has been shown to be most suitable for multiplication compared to LED sources;
 - better regeneration of regenerants and higher survival in *ex vitro* adaptation have been found in the cultivation of the last subculture on nutrient medium without growth regulators.
- ✓ Spectrophotometric analysis of *in vivo* and *in vitro* cultures of *Fabiana imbricata* Ruiz . et Pav was performed for the first time, which demonstrated high antioxidant activity of the analyzed extracts. It was found that the light regime, spectrum and photoperiod in combination with selected nutrient medium have a significant effect on the production of secondary metabolites from undifferentiated *in vitro* systems and the change of these parameters can be used to model the synthesis of target biologically active substances.
- ✓ For the first time, an experiment with digital holographic microscopy (DHM) was performed, which is easily applicable for determining the number and size of cells and cell clusters in suspension cultures of *Fabiana imbricata* Ruiz. et Pav.

Scientific and applied contributions

- ✓ A nutrient medium for micropropagation of *Fabiana imbricata* Ruiz et Pav has been developed and optimized, in which the balance of growth regulators provides a high multiplication rate.
- ✓ After induction of callus cultures of *Fabiana imbricata* Ruiz et Pav, different in morphology and development are chosen these suitable for inducing plant cell suspensions. Suspension cultures have been shown to be more suitable for the production of target metabolites due to the faster accumulation of biomass compared to callus cultures and *in vitro* plants.
- ✓ The polyphenolic profile of plants *in vivo* and *in vitro*, calluses and cell suspensions from *Fabiana imbricata* Ruiz et Pav were studied. by HPLC. The diversity of the synthesized compounds in the studied *in vitro* systems with

different degree of differentiation has been proven. Their potential as technological matrices for obtaining target metabolites has been determined.

7. Critical remarks and questions.

I welcome the idea of researching the presented topic and I cannot underestimate its merits. The dissertation is planned, structured, conducted and presented correctly and in detail. The analysis of the results is thorough and adequate. No methodological or stylistic errors were made in the implementation and description of the achievements.

I allow myself very small remarks:

Despite the detailed literature review, I find no reason to formulate the first conclusion from it. No attempts at industrial cultivation and use have been reported to prove any problems. There is no need for such a conclusion to emphasize that obtaining biologically active substances under controlled conditions of an *in vitro* regeneration system would be successful and efficient. Позволявам си съвсем дребни забележки:

The writing of the dissertation itself is the end of all efforts. It would be good to correct small mistakes related to spelling, numbering and explaining a legend to some tables.

I will also allow myself two questions:

1. Why did the germination test last 22 weeks?
2. Do you have information on whether there is a demand at the moment and if so - in which preparations are the compounds analyzed by you used?

8. Published articles and citations.

In response to the minimum national requirements for awarding the educational and scientific degree "Doctor" and the requirements of the Regulations for the development of the academic staff at the Agricultural University of Plovdiv, PhD student Pervin Halkoglu-Hristova has published her results in peer-reviewed and referenced journals in Scopus / Web of Science. The presented report contains three articles in English, published in 2019, directly related to the topic of the dissertation, as well as participation in three more publications (2015-2016) in other projects in this area.

The presented report does not contain citations of the published results.

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

CONCLUSION:

Based on the learned and applied by the doctoral student, different research methods, correctly performed experiments, summaries and conclusions, I consider that the submitted dissertation meets the requirements of the law for the development of the Academic Staff of Republic Bulgaria and the and the Regulations of the Agricultural University for its application, which gives me reason to evaluate it POSITIVE.

I dare to suggest to the venerable Scientific Jury also voting positive and awarding

Pervin Shengun Halkoglu – Hristova

The educational and scientific degree "Doctor" in scientific specialty Plant breeding and seed production (Plant biotechnology)

Date: 30. 08. 2021 г.
Plovdiv

REVIEWER:
(Assoc.Prof. Dr. Marina Marcheva)