



OPINION

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 Plant breeding, scientific specialty "Breeding and seed production of cultivated plants (Plant biotechnology)

Author of the dissertation: Pervin Shengyun Halkoglu-Hristova, full-time doctoral student at the Department of Genetics and Selection at the Agricultural University, Plovdiv

Dissertation topic: "In vitro cultures of *Fabiana imbricata* Ruiz. et Pav. as technological matrices for obtaining biologically active substances "

Reviewer: Prof. Dr. Bistra Yaneva Atanasova-Dimitrova, field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.1 Plant breeding, scientific specialty "Selection and seed production of cultivated plants", appointed a member of the scientific jury by order № RD-16-744 / 29.06.2021 by the Rector of the Agricultural University - Plovdiv.

1. Relevance of the problem.

The species *Fabiana imbricata* Ruiz et Pav. is one of the most common species and widely used in traditional medicine in Central Chile. In our country it is almost unknown as a medicinal and ornamental plant.

Plants of the species *F. imbricata* are a source of secondary metabolites, possessing various biologically active substances, possessing not only medicinal, but also important ornamental qualities.

The decorative effect of the plants is determined by their habitus - evergreen shrub with many branches, covered with small needle-like dark green leaves and numerous small, conical in shape flowers, ranging from white to violet color. Regardless of its decorative value, the use of the species in ornamental gardening in our country is not represented due to its unknown.

There are a number of problems and difficulties in the conventional propagation and cultivation of *F. imbricata*. The use of biotechnological methods for its more mass reproduction and distribution provides new opportunities, as well as for a more in-depth study of the species in order to obtain biologically active substances and its use as an ornamental plant.

The development of appropriate techniques for cell and tissue culture of *F. imbricata* are essential for its accelerated reproduction, exploration and widespread use of the species as a valuable medicinal and ornamental plant.

From the available scientific literature there are no data for: developed in vitro systems of *F. Imbricata* species, for study of the metabolic profile of in vitro cultures, for performed analyzes of biological activity of extracts from cultures with different degree of differentiation in order to use them as technological matrices for the production of biologically active substances.

All this determines the relevance and significance of the topic developed in the dissertation.

2. Purpose, tasks, hypotheses and research methods.

The purpose of the dissertation is correctly formulated, and 5 main tasks are precisely and clearly stated, the implementation of which has led to the results.

The doctoral student has successfully mastered the necessary for the purpose of the dissertation modern research methods (DPPH, ABTS, FRAP, CUPRAC, HPLC, etc.) for the determination of total phenols, flavonoids, polyphenols, antioxidants and others. biologically active substances.

3. Visualization and presentation of the obtained results.

The presented dissertation is a purposeful research work that complements and expands the knowledge about the species. It is formed according to the classical model of 118 pages adopted in the Republic of Bulgaria, as the illustration and presentation of the results is achieved with 16 tables, 31 figures and 2 appendices.

The structure of the dissertation is well balanced, as the literature review is 22 pages, materials and methods - 13 pages, results and discussion - 30 pages and conclusions - 3 pages.

4. Discussion of the results and used literature.

The obtained results are summarized and interpreted correctly, in a good scientific style. In discussing them, the sequence of the presented problem in the literature review is followed, which gives value and clarity to the exposition. All experiments were performed strictly, which makes the results reliable. The discussion on them shows an in-depth knowledge of the issue. 191 literature sources were used, 3 of them in Cyrillic and 188 in Latin.

5. Contributions to the dissertation.

From the developed dissertation work, the most significant contributions are the following:

Scientific contributions

- ✓ An optimized culture medium for micropropagation of *F. imbricata* has been developed, in which the balance of growth regulators provides a high multiplication rate.
- ✓ Callus cultures were induced, differing in morphology and growth characteristics, from which 3 cultures suitable for induction of cell suspensions were selected.
- ✓ For the first time, the number and size of cells and cell clusters in suspension cultures of *F. imbricata* were determined by digital holographic microscopy (DHM).
- ✓ The polyphenolic profile of plants in vivo and in vitro, calluses and resulting cell suspensions of *F. imbricata* were determined by HPLC analyzes. The diversity of the synthesized polyphenolic compounds in the studied in vitro systems with different degree of differentiation has been proven.
- ✓ For the first time, spectrophotometric analysis of in vivo and in vitro cultures of *F. imbricata* was performed, which demonstrated high antioxidant activity of the analyzed extracts.

Scientific and applied contributions

- ✓ In vitro material was obtained from *F. Imbricata* starting plants, suitable for use as a source of biologically active substances.

✓ The addition of activated charcoal (AC) in the nutrient medium has a stimulating effect on the shoots proliferation and leads to overcoming negative physiological conditions.

✓ The use of nutrient medium without growth regulators is a suitable approach for hardening of regenerants and ensures high survival of plants during *ex vitro* adaptation.

✓ For the growth and development of *F. imbricata* plants, white fluorescent light has been found to be most suitable for the multiplication stage compared to LED sources.

✓ Suspended cultures of *F. imbricata* have been shown as suitable for the production of target metabolites because they reach a maximum increase in biomass in a short cultivation period (8-10 days) compared to callus cultures and *in vitro* plants. 28 days).

✓ The potential of *in vitro* cultures of *F. Imbricata* as technological matrices for obtaining target metabolites was determined.

6. Critical remarks and questions.

I have no critical remarks or questions to the doctoral student.

7. Published articles and citations.

In connection with the dissertation, the doctoral student has published in indexed editions 3 scientific articles, which exceed the required number of points for obtaining the educational and scientific degree "Doctor", according to the requirements. She has published 3 more publications from participation in scientific forums.

No citations to the submitted publications are attached.

The 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 believe that the presented dissertation meets the requirements of ZRASRB and the Rules of the Agricultural University for its application, which gives me reason to evaluate it.

POSITIVE.

I allow myself to suggest to the esteemed Scientific Jury that it also vote positively and to award Pervin Shengyun Halkoglu-Hristova the educational and scientific degree "Doctor" in the field of higher education 6. Agricultural sciences and veterinary medicine, professional field 6.1 Plant breeding, the scientific field "Breeding and seed production of cultivated plants (Plant biotechnologies)".

Date: 16.08.2021

Plovdiv

OPINION PREPARED BY:

(Prof. Dr. Bistra Yaneva Atanasova-Dimitrova)