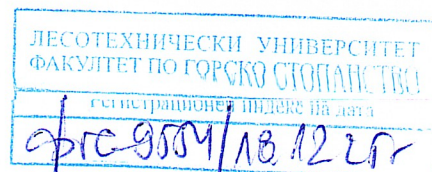


REVIEW



on dissertation for obtaining the educational and scientific degree "Doctor" in: field of higher education 6. Agrarian sciences and veterinary medicine, professional field 6.5. Forestry, scientific subject Forest plantations, improvement and seed production

Author of dissertation: Elie Antoine El Khoury – part-time doctoral student at the Department of Dendrology, University of Forestry (UF), Sofia, Bulgaria

Dissertation topic: Conservation of native pyramidal orchid *Anacamptis pyramidalis* (L.) Rich. in Lebanon through vegetative propagation

Reviewer: Assoc. Prof. Mira L. Georgieva, PhD, Forest Research Institute, Bulgarian Academy of Sciences (FRI-BAS), 04.04.03. Silviculture (incl. Dendrology), appointed as a member of the scientific jury by Order No.ZPS-666/24.11.2025 of the Rector of the University of Forestry.

1. Brief presentation of the candidate

Doctoral student Eli Antoine El Khoury was born on 18 July 1975 in the Lebanese Republic.

In the period 1996-2001 he obtained a Master's degree in Agricultural Engineering at Holy Spirit University - Kaslik-Lebanon.

From 1995 to present he is the owner of a Nursery for the production of ornamental plants in Batha-Mount Lebanon-Lebanon. He acquires knowledge and skills related to floriculture, production and cultivation of ornamental plants and cut flowers, propagation of floral material (cuttings, bulbs), technical training for trainees, production and cultivation of vegetables. From 2012 to present he is a managing partner in AGRITEAM SARL Company, Kehale-Mount Lebanon-Lebanon, where the main subject of activity is trade in agricultural machinery, agrochemicals, pesticides, fertilisers, irrigation systems, seeds, agricultural tools, landscape projects and technical training for trainees. He works together with architects, surveyors and civil engineers.

In 2017, he was enrolled as a doctoral student at the Department of Dendrology at the Faculty of Forestry at the University of Forestry with Order No. 713/22.12.2017 in an independent form of study, but in 2018 it was changed to a part-time form of study with Order No. 134/02.03.2018 and was enrolled with the right to defend with Order No. ZPS – 557/ 11.12.2020 of the Rector of the University of Forestry. The doctoral exams were successfully passed, with excellent results.

The doctoral student has 2 publications related to the topic of the dissertation. His scientific articles have thoroughly investigated problems on the topic of the dissertation, and the aforementioned contributions are also a result of the experience gained in his overall work as an owner and managing partner in the field of agricultural engineering, who seeks to apply his experience and knowledge in the field of production and cultivation of ornamental plants through scientific research.

2. Relevance of the problem

In recent years, there has been an increasing need to develop conservation strategies for preserving the habitats and genetic resources of valuable, rare, endangered and extinct plant species, which is key to preserving biodiversity as a whole. The lack of such information and studies on *Anacamptis pyramidalis* (L.) Rich. prove that the topic of the dissertation is very relevant and necessary.

In this dissertation, the preparation of a future conservation strategy and the discovery of the genomic sequence of the rare and protected species *Anacamptis pyramidalis* (L.) Rich. are based on two main methods – *in vitro* and *in vivo*.

In support of the relevance of the topic of the dissertation, the conducted research and the selected methodologies, numerous literary sources have been cited, which logically follow the main thesis of the dissertation.

2. Degree of knowledge of the state of the problem and creative interpretation of the literature review

In developing the dissertation, the author has conducted a comprehensive review of 221 literary sources in a foreign language, which demonstrates excellent knowledge of the state of the problem and a high degree of awareness, which allows him to analyse the literary data thoroughly and accurately, to clearly define the purpose and objectives of his work, and to make valuable conclusions and recommendations for future conservation actions.

3. Aim, objectives, hypotheses and research methods. Relevance of the chosen research methodology to the stated aim and objectives of the dissertation

The aim of the dissertation is to reveal the genomic sequence of the species, to study the growth and behaviour indicators and to conduct experiments for vegetative propagation of *Anacamptis pyramidalis* (L.) Rich. from bulbs with a view to create a conservation strategy using two main methods – *in vitro* and *in vivo*.

The selected methodologies are described in detail, the experiments are properly designed, and the tasks set are directly aimed at the implementation of the well-formulated goal of the dissertation, which are divided into 3 phases:

- **phase 1:** Revealing the genomic sequence of two different loci through DNA analysis performed with PCR (polymerase chain reaction);

- **phase 2:** *In vivo* propagation of orchid bulbs collected from the wild and grown in the laboratory under controlled experimental conditions: different types of substrates, mycorrhizal application and separation of the mother bulbs;

- **phase 3:** repetition of *in vivo* propagation and an *in vitro* propagation method based on micropropagation.

This experimental study aims to answer the following questions:

- What are the optimal conditions for *in vivo* growth of *Anacamptis pyramidalis*?
- Would the *in vitro* propagation method be an effective method for the future conservation of this species?
- Is there a relation between the sequence obtained from *Anacamptis pyramidalis* from Lebanon and other sequences retrieved from Genbank?

The chosen methods are fully suitable for achieving the aim and fulfilling the tasks of the dissertation. The author demonstrates a high level of knowledge of various research methodologies with the help of which important results have been achieved:

- The genomic sequence of *Anacamptis pyramidalis* has been established.
- The obtained sequences have been compared with other available sequences retrieved from Genbank.
- The behaviour of *Anacamptis pyramidalis* under experimental conditions (*ex situ*) has been studied and compared with its natural behaviour in the wild.
- The vegetative growth of orchid plants grown in different combinations of soil substrates has been compared.
- The effect of mycorrhiza on plant growth has been evaluated.
- The effect of the separation of the mother bulb on plant growth has been evaluated.
- The effect of various studied factors (soil substrates, mother bulb separation and mycorrhiza) on plant growth was compared and evaluated in order to investigate the optimal conditions for *in vivo* growth.
- The effectiveness of *in vitro* as a method for propagation of this species was evaluated.

4. Visualisation and presentation of the results

The presented dissertation is well-formed and structured in the following sections: "Introduction", "Literature Review"; "Objective and Tasks", "Materials and Methods"; "Results and Discussion", "Conclusions and Recommendations", References (a list of used literature sources) and Appendices. For a better visualisation of the information, the results are illustrated with 43 colour figures, 6 tables and photographs.

The content of the dissertation is clear and well-structured, with accurate interpretation of the data and correctly conducted statistical analyses and conclusions, written in professional academic English.

5. Discussion of results and literature used

The presented results meet the set aims. Their interpretation is clear and logical and allows the formulation of conclusions and recommendations, corresponding to the set tasks. The used literature is on the topic of the dissertation, in large volume and entirely in a foreign language. The computer literacy and excellent command of foreign languages of the doctoral student are useful in his scientific work. The dissertation work undoubtedly proves the need to prepare a conservation strategy for *Anacamptis pyramidalis* (L.) Rich.

6. Contributions of the thesis

The dissertation work lists a total of 9 contributions of a scientific and applied nature. Of these, 4 are scientific, and 5 are applied. They are correctly formulated and actually reflect the achieved results.

Scientific contributions:

1. The successful application of the DNA barcoding method for the successful identification of *Anacamptis pyramidalis* (L.) Rich has been proven.
2. The sequence of nucleotides in two regions of the species' genome has been established based on two types of markers.
3. The phylogenetic relationships of populations from Lebanon with those from other parts of the species' natural range have been established.
4. The possibilities for applying a combined approach for vegetative propagation of the species have been established.

Scientific and applied contributions

1. The optimal growth conditions for *Anacamptis pyramidalis* (L.) Rich., similar to those in its natural environment, have been established.
2. The influence of different growth substrates and the application of mycorrhiza for the propagation of the species has been established and the most suitable of them have been recommended.
3. The influence of different treatment regimes on morphological and morphometric characteristics of the species has been established.
4. It has been established that *in vitro* propagation is difficult and additional studies are necessary for its successful application.

5. It has been established that the achieved results can be used not only for the purposes of conservation of the species, but also for its mass propagation.

7. Assessment of the extent of the dissertator's personal involvement in the contributions

The independent participation of the doctoral student in the field research and in analysing and processing the results is obvious and undeniable.

The presented dissertation, abstract and publications give me sufficient grounds to believe that the work on the dissertation and the results obtained from it are the personal work of Eli Antoine El Khoury under the guidance of his scientific supervisor and his scientific consultant.

8. Critical comments and questions

Some notes of an editorial and technical nature could be made, which do not belittle the quality of the achieved results and the contributions of the dissertation work, but would only contribute to its even better appearance:

- incorrect page numbering – discrepancy between the beginning of the chapters indicated in the content of the dissertation work and the beginning of the individual chapters in the text;
- incorrect citation of some literary sources, which requires a correction of their total number
- on page 83 – references 6 and 7, describe the same publication
- on page 84 – references 20 and 21, describe the same publication
- on page 85 – references 32 and 33, describe the same publication
- on page 87 – reference 62 - incomplete spelling of the author's name
- on page 88 – references 69 and 70, describe the same publication
- on page 89 – incomplete citation of reference 80. Hassler and Rheinheimer 2019 – other information is missing except for the names of the authors.
- on page 90 – references 101 and 102, describe the same publication
- on page 94 – references 139 and 140, describe the same publication
- on page 94 – references 143 and 144, describe the same publication
- on page 97 – reference 183 was omitted, it was specified that it was a second edition
- on page 98 – inaccuracy in citing reference 184
- on page 99 – incomplete citation of reference 201. Vicente-Serrano et al. 2006, other information is missing except for the names of the authors.
- on page 100 – incomplete citation of reference 215. Wood and Ramsay 2004, other information is missing except for the names of the authors.

9. Published articles and citations

The doctoral student has cited two publications on the topic of the dissertation. On one he is single author, and on the other he is the first author.

Khoury E., Sajyan T.K., Zhelev P., Sassine Y.N., 2018. Investigating the effect of substrate, mycorrhizal application and bulb separation on the growth of the wild orchid *Anacamptis pyramidalis*. AGROFOR International Journal, 3 (3): 5-13.

Khoury E., 2023. Ex-situ propagation of *Anacamptis pyramidalis* (L.) from an agroecological perspective. International Journal of Advanced and Applied Sciences, 10(6): 201-212.

10. Assessment of the publications on the dissertation: number, nature of the publications in which they are printed. Reflections in science - use and citation by other authors

The submitted abstract objectively reflects the structure and content of the dissertation work.

The two publications submitted by the doctoral student fully meet the requirements of the Regulations for the Development of Academic Staff at the University of Forestry of 2021, according to the rules of which this procedure is carried out.

The first publication is in the International Journal AGROFOR, which is submitted for consideration in: SCOPUS, EBSCO, AGRICOLA, AGRIS, Islamic World Centre for Science Citation (ISC), Directory for Indexing Scientific Journals, Directory of Open Access Journals (DOAJ), Abstracts of Food Science and Technology, Genamics JournalSeek and the Open University Library.

The independent publication is in a scientific journal, referenced in global databases (Web of Science, Scopus and others) and has an impact factor of 0.86.

Citations of the submitted publications are not indicated.

CONCLUSION

On the basis of the different research methods learned and applied by the applicant, the correctly derived experiments, the made generalisations and conclusions, I consider that the presented dissertation work meets the requirements of the FRAADB and the Regulations of the University of Forestry for its application, which gives me the reason to evaluate it as **POSITIVE**.

I would suggest the Honourable Scientific Jury also to vote in the affirmative and award the educational and scientific degree "**Doctor**" in the scientific subject Forest plantations, improvement and seed production.

18.12.2025
Sofia

REVIEWER: 
/Assoc. Prof. Mira L. Georgieva, PhD/