

STATEMENT

regarding the PhD Thesis for awarding the educational and scientific degree "Doctor" (PhD) in field of higher education: 4. Natural sciences, mathematics and informatics; professional direction: 4.3. Biological Sciences. PhD program: Molecular Biology.

PhD Student: Nikol Slaveva Hadjieva

Title: *"Identification of specific interactions between Potato Spindle Tuber Viroid and two Bulgarian pepper varieties"*

Research supervisor: Assoc. Prof. Dr. Mariyana Hristova Gozmanova, University of Plovdiv "Paisiy Hilendarski"

Member of the Scientific Jury: Prof. Liliana Rumenova Nacheva, Ph.D., Fruitgrowing Institute - Plovdiv, Agricultural Academy (SSA), professional direction 6.1. Horticulture, scientific specialty "Fruitgrowing" (Professor) and 4.3. Biological Sciences, scientific specialty "Plant Physiology" (Associate professor).

1. General presentation of the procedure and the doctoral student

By order № PD-21-456 of 02.03.2023. of the Rector of Plovdiv University "Paisiy Hilendarski" (PU), I have been appointed as a member of the scientific jury to ensure a procedure for the defense of a dissertation on the topic "Identification of specific interactions between *Potato Spindle Tuber Viroid* and two Bulgarian varieties of pepper" for the acquisition of the educational and scientific degree "Doctor" in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, PhD program Molecular Biology.

The author of the dissertation is Nikol Slaveva Hadjieva - full-time PhD student at the Department of "Plant Physiology and Molecular Biology" supervised by Associate Professor Mariyana Hristova Gozmanova, University of Plovdiv "Paisiy Hilendarski".

The documents provided to me regarding the Procedure of Nikol Hadjieva was prepared in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Application, as well as Article 36 (1) of the Regulations on the Development of the Academic Staff of the University of Plovdiv.

The PhD student has presented three articles on the subject of the dissertation, two of which were published in journals with an impact factor and one in a journal without an impact factor.

The student completed her higher education at the "Paisii Hilendarski" University of Plovdiv with a bachelor's degree majoring in "Biology and Chemistry", after which she completed a master's degree majoring in "Molecular Biology" at the same University. She was a PhD student in the Department of "Plant Physiology and Molecular Biology". All this is a prerequisite for Nikol Hadzhieva to become a successful young researcher.

2. Relevance of the topic

Ecological approaches in the protection of agricultural production require a thorough knowledge of the causative agents of economically important diseases in cultivated plants. The study

analyzed the responses of two Bulgarian pepper varieties infected with the *Potato Spindle Tuber Viroid* (PSTVd). In addition to scientific contribution, revealing the regulatory molecular mechanisms of viroid infection would illuminate new opportunities for plant protection and production quality improvement. In this sense, the topic of the development is very relevant and would have not only a theoretical but also an applied contribution.

3. Knowing the problem

The extensive literature review presents the basic concepts, classification, distribution, hosts and modes of transmission of viroids in a consistent and logical manner. It is focused on the features of the research *Potato Spindle Tuber Viroid* (PSTVd), its genomic organization, distribution, etc. All this shows an excellent knowledge of the problem and substantiates the aims and objectives of the study.

4. Research methodology

The aim of the dissertation to identify specific interactions between *Potato Spindle Tuber Viroid* and two Bulgarian varieties of pepper is precisely and clearly formulated. The set tasks describe all directions of the research leading to the achievement of the set goal. The experimental setups are clear and logical. Analyzes are accurately presented and precisely executed.

5. Characterization and evaluation of the dissertation work and contributions

The Thesis is very well structured and the individual parts are balanced in terms of volume. The literature review has a volume of 31 pages (33%), and the results, together with the supplementary materials and the discussion, make up more than half of the volume of the dissertation work. The excellent style and language, with minor technical errors, is striking.

The materials are presented logically and sequentially. After preliminary biotests with PSTVd inoculation on main varieties of Bulgarian pepper, two varieties were selected for subsequent analysis, which showed specific phenotypic manifestations. The object of research was correctly selected - on the one hand, the most economically significant pepper varieties for Bulgaria, and on the other, with the most pronounced phenotypic expression after infection with the studied viroid.

The results are presented clearly, very well illustrated with tables, figures and photos. A tremendous amount of work has been done, from preliminary testing of individual pepper cultivars for susceptibility to the viroid to next-generation sequencing of small RNAs to study the profile of these regulatory RNAs in response to PSTVd infection in pepper. The PhD student has mastered basic molecular methods applied to plants.

The obtained results are skilfully compared with the research of leading scientists in the world literature. Of the 240 authors cited, all are in Latin, and most of the articles were published in the last fifteen years.

6. Evaluation of the publications and personal contribution of the PhD student

In connection with the dissertation, three publications are presented, two of them are in journals included in the highest quartile Q1. The personal contribution of the PhD student is demonstrated by the fact that she is the first or second author on the cited articles.

The original scientific contributions include the molecular interactions studied for the first time between PSTVd and two varieties of Bulgarian pepper through large-scale expression analyzes of small RNAs and mRNAs. Also, for the first time, differentially expressed miRNAs and differentially expressed protein-coding genes were identified in the pepper cultivars 'Kurtovska capia' and 'Julyunska shipka', which collectively determine the induction of a cultivar-specific response to PSTVd. The results of the conducted large-scale sequencing of mRNA and small RNAs obtained from infected and control pepper plants are annotated in global biodatabases and can be used in future research by scientists from all over the world.

In addition, the results of the thesis, and in particular molecular biological techniques for gene expression analysis, will be used for knowledge transfer in the training of students and specialists in Molecular Virology.

The described molecular interactions between PSTVd and pepper in the thesis could also find practical applications in the development of strategies for the prevention and control of viroid infection among cultivated plants.

7. Abstract

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

8. Recommendations for future use of dissertation contributions and results

I have no critical remarks about the dissertation. I would recommend that research continue and possibly be extended to other viroid host plants.

In this sense, I have a question for the PhD student: In the literature review, it was noted that the infection of ornamental species and grasses of the *Solanaceae* family is usually asymptomatic, but it has been reported to affect representatives of other families such as dahlia (*Asteraceae*). Is there information on infectivity and phenotypic expression of other ornamental species?

CONCLUSION

On the basis of the various research methods learned and applied by the doctoral student, the correctly performed experiments, the generalizations and conclusions made, I believe that the presented dissertation represents an original contribution to science, meets the requirements of the ZRASRB and the Regulations of the University of Plovdiv for its application, which gives me reason to evaluate it POSITIVELY.

I would suggest the honorable Scientific Jury to also vote positively and award **Nikol Slaveva Hadjieva** the educational and scientific degree "**Doctor**" in the field of higher education 4. Natural

sciences, mathematics and informatics, professional direction: 4.3. Biological Sciences, PhD Program in Molecular Biology.

06.04. 2023

Plovdiv

Prepared by:

(Prof. Dr Lilyana Nacheva)