

OFFICIAL STATEMENT

by Prof. Tsveta Hristova Hristeva, PhD

Pensioner, Tobacco and Tobacco Products Institute - Markovo, (Agricultural Academy)

Regarding a dissertation for the nomination 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

Author: NIKOL SLAVEVA HADZIEVA – Plovdiv, "Paisii Hilendarski" University

Topic: "Identification of specific interactions between Potato Spindle Tuber Viroid and two Bulgarian pepper cultivars"

Scientific supervisor: Assoc. Prof. Mariyana Hristova Gozmanova, PhD – "Paisii Hilendarski" University of Plovdiv.

1. General presentation of the procedure and the doctoral student

I was appointed by order No PD-21-456 of March 2, 2023 of the Rector of Plovdiv University "P. Hilendarski" (PU), as a member of the scientific jury to provide a procedure for the defense of a dissertation on "Identification of specific interactions between Potato Spindle Tuber Viroid and two Bulgarian pepper cultivars" for acquiring 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 Slaveeva Hadjieva - a full-time doctoral student at the Department of "Plant Physiology and Molecular Biology" with scientific supervisor Assoc. Prof. Mariyana Hristova Gozmanova, PhD at University of Plovdiv (PU).

The dissertation work was discussed and proposed for defense to the departmental council of the Department of "Plant Physiology and Molecular Biology" at the Faculty of Biology of the PU, held on February 6, 2023.

The set of paper materials presented by Nikol Slaveeva Hadzhieva is in accordance with Article 36 (1) of the Regulations for the Development of the Academic Staff of the PU and includes the following documents:

- Application to the Rector of the University of Plovdiv for disclosure of the procedure for defense of the dissertation (20 Feb, 2023);
- CV in European format;
- Protocols of departmental council related to the opening of the procedure and with the preliminary discussion of the dissertation (No 01/ 10 Jan, 2023 and No 02/ 6 Feb, 2023);
- Dissertation work;
- Summary of the dissertation;
- List of scientific publications on the topic of the dissertation;
- Copies of scientific publications;
- Declaration of originality and authenticity of the attached documents;
- Reference for compliance with the minimum national requirements

The doctoral student has attached three scientific publications directly related to the dissertation, two of which are in journals with an IF in the Q1 quartile and one in Scientific Works of USB-Plovdiv, included in the NACID database for refereed publications.

Brief biographical data of the doctoral student

Nikol Hadjieva was born on January 26, 1994. She completed her higher education at the University of Plovdiv "Paisii Hilendarski". She obtained a bachelor's degree in the specialty "Biology and Chemistry" with the professional qualification "teacher" in the period 2013-2017. She defended her master's degree in 2018, majoring in "Molecular Biology". Nikol Hadjieva was enrolled in full-time doctoral studies in 2019, in the doctoral program "Molecular Biology" at the Department of "Plant Physiology and Molecular Biology" at the Faculty of Biology, PU "P.Hilendarski". During the training process, she acquired knowledge and skills related to techniques for performing molecular genetics analyses. She has excellent command of written and spoken English as well as very good computer skills to perform bioinformatics analyses. Nikol Hadjieva currently holds the position of "Biologist" at a clinical laboratory, the city of Lovech.

2. Relevance of the topic

The presented dissertation examines current issues related to a relatively new class of plant pathogens causing viroid infections. The representatives of this class of phytopathogens are small, single-stranded, covalently closed circular RNA molecules that lack a protective protein

capsid. Despite the limited genetic information (genome of 239-401 nucleotides) and simplified structure, viroids alter the cellular mechanism and physiology of host plants and can cause severe systemic infections. Even minor changes in their nucleotide sequence are capable of inducing completely different symptoms within one host, incl. varietal variation while generating very similar molecular variants of viroids. This extremely high genome plasticity defines viroids as the most rapidly evolving biological system and an expanding potential threat to a number of agricultural crops. The emphasis of research in the current dissertation work is on the specificity of the relationships between Potato Spindle Tuber Viroid (PSTVd) and a host - pepper (*Capsicum annuum* L.). The studies on this pathosystem are limited, although the causative agent of potato tuber spindle-shaped was the first viroid identified. PSTVd is characterized by its ubiquity and wide host range, which also determines its economic importance. More detailed researches have been carried out mainly with potato and tomato hosts. In this aspect, the topic, the aim and the tasks set in the present dissertation work are particularly relevant. The obtained results represent a certain theoretical and scientific-applied contribution to clarifying the molecular mechanisms related to varietal specificity, pathogenesis, physiological-biochemical changes and the severity of symptoms in pepper infection with PSTVd.

3. Knowledge of the problem

The presented literature review contains a thorough and informative analysis of the scientific literature related to the topic of the dissertation. It was prepared on the basis of 240 literary sources presented in English. Earlier and contemporary scientific publications are listed, with about 30% published in the last five years. The information presented is systematized in six sections and covers the main aspects of viroid infections, with special attention to the studied viroid pathogen PSTVd (its genomic organization, mechanism of replication, pathogenesis, hosts, etc.). The known scientific information on the role of molecular genetic factors and the involvement of microRNA molecules in the plant defense response to PSTVd infection is comprehensively summarized. The literature review concludes with a characterization of the host used in the study - pepper and data from large-scale analyzes of expression profiles in pepper infection with various phytopathogens. In this way, the topic and purpose of the dissertation is convincingly substantiated. The structure, scope and interpretation of the presented literary material show that the doctoral student is very well acquainted with the current state of the researched problem.

4. Research methodology

A wide range of modern molecular-biological, bioinformatics and statistical methods was used to realize the set goal and tasks: bioassays; total RNA isolation, quantitative and qualitative analysis; PCR - for specific amplification; RT-qPCR to study the expression levels of microRNA genes and of protein-coding genes; next-generation sequencing of microRNA and mRNAs, etc. The techniques and procedures used are comprehensively described, allowing comparability and repeatability of results. The experimental staging is also adequate for the purpose. The choice of the two pepper cultivars (Djulyunska shipka and Kurtovska capia), with which the experimental work was carried out, was made after a preliminary screening of 14 pepper varieties of different genotypes. The correct methodical approach, the set of appropriate methods and the precision in performing the analyzes allow achieving the objective and obtaining results that provide an answer to the tasks in the dissertation.

5. Characteristic and evaluation of the dissertation work and contributions

The dissertation has a total volume of 121 pages, including a list of cited literature (23 pages). The structure is standard with a well-balanced proportion between the individual sections: introduction and literature review (27 pages); purpose, tasks, material and methods (18 pages); results and discussion(40 pages); conclusions and contributions (3 pages). The results are presented in 7 tables and 33 figures, incl. author's photo material. Five additional Excel tables are attached with some of the data related to gene-ontology analysis of overlapping and cultivar-specific differentially expressed genes and transcription factors identified in the pepper cultivars following PSTVd infection.

The aim of the dissertation is to identify specific interactions between Potato Spindle Tuber Viroid and two Bulgarian pepper cultivars. This is achieved through clearly and concretely formulated six tasks aimed at the study of molecular genetic mechanisms in this pathosystem.

The rich set of data obtained is presented in a logical consistency, and the results are adequately interpreted and discussed in the context of the latest scientific achievements on the subject. The results are summarized in 10 conclusions. The time of manifestation of a PSTVd-specific phenotype and differences in symptom intensity between the two pepper cultivars were determined. Differences in levels of accumulation and distribution of PSTVd (+) RNA in the analyzed libraries of pepper cultivars were recorded. A cultivar-specific response to PSTVd infection due to differences in gene expression was established - specific differentially expressed

genes and opposite expression profiles were identified. Small interfering RNAs have been mapped in PSTVd-infected pepper cultivars, suggesting their active involvement in plant defense through RNA silencing.

Based on the overall research and the results obtained from the dissertation, five scientific and scientific-applied contributions have been formulated. I would define the following three as **original scientific contributions**: **1)** For the first time the molecular interactions between PSTVd and two cultivars of Bulgarian pepper were investigated by large-scale expression analyzes of small RNAs and mRNAs; **2)** For the first time, differentially expressed miRNAs and differentially expressed protein-coding genes were identified in the pepper cultivars Kurtovska capia and Djulyunska shipka, which collectively determine the induction of a cultivar-specific response to PSTVd; **3)** The described molecular interactions between PSTVd and pepper, suggest their role in modulating viroid spread, replication, and development of viroid-specific symptoms, which may contribute to the development of strategies to improve the control of PSTVd infection in agricultural crops. The following can be considered as **scientific- applied contributions**: **1)** The results of large-scale sequencing of mRNA obtained from infected and control pepper plants are annotated in the NCBI bio-database; **2)** The results of the large-scale sequencing of small RNAs are annotated in the NCBI SRA bio-database.

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

Three co-authored scientific publications are presented in connection with the dissertation work. The doctoral student is first author in one research paper and second in the other two articles, which shows her active participation and personal contribution in the developed topic. Two of the articles were published in journals with a high impact factor in the Q1 quartile (*Plants* and *Scientia Horticulturae*) and one in Scientific works USB-Plovdiv, included in the NACID database for refereed publications. The doctoral student also participated in six national and international scientific forums, where a part of the results have been reported. The presented publications meet and exceed the requirements of the Faculty of Biology, adopted in connection with the Regulations of the University of Plovdiv for the implementation of the Academic Staff Development Act in the Republic of Bulgaria and the stipulated national minimum requirements for obtaining an educational and scientific degree "doctor".

7. Abstract

The abstract presented in Bulgarian and English is prepared in accordance with the requirements and accurately reflects the content and main results achieved in the dissertation.

8. Recommendations for future use of dissertation contributions and results

I have no critical remarks and recommendations to the presented dissertation work.

Given the importance of infections of viroides at a number of agricultural crops, the achievements of the dissertation work can be used and expanded in the direction of epigenetics and inclusion in a breeding plant-protection programs not only for pepper, but also for other the hosts of the viroid.

CONCLUSION

The dissertation *contains scientific and scientific-applied results that represent an original contribution to science and meet all the requirements* of the Academic Staff Development Act in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the relevant Regulations of the University of Plovdiv "Paisiy Hilendarski".

The dissertation shows that the PhD Nikol Slaveeva Hadjieva **possesses** in-depth theoretical knowledge and professional skills in the scientific specialty "Molecular Biology", **demonstrating** qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my *positive assessment* of the conducted research, presented by the above-reviewed dissertation work, abstract, achieved results and contributions, and *I propose to the honorable scientific jury to award the educational and scientific degree "doctor"* to **Nikol Slaveeva Hadjieva** in the field of higher education: 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, PhD program "Molecular Biology".

Date: April 12, 2023

Member of the scientific jury:
(Prof. Tsveta Hristeva, PhD)