

OPINION

From Prof. Dr. Teodora Atanasova Staykova – Plovdiv University „Paisii Hilendarski“

of a dissertation for awarding the educational and scientific degree “**Doctor**”

by: field of higher education 4. *Natural science, mathematics and informatics*

professional direction 4.3 *Biological Sciences*

doctoral programme *Molecular Biology*

Autor: *Nikol Slaveva Hadzieva*

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

Scientific supervisor: *Assoc. Prof. Dr. Maryiana Hristova Gozmanova – Plovdiv University „Paisii Hilendarski“*

1. General presentation of the procedure and the PhD student

By order No. ПД-21-456 from 02.03.2023 of the Rector of the Plovdiv University “Paisii Hilendarski” I have been appointed as a member of the scientific jury to ensure a procedure for the defence of a dissertation on a topic “*Identification of specific interactions between Potato Spindle Tuber Viroid and two Bulgarian pepper cultivars*” for the acquisition of the educational and scientific degree “doctor” in the field of higher education 4. Natural science, mathematics and informatics, professional direction 4.3 Biological Sciences, doctoral programme Molecular Biology. The autor of the dissertation is Nikol Slaveva Hadzieva – full-time doctoral student at the Department of Plant physiology and Molecular biology with a scientific supervisor Assoc. Prof. Dr. Maryiana Hristova Gozmanova from Plovdiv University „Paisii Hilendarski“.

The set of paper materials submitted by PhD student Nikol Hadzieva is in accordance with Art. 36 (1) of the Regulations for the Development of the Academic Staff of the PU and includes all the necessary documents - a request to the Rector to open the procedure, a protocol from the departmental council, a curriculum vitae, dissertation, abstract, declaration of originality and authenticity of the attached documents. The doctoral student has also presented three publications, two of which are in English.

Nikol Hadzieva graduated from high school for foreign languages in Lovech with a major in English in 2013. In 2017 obtained a bachelor's degree in the specialty "Biology and Chemistry", and in 2018 obtained master degree in the specialty "Molecular Biology" at PU "P. Hilendarski". She was enrolled as a full-time doctoral student in "Molecular Biology" at the Faculty of Biology of the PU in 2019.

2. Actuality of the topic

The potato tuber spindle viroid (PSTVd) disrupts the normal growth and development of plants, as it provokes changes in the shape and size of fruits, morphological changes in the stems and leaves of plants, suppresses the expression of genes responsible for the biogenesis and functioning of chloroplasts in the host plant, which is also associated with a significant reduction in yields. The response of the cultivar *Capsicum annuum* L. to PSTVd infection is understudied, which makes the subject of the dissertation work relevant and the results obtained significant, as they could be used in the development of strategies to control PSTVd infection in agricultural plants.

3. Knowing the problem

The literature review is structured in six main parts and is based on the study of a large number of literary sources. The manner in which the information is presented shows a detailed knowledge of the problem. The genomic organization of PSTVd, modes of spread, mechanism of replication, symptomatology and change in gene expression in infected plants in response to PSTVd infection

were studied. Systematized information on the role of microRNAs in the host response to infection is also presented. A special place is dedicated to the plant test object used - pepper. The summarized literature information enables a clear motivation of the purpose of the research. The doctoral student shows the ability to carry out independent and in-depth scientific analysis, which allows her to skillfully interpret the obtained results.

4. Research methodology

Based on conducted biotests, 2 of 14 pepper varieties with different phenotypic characteristics and agricultural purpose were selected. These are the Bulgarian varieties Djulyunska shipka and Kurtovska kapia, which demonstrate the most pronounced phenotypic reaction after infection with PSTVd.

An excellent impression is made by the use of a set of modern molecular methods that are adequate to the purpose of the dissertation work and include: quantitative and qualitative analysis of the isolated total RNA, next-generation sequencing of small RNAs, synthesis of complementary DNA, PCR, quantitative analysis of the relative gene expression as well as bioinformatic analyses.

The correctly selected complex of methods allows the achievement of the set goal and the adequate solution of the tasks formulated in the dissertation work.

5. Charakterization and evaluation of the dissertation work and contributions

The presented dissertation work of Nikol Hadzhieva is 119 pages long and includes 7 tables and 33 figures. The work is structured according to the requirements in the following main parts: Introduction (1 page), Literature review (24 pages), Aim and tasks (1 page), Material and methods (18 pages), Results (32 pages), Discussion (6 pages), Conclusions (2 pages), Literature (23 pages), Scientific and scientific-applied contributions (1 page). A list of publications and participation in scientific symposia and conferences is also presented. The dissertation is balanced in terms of volume and ratio between the individual parts. In accordance with the purpose of the study, 6 tasks are formulated. The obtained results are described in detail, the summarized data are presented in tables and figures. The discussion made by the PhD student shows good skills in interpreting scientific results and comparing them with those obtained by other authors. The skillful use of scientific terminology makes a good impression. Based on the obtained results and their interpretation, 10 conclusions were formulated that highlight the established dependencies. The list of cited literature includes 240 literary sources in English only.

Nikol Hadjieva's dissertation work is based on the study of a well-chosen plant test subject using appropriate methods. The obtained results and their interpretation enable findings and conclusions of fundamental and applied importance.

As a result of the carried out research work, the following contributions can be defined:

- As ***original scientific contributions***, I would point out the study of the molecular interactions between PSTVd and two Bulgarian pepper varieties through large-scale expression analyzes of small RNAs and mRNAs, as well as the establishment of differentially expressed miRNAs and protein-coding genes that determine the variety-specific infection response.

- As scientific and applied contributions, I would point out the possibility of using the detected molecular interactions in the development of strategies to control PSTVd infection in economically important plant crops, as well as the annotation of the results obtained from the sequencing of mRNA and small RNAs in the bio database NCBI.

The contributions formulated in this way outline the prospects for using the scientific results established in the dissertation research.

I have some critical notes related to the technical layout of the work and admitted inaccuracies. For example:

- two different figures (on pages 16 and 23) are marked with the same number as Fig. 5;
- figures 8, 24 and 33 are of poor quality;

- those shown in fig. 9 graphs could be differentiated as notations;
- table 5, which is cited in the text, is missing;
- the abbreviated names of the samples in the text and table 6 would be good to be uniform (written only in Latin or only in Cyrillic);
- the summary comparison made on page 71 regarding DEG in the two pepper varieties, as a result of the infection, sounds unclear.

Admitted inaccuracies do not reduce the importance of the work.

I want to congratulate the PhD student for the very good pictures of electrophoregrams.

6. Assessment of the PhD student's publications and personal contributions

Doctoral student has presented a list of 3 publications related to the subject of the dissertation research. Two of the publications were published in indexed and refereed journals in world-renowned databases with a Q1 and IF of 3.935 and 3.463, respectively. One of the publications was printed in Scientific works of the Union of Scientists of Bulgaria.

Results of the conducted research were reported at 6 conferences, 4 of which with international participation.

The PhD student is the lead author in one of the publications. The submitted declaration of originality proves that the analyzes included in the dissertation research, the results obtained and the summaries made are with the personal participation of Nikol Hadzieva.

7. Autor's abstract

The printed autor's abstract has a volume of 33 pages and is structured according to the requirements, reflecting the main results, summaries and conclusions of the dissertation research.

8. Recommendations for future use of dissertation contributions and results

I recommend the doctoral student and the scientific team in which she works to continue the work on the study of the specific interactions between PSTVd and the plant organism, expanding the spectrum of the used test objects.

CONCLUSION

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

The dissertation shows that the doctoral student Nikol Slaveva Hadzieva possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Molecular Biology" by 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 a dissertation work, an abstract and the results described in them, as well as the formulated contributions, and I propose to the honorable scientific jury to give an educational and scientific degree "doctor" to Nikol Slaveva Hadzieva in the field of higher education: 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences, PhD Program in Molecular Biology.

17.04. 2023

Prepared the opinion:

Prof. Dr. Teodora Staykova