

## REVIEW

from the DSc. Ivan Nikiforov Minkov, professor, IMBB  
of a dissertation for awarding the educational and scientific degree "doctor" on:  
field of higher education: 4. Natural sciences, mathematics, and informatics  
professional direction: 4.3. Biological Sciences  
PhD program: Molecular Biology

### **Author:**

Nikol Slaveva Hadjieva

### **Subject:**

Identification of specific interactions between Potato Spindle Tuber Viroid and two Bulgarian pepper cultivars.

### **Supervisor:**

Assoc. Prof. Dr. Mariyana Gozmanova, "Paisii Hilendarski", University of Plovdiv.

### **1. General description of the presented materials**

By order No. RD 21-456 of March 2, 2023 of the Rector of the Plovdiv University "Paisii Hilendarski" (PU), I have been appointed as a member of the scientific jury to ensure the procedure for the defence of a dissertation work on the 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" (PhD) in the field of higher education 4. Natural sciences, mathematics, and informatics", professional direction 4.3. Biological Sciences, Molecular Biology PhD Program.

The author of the dissertation work is Nikol Slaveva Hadjieva – a full-time PhD student at the Department of Plant Physiology and Molecular Biology, with scientific supervisor Associate Professor Mariyana Gozmanova, University of Plovdiv "Paisii Hilendarski".

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

- a request to the Rector of the PU to disclose a procedure for the defence of a dissertation work.
- curriculum vitae in European format.
- protocol from the departmental council, related to reporting the readiness to open the procedure and preliminary discussion of the dissertation work.
- abstract.
- declaration of originality and authenticity of the attached documents.
- certificate of compliance with national requirements
- a list of scientific publications on the topic of the dissertation.
- dissertation work.
- copies of scientific publications.
- a set of paper documents.
- document for paid fee as required

The doctoral student has attached 3 publications.

## **2. Brief biographical data for the doctoral student**

Nikol Hadzhieva completed her higher education at Paisi Hilendarski University of Plovdiv in the bachelor's program in biology and chemistry in 2017 and subsequently graduated from the master's program in molecular biology in 2018. During her studies, she acquired a significant level of knowledge and skills related to the dissertation work. She is fluent in English - she graduated from a high school teaching English and has completed a course on English academic writing in the field of natural sciences. She has basic computer skills (MS Office) as well as basic knowledge of bioinformatics, data processing and statistics necessary for manipulation of data in her research.

## **3. Actuality of the topic and appropriateness of the set goals and tasks**

The topic of the dissertation is generally very current. It covers two basic problems - viroids, as a special form of existence of genomes and of life in general, their interaction with their hosts - plants and all the molecular connections between them. Although the basic mechanisms of parasitism and other viroid-plant interactions are generally known, the finer mechanisms of mutual regulation are still being explored and there are many uncertainties in this area, especially as regards of the mechanisms of specificity of viroids development in their hosts.

On the other hand, the "existence" of viroids as an independent, "naked" RNA molecule (without the protein coat of viruses) hints at very ancient times of the origin of the genome, and their interaction with complex eukaryotic genomes - at the billions of years of evolution of life. A delicate moment in this direction is the participation of microRNAs in these interactions.

The pragmatic significance of the questions is that many hosts of viroids are cultural plants of great economic importance and viroids can cause serious damage to agriculture, and the methods of combating them are non-traditional, difficult, and expensive. On the other hand, viroids, which are most often named after their primary host, can also parasitize on other plants within the taxonomic group of the primary host, and sometimes more distant relatives. This shows that the mechanisms of infection are much more complex than known and there is a wide field of future research here.

The dissertation examines the interaction of two varieties of pepper and the Potato Spindle Tuber Viroid (PSTVd), whose main host is potatoes. More special attention is paid to eukaryotic genes - targets of viroid infection, which on the other hand is also a very important biotic factor for causing stress in higher plant hosts. And to make the picture even more complicated, another class of small RNA molecules - microRNA (miRNA) - are involved in the study, which, apart from being specific, carry out a massive process of gene expression regulation in eukaryotes (including plants), may also interact with other small RNAs, such as viroids, and substantially alter mechanisms of interaction, specificity, parasitism, and biotic stress.

All this shows that the topic of the dissertation is well thought out and composed and has significant theoretical and practical significance.

## **4. Knowing the problem**

The extensive overview of the problems mentioned in the previous section of this review, made in the dissertation, shows that the doctoral student has done a very substantial study of the existing published research in this field and as a result knows the state of the problem, and the literature review is made with a creative appreciation of the written sources.

## **5. Research methodology**

To achieve the set goals and objectives of the study, two Bulgarian varieties of pepper - Julyunska shipka (DSh) and Kurtovska capia (KK) (*Capsicum annuum* L.) were used as objects, which have a different phenotype when infected with the PSTVd isolate KF440-2 for challenge purposes.

In general, it can be said that the chosen research methodology allows achieving the set goal and obtaining an adequate answer to the tasks set in the dissertation work. These are a large group of methods related to bioassays on pepper cultivars with in vitro synthesized PSTVd (+) RNA, isolation, and quantitative and qualitative analysis of total RNA, NGS and analysis of mRNA and small RNAs, synthesis of complementary DNA, PCR for amplification of the viroid or host genes.

An essential part of the methodological approaches is the use of RT-qPCR with the fluorescent dye SYBR Green I, with the aim of analysing the expression levels of miRNA genes and protein-coding genes, as well as the bioinformatics analysis of the NGS data for the different sequenced RNAs and the differential expression analysis of genes involved in interaction with viroid RNA. Data from NGS performed on small RNAs have been deposited at NCBI data base.

## **6. Characterization and evaluation of the dissertation work**

The entire dissertation is presented in 94 standard "typescript" pages (~30 lines/65 characters) and is classically structured in several sections - "Literature review", ending with the formulation of the research goals and objectives, such as the purpose of the work is defined quite generally. The objects and research approaches used are presented in the Material and Methods section. The obtained results and their discussion are given in two separate sections ("Results" and "Discussion"), which is a more appropriate way of presentation. Brief sections follow - 'Conclusions', 'References', 'Contributions' and 'Publications'. I believe that it was appropriate, after the literature review, to define the overall hypothesis of the research more clearly, which is not expressed very clearly in the set tasks.

The literature review is well structured in terms of the planned work, its individual parts are well balanced in terms of their importance for the dissertation. Mostly new research on the problem is presented, which ensures that the PhD student is well informed about the current state of the problem and avoids "discovering hot water". The dissertation student shows a good knowledge and good handling of the known data on viroids in the literature, specifically on PSTVd and the interaction of the viroids with plants. Overall, this section is a good overview of the overall viroid-host problem, specificity, molecular mechanisms of viroids development and plant resistance, and their economic importance. The main object of research - pepper and the specific varieties used - is also characterized.

I consider the highlighted hypothesis for the modern origin of viroids to be highly improbable, since the enzymatic activity of viroid RNA is a classic "molecular fossil" from the earliest stages of evolution, especially if the proposed hypothesis of the existence or a real micro-RNAs and viroid RNAs interaction is true.

The results presented in the mentioned section are logically arranged according to the set tasks and follow the logical course of plant infection with PSTVd: analysis of levels of viroid RNA accumulation, expressed viroid specific small RNAs, mRNAs and micro RNAs. An essential place is given to the analysis of micro-RNA in the infected plant by NGS - mapping, change during viroid infection and possible effect on silencing of target genes (mRNA) for resistance of the plant to the viroid. The data on differential expression of transcription factors and the expression analysis of selected genes in the viroid infection are also interesting.

Discussion of the resulting studies is almost evenly split between commenting on the influence of microRNAs on pepper infection and resistance (hosts), expression of transcription factors and differential expression of genes conferring some resistance to the viroid(s). In all three mentioned groups,

the PhD student comments on her own data, which indicate a certain involvement of the three systems in infection and resistance to PSTVd. In this section, the comments of literature data somewhat prevail over the discussion of the own results, which to some extent turns it into an additional literature review.

The conclusions drawn well cover the results obtained and their discussion, with an essential part following the varietal differences in the molecular mechanisms of the host response to the viroid. Some of the conclusions are more descriptive and some are more analytical.

## **7. Contributions and significance of the development for science and practice**

The contributions are well described in the dissertation, so I will not detail them in the review. They mainly refer to proving by new means essential new sides and obtaining corroborating facts in existing scientific problems and theories. The more original and newer contributions are related to the varietal resistance of the investigated pepper varieties, and they have significance for practice as well.

By itself, the annotation of the obtained NGS results for mRNA and microRNA obtained from infected and control pepper plants in the NCBI bio database is a substantial contribution to the thesis.

## **8. Evaluation of publications on the dissertation work**

A total of three publications are presented, two of which have a good impact factor (Q1) and one - without an impact factor, in a Bulgarian edition. One of the publications - in Plants (MDPI) is open access, which is definitely positive, as it increases the quick and detailed familiarization with the research done and the quick response as citations. All publications have more than three authors. In one of the presented publications, the doctoral student is the first author. All submitted publications note her "equal participation" with the first/second author. In this way, the publication activity of the doctoral student fully satisfies the requirements of the law and the regulations for its implementation for the scientific and educational degree "doctor".

The publications are of very good quality, with the first two being published in English in renowned and well-referenced international editions, and they sufficiently represent the research done, the results obtained and their discussion, and they match well with the material presented in the dissertation and the abstract.

The presented publications have found application as the implementation of funded scientific projects, which is duly noted.

The presence of the PhD student in Scopus is also a good achievement, where a total of 5 citations of two of the publications on the dissertation are shown, and she herself forms a complete h-factor - 2 out of a total of three publications, which is largely due to her supervisor.

## **9. Personal participation of the doctoral student**

Partly knowing the PhD student, the excellent scientific group in which she has worked, the context of the overall research, as well as from conversations with her supervisor, I can judge that the dissertation research conducted, results obtained, and the contributions formulated are to a large enough extent her merit. Despite the relatively large number of co-authors in the presented publications, the individual participation of Nikol Hadjieva is clearly visible.

## **10. Abstract**

The abstract well reflects the main results achieved in the dissertation and is made very qualitatively in terms of layout, figures and tables and high-quality Bulgarian language. In addition, it is made according to the requirements of the relevant regulations and can serve as a quick introduction to the dissertation work.

## **11. Critical remarks and recommendations**

It would be good not to use abbreviations in formulating the conclusions of the dissertation research. This makes it difficult to understand the conclusions drawn, as it forces the reader to translate each abbreviation (although they are well described in the dissertation and the abstract).

The abstract refers to "selected" pepper cultivars for research, implying selection, while it is obvious that it is a "choosing" of cultivars for analysis.

There are some neologisms in the dissertation that I personally don't like: " novogenerationno", "verticilijno" and " vdgudgechvane ".

## **CONCLUSION**

The dissertation contains scientific, scientific-applied, and 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 Hadjieva possesses in-depth theoretical knowledge and professional skills in the scientific area "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 honourable scientific jury to award the educational and scientific degree "doctor" to Nikol Slaveva Hadjieva in field of higher education: 4. Natural sciences, mathematics, and informatics; professional direction: 4.3. Biological Sciences; PhD program "Molecular Biology".

12/04/2023

Reviewer:

Prof. DSc. Ivan Minkov