

EVALUATION REPORT

by **Dr. VALYA NIKOLOVA VASSILEVA**, Professor at the Research Department 'Molecular Biology and Genetics', Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences on the materials submitted for receiving the academic degree '**DOCTOR OF SCIENCES**', Sofia University 'St. Kliment Ohridski'

AREA OF HIGHER EDUCATION: 4. Natural sciences, mathematics and informatics;

PROFESSIONAL FIELD: 4.3 Biological sciences;

SPECIALITY: Molecular Biology

AUTHOR OF THE DSc THESIS: **Dr. LYUBEN IVANOV ZAGORCHEV**, Associate Professor at the Department of Biochemistry, Faculty of Biology, Sofia University 'St. Kliment Ohridski'

1. GENERAL OVERVIEW OF THE PROCEDURE AND THE CANDIDATE

By order No. *RD-38-157 of 03/04/2023* of the Rector of Sofia University (SU) 'St. Kliment Ohridski', I have been appointed as a member of the Scientific jury for the defence of the thesis for obtaining the academic degree of 'Doctor of Sciences' in the *Area of higher education: 4. Natural sciences, mathematics and informatics; Professional field: 4.3. Biological Sciences; Speciality: Molecular Biology.*

Dr. Lyuben Ivanov Zagorchev, the author of the DSc thesis, is an Associate Professor at the Department of Biochemistry, Faculty of Biology (BF) at SU 'St. Kliment Ohridski'. The submitted documents fulfil all the requirements stipulated by the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB) and the Regulations for its implementation. The documents are appropriately formatted, adhering to the prescribed guidelines regarding structure, content and volume. Furthermore, Assoc. Prof. Zagorchev research achievements satisfy the national requirements and criteria for the acquisition of the academic degree of 'DOCTOR OF SCIENCES'.

Assoc. Prof. Lyuben Zagorchev completed his secondary education at the National High School of Mathematics and Natural Sciences 'Akademik Lyubomir Chakalov' in Sofia in 2000, specialising in 'Biology and biotechnology, and advanced study of English'. He obtained his Bachelor (BSc) degree in 'Molecular Biology' in 2004, and his Master (MSc) degree in 'Biochemistry' in 2006 from the Faculty of Biology (BF) at SU 'St. Kliment Ohridski'. In 2012, he successfully defended his PhD thesis on the topic: 'Biochemical changes in the composition and structure of the cell wall of embryogenic callus cultures of *Dactylis glomerata* L. treated with NaCl'. Following the completion of his PhD, he served as a Research assistant from 2013 to 2017, and was subsequently appointed as an Associate Professor in the Faculty of Biology at 'St. Kliment Ohridski' University, a position he has held since 2017.

Dr. Zagorchev has extensive teaching experience, mainly at SU 'St. Kliment Ohridski', who at an earlier stage of his career was associated with leading practicals in Biochemistry and Molecular Biology for BSc students, and after his habilitation in 2017, with lectures in Biochemistry for BSc in Agrobiotechnology and Biotechnology specialities at BF, and in Chemistry and Ecochemistry specialities at the Faculty of Chemistry and Pharmacy, and in the Physical Education and Sports specialities at the Faculty of Educational Sciences and Arts.

Under the Erasmus+ programme, he gave a series of lectures at the Department of Agriculture at the Mediterranean University of Reggio Calabria, Italy. He leads lectures and practicals on discussion problems in molecular biology, omics technologies, etc., for MSc students at BF. He was the supervisor of 7 graduate students (5 in BSc and 2 in MSc), and another 4 graduates defended their degrees on projects led by Assoc. Prof. Zagorchev, on which the diploma work was financed.

Since 2020, Assoc. Prof. Lyuben Zagorchev has taken on the role of Deputy Dean for quality management, scientific and project activities, at BF of SU 'St. Kliment Ohridski'. Additionally, starting from 2021, he has been appointed as a 'Recognised Researcher R2' at the Competence Center

'Sustainable utilization of bio-resources and waste from medicinal and aromatic plants for innovative bioactive products'.

In addition to his academic achievements and positions held at SU 'St. Kliment Ohridski', Assoc. Prof. Zagorchev has also engaged in research specialisations in Australia, Austria, Great Britain and China. These international experiences have contributed to his professional development and enhanced his expertise in the field of molecular biology and biochemistry. All of these activities demonstrate that Prof. Zagorchev is an engaged, capable and promising scientist, who possesses the ability to effectively share his experience and expertise with the younger generation.

2. GENERAL EVALUATION OF THE DSC THESIS

The presented DSc thesis fully meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria. The total volume is 277 pages, and the text is organised in a standard way into several main sections: it begins with an Introduction (2 pages), Literature review (48 pages), Aim and objectives (1 page), Materials and methods (15 pages), followed by Results (73 pages), Discussion (20 pages), Conclusions (2 pages), Achievements (1 page) and References (14 pages). Eighty-seven pages of Supplementary Materials are also included. The literature overview and the Results are structured logically in relevant subsections. The thesis ends with 337 references, which includes both older developments and research from recent years. The DSc thesis leaves an overall impression of a well-structured work, demonstrating a high level of thoroughness and organisation.

The Introduction part briefly presents the significance of the problem of interest and the need to expand knowledge about the distribution, taxonomic status and molecular mechanisms of parasitism of the genus *Cuscuta*, as well as the influence of abiotic and biotic stress factors on these parasitic plants. Emphasis is placed on the fundamental and applied importance of this knowledge, which will enable predicting the effects of climate change stressors.

In *the Literature Overview*, a comprehensive review of the available literature has been made. The information on the classification and role of parasitic plants, their influence on host plants, the mechanisms of parasitism in the genus *Cuscuta*, the effect of abiotic stress factors on parasitic plants (drought, salinization, heavy metals), as well as biotic factors, emphasising the relatively small number of studies on the effects of biotic stress on representatives of the genus *Cuscuta*. The literature overview is well illustrated with 11 figures and 3 tables, and ends with a brief conclusion that justifies the need for research on the problem.

The main goal and tasks of the thesis are clearly formulated, and the main goal is based on three working hypotheses, for the proof of which several basic research tasks are set.

The Materials and Methods section describes in detail the plant material used and the experimental approaches, which provide the opportunity to examine the problem from different sides and ensure the reliability of the obtained results. Innovative approaches, such as genomic, transcriptomic and metabolomic analysis, as well as biochemical, physiological and statistical analyses were used. Dr. Zagorchev also presented the software products used to create and design the DSc thesis. All materials, methods and procedures are described in detail and comprehensively.

The Results section is the most extensive section detailing the new experimental data obtained. The results are logically divided into four main subsections and illustrated with 76 figures and 13 tables. Furthermore, the thesis includes a comprehensive set of 87 appendices, offering significant supporting evidence and supplementary information on the subject matter. These appendices can serve as valuable resources for fellow scientists engaged in research within the field of parasitism.

In *the Discussion* section, Prof. Zagorchev provides a comprehensive summary and critical analysis of the results obtained, placing them in the context of existing literature. The discussion is divided into

three sub-sections, skilfully addressing the distribution, host range, and impact of *Cuscuta* spp. on host plants, as well as the effects of abiotic and biotic stress on the parasite-host interactions. To enhance the comparability and categorisation of various hosts in terms of their susceptibility to *Cuscuta* spp., the author has thoughtfully included an algorithmic scheme (Fig. 85) to address any potential inconsistencies in host classification terminology. This demonstrates a meticulous approach to ensure accuracy and clarity in the analysis. The discussion is comprehensive, effectively aligning with the findings obtained, and provides a thorough exploration of the implications and significance of the results.

The dissertation culminates with 10 *Conclusions*, organised according to the three formulated hypotheses. Additionally, the author presents 8 *Achievements*, with 4 being fundamental in nature, 3 being scientific-applied, and one being methodological. I fully agree with the formulated conclusions and achievements, as they are in line with the objectives of the study and supported by a significant body of experimental data.

A comprehensive originality check was performed on the DSc thesis using a plagiarism prevention system, which conclusively determined that the identified similarities do not display any indications of plagiarism.

3. RELEVANCE OF THE SUBJECT AND EVALUATION OF THE RESULTS

The DSc thesis is dedicated to an important and understudied problem that focuses on the distribution, taxonomic status, and molecular mechanisms of parasitism in species belonging to the genus *Cuscuta*. The study possesses a distinctiveness that sets it apart both nationally and internationally. Built upon three core hypotheses, the successful validation of which contributes to the attainment of the primary objective of the DSc thesis. The findings, presented comprehensively, are articulated in a clear, accessible, and persuasive manner, further bolstered by excellent visual representations and illustrations.

The research conducted in relation to the *First Working Hypothesis* provides evidence that the introduced species *Cuscuta campestris* exhibits a higher invasive and parasitic potential compared to native species. The study encompasses an assessment of species and genetic diversity within the genus, focusing on representatives found in Bulgaria. The research is based on plant material collected from 54 locations covering four common species in Bulgaria (*Cuscuta approximata*, *C. campestris*, *C. epithymum* and *C. europaea*). To evaluate genetic diversity, ITS sequences were utilised, while a phylogenetic tree was constructed using available rDNA sequences. While RAPD markers were found to be suitable for species identification, their efficacy in characterising intraspecific diversity of *Cuscuta* spp. was limited. These findings significantly enhance the understanding of the distribution of the *Cuscuta* genus and its host spectrum. An important scientific and applied contribution of this research is the expansion of the Herbarium collection at the SU 'St. Kliment Ohridski' with additional materials.

Research pertaining to the *Second Working Hypothesis* reveals the impact of biotic and abiotic stressors on the parasite-host interaction within *Cuscuta* spp. Furthermore, the influence of host species on *Cuscuta* spp. parasitism, and the effect of salinity on various stages of parasitism, was examined. This section provides compelling evidence of the detrimental and dose-dependent consequences of salinity on all developmental stages of *Cuscuta*, and the influence of host species on parasitism in *Cuscuta* spp., as well as the influence of salinity on different stages of parasitism was examined. This section convincingly demonstrates the negative and dose-dependent effect of salinity on all developmental stages of *Cuscuta*. The response to salt stress is shown to be contingent upon the specific host species, and salinity can induce alterations in the host itself. An intriguing finding is the pronounced influence of host species on the *Cuscuta* metabolome, surpassing that of abiotic stressors.

Additionally, an important result with both scientific and practical implications is the observed impact of aboveground parasitism on the interaction between the root system and the rhizosphere microbiome. *Cuscuta* parasitism disrupts the host ability to adapt to both salinisation and insect herbivory. Through transcriptome analysis of *Trifolium repens* plants infected with *Cuscuta australis*, differentially expressed genes were identified between the infected hosts and the control group. The conducted numerous studies demonstrated that environmental and host conditions influence the viability of different parasite populations, implying that parasitic plants suppress host defence mechanisms. Many of these studies stand out as unique contributions, both nationally and internationally.

The investigation pertaining to *Working Hypothesis 3* provides compelling evidence that parasites belonging to the *Cuscuta* genus are susceptible to the influence of biotic factors, which significantly impact their metabolism. The study explores the influence of plant pathogens on the parasitism of *Cuscuta* spp., as well as the potential role of these parasites as hosts and vectors of plant pathogens. Despite limited available data on pathogens or parasites affecting *Cuscuta* spp., the DSc thesis convincingly demonstrates that members of the *Smicronyx* genus exhibit specific preferences for *Cuscuta* spp. as hosts. Infestation by these parasites results in substantial growth retardation, prevents flowering and seed formation, and ultimately leads to the death of infected plants.

From the obtained results, it is deduced that the parasitism of *Cuscuta* spp. hampers the host ability to adapt to biotic stress. Another noteworthy finding with both scientific and practical implications is the established role, particularly of *Cuscuta campestris*, as a reservoir and vector of plant viruses. Among the four agriculturally significant viruses tested, two were detected within *C. campestris* populations. This demonstrates that parasitic plants of the *Cuscuta* genus can serve as active vectors of viruses, transmitting them to the host without exhibiting visible symptoms of virus infection themselves.

Lastly, an important methodological accomplishment is the successful optimisation of a parasite/stress-sensitive host and parasite/stress-tolerant host model system. This system enables comparative analyses of various stress factors on *Cuscuta* spp. Such optimised methodology holds significance not only for colleagues in the field but also for university students and PhD candidates, as it can serve as a valuable resource for conducting similar studies and furthering the understanding of stress responses in *Cuscuta* spp.

4. EVALUATION OF THE RESEARCH INDICATORS

Prof. Zagorchev presents comprehensive and very well-structured information about his research activity, which not only meets the minimum national requirements for the academic degree 'Doctor of Sciences', but in some groups of indicators significantly exceeds them. A list of 20 co-authored research publications is provided, most of which possess an impact factor (IF) and quartile (Q). Among them, 14 publications have an IF (11 - Q1, 2 - Q2 and 1 - Q3) and publications have an SJR (1 - Q1, 1 - Q2 and 1 - Q3). Additionally, 3 book chapters and 4 review articles are provided. The cumulative IF of these articles reaches 51.88. Prof. Zagorchev is first and corresponding author in 15 publications (13 - first author, 11 - corresponding author). The total number of citations of his papers is 351. He has reported his results at 9 international and 10 national scientific forums. A large part of the publications are on topics related to projects he has led. Furthermore, Prof. Zagorchev demonstrates very good management and coordination skills by overseeing 7 research projects and participating in an additional 13 projects, including one COST action (FA0901 - Putting Halophytes to Work - From Genes to Ecosystems).

In terms of meeting the minimum national requirements, according to the information presented, the indicators from **group A** are covered by 50 scores for a defended PhD thesis; 100 scores for **group**

B indicators, 405 scores in **group G** (out of the required 100), and 556 scores in **group D** (out of the required 100). Consequently, Dr. Zagorchev has amassed a total of 1111 scores, significantly exceeding the minimum national requirements of 350 for acquiring the academic degree of 'Doctor of Sciences'. According to the specialised Scopus database, Prof. Zagorchev has an H-index of 8, indicating the recognition of his research within the international scientific community. This signifies the positive reception of his work and the impact he has made in his field.

5. AUTHORISED SUMMARY

The Authorized Summary of the DSc thesis diligently follows the prescribed length and content guidelines, effectively summarising the principal experimental findings and theoretical interpretations. Its structure, comprising 53 pages, adheres to established standards. The Summary also incorporates a list of publications related to the DSc thesis, alongside the author active participation in scientific forums and supervision of BSc and MSc theses. The summarised data leave a favorable impression and effectively highlights his personal contributions to the publications under consideration.

CONCLUSION

The presented DSc thesis delves into a timely topic concerning the exploration of species diversity within the genus *Cuscuta* in Bulgaria, along with investigating the influence of abiotic and biotic stress factors on the relationships between parasites and hosts. In addition to its fundamental significance, the research also has practical importance associated with predicting and mitigating the effects of climate changes.

Prof. Zagorchev presents original and substantial scientific results, laying the basis for well-founded conclusions and contributing to the field with original scientific findings. The utilisation of advanced experimental approaches and the competent interpretation of the results leave a positive impression. The considerable number of high-quality research papers, published in reputable journals and significant citation rates, further supports the quality of the research. The investigated topics are highly relevant and will remain so in the future, with some directly applicable to Prof. Zagorchev educational activities. These factors collectively establish Prof. Zagorchev as a capable and promising scientist in the field of biochemistry and molecular biology, possessing necessary qualities to advance and excel in his academic career.

Based on this analysis, it is evident that the submitted documents fully comply with the requirements stipulated in the Law on the Development of Academic Staff in the Republic of Bulgaria (ZRASRB), and the accompanying regulations for obtaining the academic degree of "Doctor of Sciences." As a member of the Scientific Jury, I confidently vote in favour and strongly recommend to the esteemed members of the Scientific Jury and the Scientific Council of the BF of SU 'St. Kliment Ohridski' to also support DR. LYUBEN IVANOV ZAGORCHEV and award him the academic degree of "DOCTOR OF SCIENCES" in the Professional field 4.3. Biological Sciences, Speciality: Molecular Biology.

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Sofia

Evaluation report prepared by:
(Prof. Dr. Valya Vasileva)