

REVIEW

by **Prof. Ivan Georgiev Ivanov, PhD, DSci**

of the thesis of **Assoc. Prof. Dr. Lyuben Ivanov Zagorchev** entitled "*Influence of biotic and abiotic factors on the parasitism of stem holoparasitic plants of the genus *Cuscuta**", presented for the awarding of the scientific degree "**Doctor of Sciences**" in the Area of Higher Education 4. "Natural sciences, mathematics and informatics", Professional direction 4.3. Biological Sciences, Scientific speciality "**Molecular Biology**"

1. Brief biographical data

Lyuben Ivanov Zagorchev was born on 05/04/1981 in Sofia. In 2004, he graduated from the Faculty of Biology of the Sofia University "St. Climent Ohridski" with a Bachelor degree in "Molecular Biology", and in 2006 acquired a MS in "Biochemistry". During 2007-2012 he was a PhD student at the Department of Biochemistry. After defending his doctoral thesis in plant biochemistry, he took the position Assistant Professor at the same department, later on growing to Chief Assistant Professor (2013-2017) and Associate Professor (since 2017). During his professional development Dr. Zagorchev held short-term internships at a number of outstanding laboratories and world-renowned plant molecular biology research centers in Austria, Australia, the United Kingdom and China. He participated also in 8 training courses organized by various national and international scientific organizations.

Currently Assoc. Prof. Zagorchev is a Deputy Dean of the Faculty of Biology. He lectures Biochemistry, Molecular Biology of the Plant Cell, Omics Technologies and Molecular Biology methods. He has supervised one PhD, 5 MS and 9 BS students.

Prof. Zagorchev is a Principal Investigator of 7 research projects and participant in 3 others. He is the author of 44 scientific papers (of which 33 in IF journals), 5 chapters of monographs and 4 textbooks. The results of his research have been reported at 70 scientific forums, 22 of which abroad. According to Google Scholar, Scopus, Web of Science and Research Gate, his papers have been cited 475-711 times so far and his h-index is 7-12.

2. General overview of the documentation related to the dissertation defense

The documents relating to the defense procedure, as well as the dissertation itself, have been prepared in accordance with the requirements of the Law on Development of the Academic Staff in the Republic of Bulgaria (LDASRB) and the Rules for its implementation. The thesis covers 189 standard pages, contains 337 references and is illustrated with 86 figures, 14 tables and 7 appendices presented on 100 pages. It is structured in a classic style: Literature review, Aims and Tasks, Materials and Methods, Results, Discussion, Conclusions, Contributions and References.

3. Relevance of the thesis topic

The Dr. Zagorchev's thesis is dedicated to the clarification of the biochemical and molecular-biological bases of parasitism in holoparasites of the genus *Cuscuta*, as well as to the clarification of the influence of biotic and abiotic factors on the parasite-host relationship. Taking into account the widespread distribution of *Cuscuta* in Bulgaria and the damage these parasites cause to the domestic crops, the relevance of the thesis topic is obvious.

4. Evaluation of the dissertation work

Literature review. The literature review covers 40 pages and is based on over 300 references. It looks like a monographic study on the molecular basis of plant parasitism and particularly on the biotic and abiotic factors affecting the parasite-host interaction in *Cuscuta* species. Although the review contains several sections, two main parts are well distinguishable: General and Special.

The first (general) part of the review represents an introduction to the nature of plant parasitism, classification of parasitic plants and their significance for agriculture and ecology, with the emphasis on *Cuscuta* stem holoparasites. The parasitism is regarded at organismal level, starting with germination, initial development of *Cuscuta* plants, their photosynthetic ability, host localization, haustoria formation, effects on photosynthetic activity, host biomass accumulation, etc.

The special part of the review is devoted to the influence of abiotic and biotic stress factors on the parasitic plants life, stress and adaptation, as well as stress response and stress tolerance. Special attention is paid to the molecular basis of the pathogenesis associated with plant parasitism, as well as to the proteins associated with this process.

Based on the in-depth analysis of literature data, the author concludes that suboptimal conditions can alter host preferences of *Cuscuta* parasites. He found large gaps in the knowledge on the distribution, taxonomy and molecular mechanisms of parasitic plants. As for the influence of abiotic and biotic stresses (well studied in host plants), they are almost unexplored in plant parasites. Dr. Zagorchev concludes that for their investigation new models of resistant and stress-sensitive pairs of hosts and parasites are required.

Goals and tasks. The profound literature analysis allowed Dr. Zagorchev to find his research niche and formulate the aim and tasks of his thesis. The aim is to characterize the species diversity of the genus *Cuscuta* in Bulgaria and to investigate the influence of biotic and abiotic stress factors on the parasite-host relationship.

Methodology. All experiments are conducted by modern and diverse methodology. The employed methods vary from routine plant cultivation in laboratory conditions to state-of-the-art molecular biological and genomic methods such as DNA sequencing, transcriptomic and metagenomic analyses, proteome analysis, metabolome analysis, spectroscopic measurements, computer processing of experimental data, etc.

Results. The obtained results are presented on 70 pages. They are designed to check/verify three working hypotheses, namely: 1) *The introduced species Cuscuta campestris has a higher invasive and parasitic potential than the native species*; 2) *The parasite-host interaction is influenced by abiotic and biotic stress factors*; 3) *Parasites of the genus Cuscuta are influenced by biotic factors changing their metabolism*.

To test his hypotheses, Prof. Zagorchev designed and successfully conducted numerous experiments, whose results are presented in five subsections: 1) Distribution and genetic diversity of *Cuscuta* spp. in Bulgaria; 2) Influence of abiotic and biotic factors on seed germination and developmental stages prior to host infection; 3) Influence of abiotic and biotic factors on the development of the parasite after infection; 4) Influence of *Cuscuta* parasitism on host metabolism and soil microcommunities; 5) Interaction of the parasite-host pair with other biotic factors.

Dr. Zagorchev's results convincingly show that the introduced and invasive species *Cuscuta campestris* has a wider distribution under diverse climatic conditions and infects more hosts compared to the local Bulgarian parasitic species. They are also characterized by a greater genetic diversity, which does not correlate with their distribution or host range. Thus, these results support the first working hypothesis.

Dr. Zagorchev also shows that soil salinization has an adverse effect on all stages of *Cuscuta* development and that the response to the salt stress is host dependent. The host type determines also the type of metabolism/metabolome of *Cuscuta*, and this factor is even more significant than the abiotic stressors themselves. In turn, *Cuscuta* interferes with the hosts' ability in adapting to both salinization and herbivory insects. It has an adverse effect on both phases of host photosynthesis, even with suboptimal parasite development. *Cuscuta* also affect the interaction between the host root system and soil microbial communities, thus impairing the exchange of mineral and organic substances. These results support the second working hypothesis, namely, *the parasite-host interaction is affected by abiotic and biotic stressors*.

I highly appreciate Dr. Zagorchev's studies on the influence of various biotic factors on *Cuscuta* metabolism. He found that certain factors, such as the galls of the *Smicronyx* beetles, can activate the light phase of photosynthesis in the non-photosynthetic species *Cuscuta campestris*, although the *Smicronyx* galls themselves do not negatively affect the growth and development of *Cuscuta*. An important scientific result is the observation that parasitic plants of the genus *Cuscuta* are good carriers of the Cucumber Mosaic Virus, although they themselves do not show visible symptoms of virus infection. These studies confirm the validity of the last (third) working hypothesis.

Discussion. The voluminous experimental data are discussed in the section Discussion. The emphasis of the discussion falls on the distribution of plant parasites of the genus *Cuscuta* and their interference with the host plants, the influence of abiotic stress on the parasite-host interaction, the interaction of parasites with the host under conditions of biotic stress, etc.

5. Contributions and significance for science and practice

Dr. Zagorchev's achievements have scientific, applied and methodological significance. In his thesis he discussed for the first time the problems related to the influence of abiotic stress on the parasitic plants of *Cuscuta* spp. and highlighted the effect of host species on their adaptation to abiotic stress. It confirms the involvement of specific enzymes and glycoproteins in haustoria formation and identified new glycoproteins taking part in this process. Another contribution of his research is the proof of the role of gall-forming insects of the genus *Smicronyx* on the photosynthetic apparatus of *Cuscuta campestris*. It should be noted also that Dr. Zagorchev's studies shed more light on the distribution of *Cuscuta* species in Bulgaria and enriched the Herbarium of the Sofia University with new specimens. He optimized also the model systems parasite/stress-sensitive hosts and parasite/stress-tolerant hosts thus enabling future comparative studies of diverse stress factors on *Cuscuta* life and development.

6. Evaluation of the scientific papers related to the thesis

Twenty scientific articles, 14 of which in journals with IF (total IF 51.88), 3 in journals with IF-rank and 3 chapters of monographs have been published in connection with the reviewed thesis. In 16 of them Dr. Zagorchev is a leading (first or last) author. Of the papers 12 are in Q1, 3 in Q2 and 2 in Q3 journals. His papers have been cited 351 times so far. The dissertation results have been also reported at 19 scientific forums, of which 9 international.

7. Abstract, conclusions and contributions

The thesis abstract adequately reflects the content and achievements of the dissertation. Based on the obtained results, 10 conclusions and 8 contributions are formulated. They all objectively reflect the scientific achievements.

8. Personal involvement of the author

The dissertation of Assoc. Prof. L. Zagorchev has been developed in a relatively short period of time (5 years) in the implementation of 10 research projects in 7 of which he was the principal investigator. The fact that he is also the leading author in 16 (out of 20) scientific papers related to his thesis gives me reason to conclude that both the scientific ideas and the majority of the thesis achievements are his own credit.

9. Critical Notes

I have no substantive comments on the reviewed version of the dissertation.

10. Summary assessment

The summary assessment according to the Regulations for the implementation of the LDASRB for the scientific degree "Doctor of Sciences" in the field of higher education 4. "Natural sciences, mathematics and informatics" is presented in the following table.

Indicator	Required minimum	Actual number of points
A	50	50
B	100	100
C	100	450
D	100	556
Total	350	1156

Presented data show that Dr. Zagorchev's indicators exceed about three times the officially acquired ones for the scientific degree "Doctor of Sciences".

CONCLUSION

The thesis of Assoc. Prof. Lyuben I. Zagorchev submitted for the acquisition of the scientific degree "Doctor of Sciences" is a result of a five-year extensive research in the field of plant parasitism. His thesis aims to shed more light on the influence of biotic and abiotic stress factors on the parasite-host interactions, and to clarify the biochemical and molecular biological bases of these interactions. All experiments are conducted at a high scientific level employing the modern methodology of molecular biology, genomics, transcriptomics and proteomics. As a result, significant results have been obtained with a visible impact to both basic science and practice. The thesis results are published in 20 scientific papers in refereed journals and reported on 19 scientific forums. Thus Dr. Zagorchev can be defined as a respectable researcher in the field of plant molecular biology, well established at both national and international level. His overall scientific indicators exceed three times those officially required by the LDASRB for the acquisition of the scientific degree "Doctor of Sciences" in Higher Education Region 4. "Natural Sciences, Mathematics and Informatics", Professional Direction 4.3. Biological Sciences, Science Specialization: "Molecular Biology". This gives me the reason to confidently recommend to the respected Scientific Jury to award it to him.

22.05.2023 г.

Reviewer:

/Акад. Иван Г. Иванов/