

OPINION

from Professor Spassimir Borissov Tonkov D.Sc., member of scientific jury pursuant to order
№ ПД-38-157/03.04.2023

for obtaining the scientific degree “Doctor of Sciences” in Professional field 4.3. Biological sciences (Molecular biology) with a dissertation on the topic: “**Influence of biotic and abiotic stresses on the parasitism of stem holoparasitic plants of genus *Cuscuta* L. (family Convolvulaceae)**”, presented by Assoc. Prof. Lyuben Ivanov Zagorchev Ph.D., from the Department of Biochemistry of the Faculty of Biology at Sofia University “St. Kliment Ohridski”

Brief biographical data

Assoc. Prof. Lyuben Ivanov Zagorchev completed his higher education with a Master's degree in molecular biology at the Department of Biochemistry, Faculty of Biology at Sofia University “St. Kliment Ohridski” in 2006. In the same department he successively held the academic positions of assistant (2010), chief assistant (2013) and was habilitated as an Associate Professor of biochemistry in 2017. He obtained the educational and scientific degree "Doctor" (Ph.D.) in 2012 with a dissertation entitled "*Biochemical changes in the composition and structure of the cell wall of embryogenic callus cultures of *Dactylis glomerata* L. treated with NaCl*". In 2020 he was elected Vice-Dean of the faculty responsible for scientific and project activities. He speaks English and French fluently and has realized short-term specializations in Austria, Australia, China and England. Assoc. Prof. L. Zagorchev was scientific supervisor of 14 successfully defended diploma students (bachelor's and master's) and currently he is scientific supervisor of two Ph.D. students. He managed 7 scientific projects funded by various institutions and is a holder of several prestigious awards for successful scientific activity and achievements.

General characteristics of the dissertation, aim and objectives

The dissertation is written in a volume of 277 pages (190 pages of text and 87 pages of appendices). The results are presented in 76 figures and 13 tables, using 337 literary sources. The dissertation is composed of the following sections: Introduction and Literature Review (6-49 pp.), Aim and Objectives (50 pp.), Materials and Methods (51-67 pp.), Results (68-142 pp.), Discussion (143-163 pp.) Conclusions and Contributions (164-166 pp.), Additional information - list of publications, participations in scientific meetings, and literature used (167-194 pp.), Appendices (195-277 pp.). The illustrations are comprehensive, precisely prepared and include abundant visual information.

The dissertation is of original character with a marked scientific-applied focus related to a current problem of the role of stem plant holoparasites of the genus *Cuscuta* (family Convolvulaceae), which have a significant impact on native and agricultural plant species, thus causing significant harvest losses worldwide. There are still many uncertainties regarding the biochemical and molecular mechanisms of parasitism, especially under the influence of abiotic and biotic stresses. In this context, the main aim of the study is to trace the influence of biotic and abiotic factors in representatives of the genus *Cuscuta* in Bulgaria on the parasite-host relationship. Three main hypotheses are proposed for testing with the implementation of six main objectives. The literature review reflects the current available studies and data on the selected topic and outlines the directions of future investigations.

Research methodology

Altogether nine modern research methods were applied for this complex study such as *molecular taxonomy and phylogenetic analysis, metagenomic analysis, transcriptome analysis, enzyme activities, proteome analysis*, etc. on seed and vegetative material collected by Assoc. Prof. L. Zagorchev from the species *Cuscuta campestris*, *C. europaea*, *C. approximata* and *C. epithimum* distributed on the territory of our country. Seeds of the remaining studied species were provided free of charge by researchers from China, Serbia and England, with whom Assoc. Prof. L. Zagorchev maintains active scientific contacts. It is necessary to point out the good background of the candidate in the field of plant taxonomy and phylogenetics of the studied group, which is a crucial prerequisite for the reliability of the obtained results. The applied complex research methodology reflects his in-depth knowledge of the selected issues, which is evident from the comprehensive and up to date literature review.

Evaluation of the results and contributions

The successful acquirement and application of the molecular-biochemical methods allowed Assoc. Prof. L. Zagorchev to realize the complex nature of his research. Important and interesting scientific results, statistically treated, and supported by appropriate illustrations were obtained in the following directions:

1. The distribution (54 localities), the spectrum of plant hosts (114 species) and the genetic diversity of the studied *Cuscuta* species in Bulgaria were established. An important achievement is the construction of a phylogenetic tree by the *maximum likelihood* method using the available sequences from the rDNA region. The differentiation of the invasive species *C. campestris* from the other three species, which also group in well-formed clusters, is demonstrated. Regarding crop plants, neither maize nor tomato was found to be suitable primary hosts for *C. campestris*.

2. With regard to abiotic stress, studied mainly in the form of salinity, it is proven that it affects every stage of the development of the parasitic plant - from seed germination, through haustoria formation, to successful parasitism and development.

3. Parasitism in the genus *Cuscuta* is related to the host's ability to adapt to both salinity and insect herbivory, and negatively affects host photosynthetic activity even when the parasite lacks optimal development.

4. The above-ground parasitism exerts a significant effect on the interactions between the root system and the rhizosphere microbial communities leading to a change in the metabolism of the host's roots.

5. Under certain conditions (the galls of the beetles of the genus *Smicronyx*), an active light phase of photosynthesis is initiated in the species *C. campestris*.

6. The parasites of the genus *Cuscuta* are also active carriers of viruses, which they transfer to the host without showing themselves virus infection.

The profound analyses, precise processing and summary of the results allowed Assoc. Prof. L. Zagorchev to formulate the main conclusions in confirmation of the three hypotheses proposed, and the contributions of his dissertation in the following categories: fundamental, scientific-applied and methodological. Among the fundamental contributions is necessary to note the established significant effects of the host species on the adaptation of the parasite to abiotic stress, as well as the established enzymes and glycoproteins involved in the formation of the haustoria. Of scientific and applied point of view is the update of the information about the current distribution of *Cuscuta* species in Bulgaria and the establishment of a rich spectrum of hosts, as well as the role of the holoparasites as a depot and vector of plant viruses.

Assessment of the publication activity and the personal participation

The dissertation is based on 20 co-authored publications in English in which Assoc. Prof. L. Zagorchev is the first author in 13. In peer-reviewed journals with impact factor such as *Plants*, *Phytoparasitica*, *Journal of Molecular Sciences*, *Cells*, *Applied Soil Ecology*, *Plant Physiology and Biochemistry* etc. are published 14 papers, 3 in journals with an impact rank and 3 as book chapters. The total impact factor is 51.88. Research results were reported at 19 scientific forums (9 of them international in Serbia, Cyprus, Slovenia, the Netherlands, Kenya, etc.). The leading role of Assoc. Prof. L. Zagorchev in the presented scientific production is supported by 278 citations found in the SCOPUS database, and only for the publication №19 in the *International Journal of Molecular Sciences* (Zagorchev et al., 2013) they are 194. The author's abstract correctly reveals the main sections of the dissertation. In fulfillment of the

requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation for the acquisition of the scientific degree "Doctor of Sciences", Assoc. Prof. L. Zagorchev scores 1111 points which significantly exceeds the required minimum of 350 points.

Conclusion

The dissertation of Assoc. Prof. Lyuben Ivanov Zagorchev is a personal work carried out in scientific teams under his leadership with the application of modern molecular-biochemical methods and in the frame of fruitful international cooperation as well. The dissertation represents a significant contribution in revealing the molecular basis of the complex parasite-host relationship of the example of plant stem holoparasites of the genus *Cuscuta*. The scientific results have also an important applied significance in relation to the struggle against plant parasites causing significant losses in yields of various cultural plants.

The dissertation is written in a very good scientific style and the results present Assoc. Prof. L. Zagorchev as an established researcher among our biological community. Also, the candidate fulfills and significantly exceeds the quantitative criteria in the Law on the Development of Academic Staff in the Republic of Bulgaria for acquiring the scientific degree "Doctor of Sciences" in Professional field 4.3 Biological sciences (Molecular biology).

Based on the above statement, I recommend the members of the scientific jury to give their positive vote for awarding this scientific degree to Assoc. Prof. Lyuben Ivanov Zagorchev.

Sofia

06-06-2023

Jury member:

Prof. Spasimir Tonkov D.Sc.