REVIEW

by Prof., Doctor Elena Ivanova Georgieva

of the dissertation work of Assoc. Dr. Lyuben Ivanov Zagorchev on the topic: "Influence of biotic and abiotic factors on the parasitism of stem holoparasitic plants of the genus *Cuscuta* L. (family Convolvulaceae)" submitted for the award of the Scientific Degree "Doctor of Sciences", in the Higher Education Region 4. "Natural Sciences, Mathematics and Informatics", Professional Direction 4.3. Biological Sciences, PhD Program "Molecular Biology"

1. General description of the presented materials

Assoc. Dr. Zagorchev participated in the competition with all the necessary documents presented in paper and electronic media, copies of the scientific works, as well as an approved protocol of a preliminary discussion of the dissertation work before an extended scientific staff of the "Biochemistry" department. The scientific output of the candidate fully matches the profile of the announced competition and all materials are prepared in full accordance with the Law on the Development of the Academic Staff of the Republic of Belarus, the Rules for the Development of the Academic Staff of the University of St. Kliment Ohridski" and the adopted internal regulations and rules of the Faculty of Biology.

2. Biographical and scientometric data

After completing his secondary education at the National Science and Mathematics High School "Acad. Lyubomir Chakalov", profile Biology and biotechnology and advanced study of English, with an excellent GPA of 5.78, Dr. Zagorchev was accepted as a student at SU "St. Kliment Ohridski", Faculty of Biology. In 2004, he received the Bachelor's degree in Biology - Molecular Biology. Two years later, in 2006, he graduated with a Master's degree in Molecular Biology - Biochemistry, again with excellent results. In the period 2010-2013, he was appointed as an assistant in the "Biochemistry" department of the Faculty of Biology, SU "St. Kliment Ohridski". In 2012, after a successful defense of a dissertation 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", he acquired the ONS "Doctor", in direction 4.3 Biological Sciences Doctoral Program "Biochemistry". From 2013-2017, after acquiring the ONS "Doctor" he was the chief assistant, and from 2017 until now he held the position of "Associate Professor" in the same scientific institution. From 2018 to the present, he also works as a translator (from English to Bulgarian) of European patents in the field of Biology, Pharmacy and Medicine. In 2020, he was elected as the deputy dean for quality management, scientific and project activities and accreditations at the Faculty of Biology of SU "St. Kliment Ohridski". From 2021 to the present, he is in the position of "Researcher R2" under Contract BG05M20P001-1.002-0012, at the Competence Center "Sustainable utilization of bio-resources and waste from medicinal and aromatic plants for innovative bioactive products". The professional growth of Dr. Zagorchev and his entire internship took place entirely in the Faculty of Biology of the SU "St. Kliment Ohridski", where he established himself as an erudite teacher and expert in the field of Molecular Biology and Biochemistry. The academic profile of Assoc. Dr. Zagorchev has been approved by NACID and published in the register for scientific activity.

The documents presented by Dr. Zagorchev prove that he won short-term post-doctoral specializations in prestigious scientific institutes in China, Australia, Austria and England, financed by various won projects and concluded contracts, which undoubtedly contributed to his scientific growth and international prestige. The scientific competences of the author are highly appreciated and he deservedly received a prestigious award from the Sofia Municipality for the best young scientist of the SU, 2014, as well as first prize for an essay on "Genetically modified organisms - hope, fear, choice", 2006, from the Bulgarian Biotechnology Information Center, AgroBioTechPark, Sofia, Bulgaria. The applicant's creative scientific abilities are also supported by 1) his appearances as the founder of Scientia Supernaturalis (2022), a fan fiction journal of speculative biology; 2) with two poster presentations dedicated to parasitic plants of the genus Cuscuta and salt tolerance; 3) awarded fifth place in a competition for a fantastic story published in "Thalasumia 2006-2007" (anthology), Kvazar publishing house, 2008, ISBN: 978-964-8826-99-0; 4) author in online magazines Starligher and Sivosten - music, cinema, popular science and 5) participation in Kindle Direct Publishing, Amazon (2016). He is the editor-in-chief of the Yearbook of the University of St. Kliment Ohridski", Faculty of Biology, Booklet 4.

Dr. Zagorchev's high professional and managerial qualities are evident from his participation in numerous scientific organizations and committees such as: International Parasitic Plant Society; Scandinavian Plant Physiology Society, part of FESPB; vice-chairman of VNEK for Biological Sciences at FNI/MES; member of the National Commission for the Olympiad in Biology and Health Education; L'Oreal Women in Science Jury Member; chairman of the Scientific Commission of the BF; chairman of the Quality Commission of the Bulgarian Academy of Sciences, member of the Management Board of the National Institute of Scientific Research, SU; member of the Faculty Council of BF, SU. He has been repeatedly selected as a reviewer of scientific articles published in international journals with an impact factor, of which I have chosen to name only a few of them: International Journal of Molecular Sciences; Plants; Journal of Saudi Society of Agricultural; Sciences; Biochemical Journal; Annals of Botany: Plants; Ecotoxicology and Environmental Safety; PloS One; Plant Physiology and Biochemistry; Acta Physiologiae Plantarum; Annual Review & Research in Biology and many others. In the period from 2008 to 2022, 8 practical courses were held in spectroscopic studies, molecular biology and biochemistry, designing competence models in electronic learning systems, etc.

Associate Professor Dr. Zagorchev also has remarkable teaching experience. In 2018 he delivered a series of lectures at the Dipartimento di Agraria, Universita Mediterranea Reggio Calabria, Italy, funded by Erasmus+. From 2017 until now, he has been conducting lectures on Biochemistry for students at the Bachelor's College of Economics and Business Administration (majors in Agrobiotechnologies and Biotechnologies - regular and part-time), FHF (majors in Chemistry and Ecochemistry) and FNOI (majors in Physical Education and Sports) of the SU. Leads lectures and exercises on discussion problems in Molecular Biology, Molecular Biology of the Plant Cell, Omics Technologies, Molecular-Biological Methods with Application in Biotechnological Production for students in the OCS "Master" from BF. From 2007 to 2017, he conducted practical exercises in Biochemistry and Molecular Biology for students in the "Bachelor" OCS of BF, SU. Possesses a language certificate: TOEFL, Computer-based with a score of 270 (out of 300).

For his entire scientific experience, Dr. Zagorchev presents a list of 44 scientific publications with high citations, which according to Google Scholar is over 700 (without cleared auto-citations), h-index 11, i10-index 12; 70 participations in international (22), in Bulgaria with international participation (19) and national (29) scientific forums; 5 book chapters and 3 study aids.

For the "Doctor of Sciences" competition, Assoc. Dr. Zagorchev participated with impressive scientometric indicators, fully covering the requirements for this Scientific Degree. Presents a list of 20 scientific publications - 4 from the competition for associate professor, 8 entered in NACID for associate professor, of them: with IF 14 (11 Q1; 2 Q2; 1 Q3), with Impact Rank 3 (1 Q1; 1 Q2; 1 Q3), 3 book chapters, one of which is a collective monograph in Chinese; 4 review articles, first author in 13, corresponding author in 11, and first and corresponding in 15 publications with a total IF=51.88. The noticed citations of these 20 publications on Scopus are 351. He is the supervisor of 8 protected diploma theses in OKS Bachelor and OKS Master. His results have been reported at 9 international and 10 national scientific forums. His scientific skills and abilities are also reflected by the large number of international and national scientific projects won with very good funding. For the competition, Dr. Zagorchev participated as the head and coordinator of 7 scientific projects, one of which was worth BGN 1,100,000. He is a participant in COST Action FA0901 Putting Halophytes to Work - From Genes to Ecosystems, in 1 project at the National Center for Polar Research, 1 in National Scientific Programs, 5 at the National Research Institute/Ministry of Education and Science and 5 at the National Research Institute of the SU "St. Kliment Ohridski".

The report on the fulfillment of the minimum national requirements under Art. 2b from ZRASRB for scientific area 4. Natural sciences, mathematics and informatics professional direction: 4.3 Biological sciences, presented by Dr. Zagorchev forms the following indicators: group A - 50 points; group B - 100 items; according to indicator D - 405 points with a required minimum of 100 points, and according to indicator D (cited) it collects 556 points with a minimum of 100 points. Thus, with a required minimum of 350 points for the National Doctor of Sciences, according to PPZRASRB, Dr. Zagorchev formed 1,111 points, which exceeds the minimum national requirements necessary for this NS.

The protocol of the originality check of Dr. Zagorchev's dissertation in the plagiarism prevention system and the generated text similarity report show that the detected similarities are regulated, legitimate and do not bear signs of plagiarism.

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

For many years, the efforts of classical genetics and post-genomic omics technologies have been primarily focused on analyzing and assessing the vulnerability of crop plants and soils, to mitigating the impact of climate conditions and adapting modern agriculture to these changes. The influence of biotic and abiotic factors on parasitic flower plants leads to a decrease in yields of cultivated plant species, poses a serious threat to global food production, increases the risk of famine and causes significant economic losses. Overcoming the nonspecific changes and rearrangements of the defense forces of the plant organism occurring under the influence of stress factors has not been fully established and is still a challenge for modern agrobiology. Despite the accumulated data, the mechanisms of regulation and the role of metabolic processes at the cellular level, such as regulators of plant growth and modulators of gene expression under stress conditions, are still not clear. Resistance to biotic and abiotic stress is controlled at the molecular level by multiple mechanisms, and the understanding of these mechanisms is incomplete. The influence of abiotic and biotic stressors, however well studied in plants, has hardly been addressed in the genus *Cuscuta*, which infect a large number of plant species and a single plant can simultaneously parasitize several different hosts. Parasitic plants are not uncommon in challenging environments with increased salinity or water scarcity, pollution or adverse temperatures. Suboptimal conditions can alter their host preferences as well as their susceptibility to parasitism. On the other hand, the additional stress of *Cuscuta* infection can have a substantial impact on the ability of hosts to adapt to other stressors.

This short analysis demonstrates that the topic of the dissertation work of Associate Professor Dr. Lyuben Ivanov Zagorchev for the acquisition of the Doctor of Science degree is more than timely, current and focused on insufficiently well-researched questions and a wide range of problems related to clarification the molecular mechanisms of stress tolerance and adaptation, influence of Cuscuta spp. on the host plants as well as the application of biochemical and molecular markers as evaluation factors on the parasitism of stem holoparasitic plants of the genus Cuscuta. The dissertation student applied targeted complex analysis to study the influence of biotic and abiotic factors on the parasitism of stem holoparasitic plants of the genus Cuscuta. The indisputable results reflected in this dissertation provide a clear idea of the importance of the studied parasitic plants, for understanding their biological role and possibilities to extrapolate the results for new strategies to improve the agronomic characteristics of the hosts, as well as to deepen our knowledge of the distribution of these parasites in RBulgaria, their host range and their genetic diversity. The clearly formulated goal of this scientific work has been fully achieved by setting and successfully deriving three research hypotheses and several main tasks related to them. A systematic approach was applied, enabling appropriate models of resistant and stress-sensitive hostparasite pairs to be developed.

4. Knowledge of the problem and research methodology

The dissertation is structured according to the accepted requirements, is perfectly formed and is a complete study that provides valuable scientific information with opportunities for practical application. The literature review is professionally written and covers all aspects of the research problem, supported by over 330 literary sources. The dissertation is written on 277 pages (190 pages of main text and 87 pages of appendices). In the literature review, 10 author figures and 3 author tables are used, which clearly demonstrates in-depth knowledge and a marked interest in the problem. On 48 pages, the literature reference is presented coherently, concisely and critically. The available literature is summarized with knowledge covering what has been published to date and the possibilities for future scientific applications and perspectives for new studies in light of climate change on the distribution, agricultural and ecological impact of these widespread parasitic plants are outlined.

The results are illustrated with representative 84 figures and 15 main tables. In order not to complicate the exposition and the large volume of information received, important additional and evidentiary results are given as appendices in a separate chapter in tabular form on more than 70 pages, which clearly shows the personal contribution of the doctoral student. Dr. Zagorchev's high scientific skills are also evident from the thorough comparison of his own results with published ones, and it is clear that the achieved original scientific contributions have a worthy place in the rich literary analysis.

Almost all modern methods covering almost the entire spectrum of molecular biological, molecular genetics, biochemical, microscopic and statistical analysis are covered in this scientific work. By using them and with scientific precision and criticality, Dr. Zagorchev managed to publish reliable evidentiary data confirming the set goal and hypotheses. Methodological approaches used include: molecular taxonomy and phylogenetic analysis; metagenomic analysis to study the diversity of soil microbial communities - by PCR and NGS sequencing on the Illumina platform; transcriptome analysis; biochemical analyzes for the study of enzyme activities by spectrophotometric or zymogram methods; metabolomic and proteomic analysis; photosynthetic measurements and statistical analyses; DNA isolation; native and denaturing electrophoresis.

The main goal of the dissertation is to characterize the species diversity of the genus *Cuscuta* in Bulgaria and to investigate the influence of biotic and abiotic factors on the parasite-host relationship. The goal thus set was tested with the formulation of three main hypotheses and was fully achieved by solving the tasks set for them and with the correctly selected plant material.

In a period of 5 years, seed and vegetative material of *Cuscuta campestris, C. europaea, C. approximata and C. epithymum* were collected in Bulgaria. Seeds of reference C. campestris were provided by the Seed Conservation Department, Royal Botanic Gardens, Kew. For part of the experiments, seeds of 26 populations of C. campestris were provided by the Republic of Serbia, and seeds of *C. australis, C. chinensis and C. japonica* were provided by China. Host plant seeds of *Arabidopsis thaliana* L. ecotype Columbia (Col-0) and *Eutrema salsugineum (Thellungiella salsuginea)* were purchased from the Nottingham Arabidopsis Stock Centre, and those of *Calystegia sepium* and Convolvulus arvensis were provided by Assoc. Dr. Anita Tosheva. BF/SU. Lentil seeds were provided by Prof. Adele Muscolo, (Reggio Calabria). All other host plant seeds were purchased from commercial sources - Sortovi Semena i Rastitelna Zashchita EOOD, Plant World Seeds and similar.

For the studies carried out, cultivation methods, germination tests and infection with *Cuscuta* spp. of host plants are described in detail.

5. Characterization and evaluation of the dissertation work

The largest share in the dissertation work is occupied by the results, supported by representative figures and tables, giving me full reason to assume that Associate Professor Dr. Zagorchev has not only an indisputable personal contribution, but also an indisputable leading role in scientific developments. A large part of them have already been published in prestigious journals with IF and Impact Rank. An excellent impression is made by the systematized discussion in which Dr. Zagorchev summarized and compared the results obtained by him and the conclusions reached in the scientific search with those reflected in the world database.

There are relatively few studies covering the distribution, taxonomic status and molecular mechanisms of parasitism in representatives of the genus *Cuscuta*. In this thesis,

the distribution and host range of *Cuscuta* spp. Plant material was collected from 54 localities of four of the species common in Bulgaria: *Cuscuta approximata, C. Campestris, C. epithymum and C. europaea.* The largest variety of sites is covered by the invasive species C. campestris - 35 pcs. and the largest number of hosts were established - 52 species from 19 families. For the studied species, 114 host species were identified, assigned to 87 genera and 33 families, all from the department of angiosperms (*Magnoliophyta*). The greatest diversity of host species was found in *Fabaceae*, followed by *Asteraceae*, *Lamiaceae* and *Rosaceae*. These data indicate that the introduced and invasive species *Cuscuta* campestris has a wider distribution, in more diverse climates and a wider range of hosts than native species and is of greater importance as a vector and reservoir of plant viruses.

In the literature, there are quite a few inaccuracies in the nomenclature used regarding the classification of hosts. In an attempt to systematize these differences, an algorithm has been proposed to categorize different hosts in terms of their susceptibility to parasitism by *Cuscuta* spp.

Genetic diversity was assessed by ITS sequences. A phylogenetic tree was constructed using the maximum likelihood method using the available sequences from the rDNA region and a clear distinction of *C. campestris* from the other three species was established. It is noteworthy that *C. europaea* is relatively close to *C. approximata*. The genetic diversity of *Cuscuta* campestris is relatively high, but does not correlate with the distribution or host range of the parasite. Significant interspecies differences in RAPD profiles were observed and it was shown that a combination of primers can be used for successful species identification. RAPD markers have been confirmed to be suitable for species identification, but not as successful for characterizing the intraspecific diversity of *Cuscuta* spp.

The distribution, host range and genetic diversity of *Cuscuta* spp have been demonstrated. in Bulgaria. The information on the distribution of the genus *Cuscuta* in Bulgaria, the range of hosts has been significantly enriched, and the collection of herbarium materials in the Herbarium of the University of Warsaw has been upgraded. These convincing data obtained by Associate Professor Dr. Zagorchev are a scientific and scientifically applied contribution in proving the first formulated hypothesis that the introduced species *Cuscuta* campestris has a higher invasive and parasitic potential than the local species.

Stem holoparasitic plants of the genus *Cuscuta* can cause severe losses to agricultural crops, ranging from negligible to over 80% of expected yields. Although the influence of abiotic and biotic stressors is relatively well studied in plants, it is hardly touched upon in parasitic plants, which are not rare in environments with increased salinity, lack of water (drought), polluted sites or unfavorable temperatures. In times of constant climate change, an approach requiring the development of appropriate models of resistant and stress-sensitive host-parasite pairs must be implemented. In the dissertation of Associate Professor Zagorchev, many analyzes were made and important conclusions reached at the highest experimental level.

The next tested and proven hypothesis concerns important questions related to the parasite-host interaction and the influence of abiotic and biotic stressors. To test this hypothesis, the influence of biotic factors – host species on parasitism in *Cuscuta* spp. and the influence of abiotic factors – salinity on different stages of the parasitism of *Cuscuta* spp. In

this section, a large part of Dr. Zagorchev's achievements are pioneering and impress with their quantity and variety. Analyzed 1) the influence of abiotic and biotic factors on germination and developmental stages before host infection; 2) the development of the parasite after successful infection; 3) the interaction of the parasite-host pair with other biotic factors; 4) host metabolism and soil microcommunities - photosynthetic activity and host transcriptome. For the first time, the problem of the influence of abiotic stress on parasitic plants was defined. Prof. Zagorchev proves that salinization negatively affects all stages of *Cuscuta* development, as the response to salt stress depends on the host species and salinization can lead to a change in the "quality" of the host, as well as that the *Cuscuta* metabolome strongly depends on the species of the host and to a lesser extent by abiotic stressors. Above-ground parasitism of *Cuscuta* exerts a significant effect on interactions between the root system and rhizosphere microbial communities by altering root metabolism and soil enzyme activity.

Analyzes of abiotic stress, mainly studied in the form of salinity, have shown that it affects every stage of development of *Cuscuta* spp., from seed germination, through haustoria formation, to successful parasitism and development. Enzymes and glycoproteins involved in the process of haustoria formation have been confirmed and identified. Prof. Zagorchev has shown that the parasitic *Cuscuta* are much more sensitive to salinity than their non-parasitic relatives. Increased salt concentration exhibits a negative and dose-dependent effect on parasite growth.

By means of zymogram analyzes of proteins, two main classes of hydrolytic enzymes with a possible role in the mobilization of reserve substances in the seeds were investigated, and a total of 5 bands differing in molecular mass with amylolytic activity were found in all investigated *Cuscuta* species. The obtained results on the influence of abiotic and biotic factors on the development of the parasite after successful infection are supported by sufficient analyses. Changes in the host proteome in *Arabidopsis thaliana* infected with *C. australis* were investigated.

Another important conclusion reached by Dr. Zagorchev, supported by numerous experiments and obtained results, is the finding that the parasitism of *Cuscuta* interferes with the hosts' ability to adapt both to salinization and herbivorous insects and negatively affects the photosynthetic activity of the host. The influence of parasitism on *Cuscuta* spp. on the hosts is significant and this is shown by studying photosynthesis and the transcriptome of the hosts. Transcriptome analysis was performed on Trifolium repens plants infected with *Cuscuta* australis. 1601 differentially expressed genes (DEGs) were identified between infected and control host, of which 945 DEGs were up-expressed and 656 DEGs were down-expressed. Depending on environmental conditions and hosts, different populations have shown different viability when tested on a single host, with no clear relationship found between this and genetic characteristics. Similar results are also obtained for *Arabidopsis* upon *Cuscuta* chinensis infection, suggesting that parasitic plants actively suppress defense mechanisms to prevent active defense responses.

Using next-generation sequencing, parasitism was found to alter the composition and diversity of the microbial environment in the rhizosphere of the invasive *Alternanthera philoxeroides* infected by *C. australis*.

The next formulated hypothesis addresses whether parasites of the genus *Cuscuta* are subject to the influence of biotic factors that significantly alter their metabolism.

In this regard, research has been carried out on interactions with hosts under conditions of biotic stress. There are relatively few literature data on pathogens or epiparasites on representatives of the genus *Cuscuta*. One such example is the gall-forming octopus beetles of the genus Smicronyx. Several of these species express specific preferences for *Cuscuta* spp. as hosts and Dr. Zagorchev has shown that they cause significant growth retardation, prevent flowering and seed formation, and ultimately lead to the death of infected plants. No active antioxidant response was observed in *Smicronyx* galls and there was no evidence that they negatively affected the growth and development of *Cuscuta*. From the obtained results it was concluded that the parasitism of *Cuscuta* spp. interferes with the ability of the hosts to adapt to both salinization and herbivorous insects. An important scientifically applied contribution was also achieved, namely, the data on the changes that occur as a result of the epiparasitism of *Cuscuta* campestris were confirmed and enriched.

An essential result of a contributing nature is the finding, mainly in *Cuscuta campestris*, as a reservoir and vector of plant viruses. Four viruses selected on the basis of their agricultural importance were tested: AMV - alfalfa mosaic virus, CMV - cucumber mosaic virus, TMV - tobacco mosaic virus, TYLCV - tomato yellow leaf curl virus, but so far there is no data on the presence of these viruses in cuckoos yarns, although these parasitic plants are known hosts and vectors of other viruses. Among *Cuscuta* populations. campestris only two have been identified - TYLCV and CMV. Parasitic plants of the genus *Cuscuta* can be active vectors of viruses (Cucumber Mosaic Virus) that they transfer to the host without themselves having visible symptoms of virus infection. An important applied contribution was achieved confirming the role of *Cuscuta* spp. as a reservoir and vector of plant viruses.

All the numerous data obtained show that the set hypotheses have been proven and the goal set in this scientific work has been fully achieved.

CONCLUSION:

Assoc. Dr. Zagorchev participated in the competition with impressive scientometric indicators fully covering the requirements for the National Doctor of Sciences.

The described conclusions and original scientific achievements are of high scientific value and fill a gap in this field. Some of them are new, pioneering and published for the first time. They are important and can have a tangible impact on our knowledge of the effects of biotic and abiotic stress on the parasitism of stem holoparasites of the genus *Cuscuta* and be a tool for defining future breeding practices aimed at improving yields under different abiotic and biotic stressors. as well as to obtain high-yielding cultural varieties with increased adaptive qualities and with an improved ideotype in changing climatic conditions.

I accept the conclusions and achievements that logically follow the results, as well as that the set goal is fulfilled and the three hypotheses are confirmed. Of these, the results obtained for the first time with modern analyses, constructs and techniques deserve special attention, which are undoubtedly the basis for future application in agricultural practice. The abstract is prepared according to the accepted standards and gives a complete picture of the main achievements and contributions of the dissertation.

From the overall review of the data presented in the dissertation work of Associate Professor Dr. Zagorchev, I can unconditionally say that they are his real achievements. A large amount of information has been summarized and evaluated. The results obtained by the dissertation student are summarized in 10 conclusions, 4 fundamental contributions, 3 contributions with scientific applied value and 1 with methodological significance, which are in agreement with the obtained results and reflect the originality of the development.

Active publication activity, high citation rate, successful management of graduate students, participation in international and national projects characterize Associate Professor Dr. Zagorchev as a prestigious scientist and undisputed expert in the field of molecular biology, with an interesting flair for the future, provoking new, important ideas. The scientific achievements of Assoc. Dr. Zagorchev fully satisfy the requirements for the Doctor of Sciences degree, both under the Law on Academic Development in the Republic of Bulgaria and National Centre for Information and Documentation (NACID), as well as under the internal regulations of the University of St. Kliment Ohridski", Faculty of Biology.

Based on the complete analysis, I allow myself to recommend with complete conviction to the respected Scientific Jury and the Scientific Council of the BF of SU "St. Kliment Ohridski" to award Assoc. Dr. Lyuben Ivanov Zagorchev "Doctor of Sciences" in the professional field 4.3. Biological Sciences, scientific specialty "Molecular Biology".

06/06/2023

Reviewer:

/prof. Dr. Elena Georgieva/