REVIEW

by Assoc. Prof. Dr. Kiril Mihaylov Mishev (Institute of Plant Physiology and Genetics - Bulgarian Academy of Sciences, IPPG-BAS)

for a competition for the academic position "Associate Professor" in professional field 4.3. Biological Sciences, scientific specialty "Biochemistry," for the needs of the "Photosynthesis – Activity and Regulation" Laboratory at IPPG-BAS, announced in issue 62 of the State Gazette on July 23, 2024.

General Information on the Candidate's Career and Research Development

In the current competition for the academic position "Associate Professor" in the "Photosynthesis – Activity and Regulation" Laboratory at IPPG-BAS, documents were submitted by a single candidate – Dr. Gergana Kirilova Mihailova. Currently, Dr. Mihailova holds the position of "Senior Assistant" in the same laboratory. She completed her higher education in 2006 at the Faculty of Biology of Sofia University "St. Kliment Ohridski," with a Bachelor's degree in Molecular Biology and a Master's degree in Biochemistry. Shortly after graduation, she joined the research team at IPPG-BAS as a doctoral student in the "Photosynthesis" section, under the supervision of Prof. Dr. Katya Georgieva. In 2012, Dr. Mihailova successfully defended her dissertation, which focused on studying the adaptation mechanisms of the resurrection plant *Haberlea rhodopensis* to drought under high temperature and varying light conditions. Throughout her career, she has successively held the positions of "Assistant" and "Senior Assistant" at IPPG-BAS. During ongoing bilateral research projects from 2007 to 2023, Dr. Mihailova conducted short-term research visits to partner scientific laboratories in Hungary, Germany, Italy, and Spain.

After obtaining her Ph.D., Dr. Mihailova maintained her scientific interest in the physiology and biochemistry of drought-tolerant plant species. In most of her scientific publications, the main model organism is the resurrection plant *Haberlea rhodopensis*, which has the unique ability to fully recover after losing almost all water content to an air-dry state. This plant species is a Balkan endemic and, unlike most resurrection plants found in warmer climates, is annually exposed to significant temperature fluctuations. The publications submitted for the competition broadly focus on studying the physiological mechanisms in *H. rhodopensis* in

response to varying temperatures and light levels combined with drought. Changes during the subsequent rehydration period have also been investigated. Experiments were conducted with plant material from both natural habitats and controlled laboratory conditions. Overall, the candidate's research in this area is of substantial importance for the field of plant stress biology and makes a valuable contribution that could form the basis for future strategies to enhance plant tolerance to adverse climatic conditions. Dr. Mihailova's long-standing interest in studying plant responses to abiotic stress is also reflected in her collaborative research with other laboratories in Bulgaria, using wheat and tomato as model systems.

Evaluation of the Submitted Compliance Report with the Requirements of Article 26, Paragraph 1 of the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB) and the Specific Requirements for the Academic Position "Associate Professor" as Reflected in the Rules of IPPG-BAS

An analysis of the information available in the Web of Science and Scopus databases, along with the candidate's provided reports and supporting documents, leads to the conclusion that Dr. Mihailova's scientific achievements meet the minimum requirements of the ADASRB and the IPPG-BAS regulations for the academic position of "Associate Professor." The points accumulated for each criterion (А, В, Г, Д, and Е) in the author's report are accurately calculated based on the criteria and conditions specified in Appendix 1 of the IPPG-BAS Rules. Notably, the candidate exceeds the minimum requirements for criteria groups Г, Д, and Е.

For criterion group **А**, Dr. Mihailova accumulated 50 points with her successfully defended dissertation for obtaining a Ph.D. in 2012. For criterion group **В**, she earned 100 points with four scientific publications in Q1 journals. Criterion group **Г** includes eight Q1 publications, two Q2 publications, and one Q3 article, all indexed in both Scopus and Web of Science. Additionally, the candidate submitted one Q3 article without an IF from WoS, one article without SJR and JCR, and one co-authored book chapter of a review nature. The total points in this criterion group amount to 280, exceeding the minimum requirement of 220. In seven of these publications, Dr. Mihailova is the first author (in two, she is both the first and corresponding author); five of these are in Q1 journals, one is in a Q3 journal, and the seventh publication is in the IPPG-BAS journal *Genetics and Plant Physiology*, which has no IF or rank. The total points for her first-author publications is

140, surpassing the required minimum of 110. The cumulative IF of the articles from groups B and Г, according to the publication year, is nearly 44, demonstrating the high quality of the candidate's scientific output and that of her co-authors. Dr. Mihailova has co-authored a total of 43 publications throughout her career, with the JCR IF of the journals totaling nearly 96. Her H-index as of October 2024 is 12, based on Scopus data. For criterion group Д, the candidate earned 392 points from 196 citations in Web of Science and Scopus, against the minimum required 100 points. Concerning criterion group E, there is evidence of Dr. Mihailova's participation in six nationally funded projects, one bilateral project funded by Bulgaria and Germany, and two projects funded by the International Atomic Energy Agency, based in Austria (totaling 120 points with a minimum threshold of 70). Although the candidate has notable project activity throughout her career, participating in a total of 23 projects, she is not listed as the project leader or coordinator for any entire contract period.

Analysis of the Main Directions in the Candidate's Research and Key Results in Each Area

From the submitted publications for the competition and the author's report on contributions, it can be concluded that Dr. Mihailova's research is focused on studying the physiological and biochemical mechanisms underlying the high ecological plasticity of resurrection plants. In most studies, the model organism is *Haberlea rhodopensis*, and one publication includes a comparative analysis with two other members of the Gesneriaceae family. The candidate's summary of contributions accurately encapsulates the scientific achievements reflected in the published articles. It should be noted that, while presenting the main findings, Dr. Mihailova did not specify the particular activities through which she contributed to the co-authored research publications. Her personal contribution is described briefly and in a generalized way across the group of articles for the competition. However, a separate paragraph is included at the end of most publications, outlining the activities of each co-author. Dr. Mihailova's published studies can be grouped into two closely related areas: analyzing the response of resurrection plants to drought combined with (1) high light conditions or (2) low environmental temperatures.

The results in the first area were obtained as a continuation of her Ph.D. work, which aimed to investigate the mechanisms of extreme drought tolerance in *H. rhodopensis* under optimal and high temperatures and under low or high light intensity. These studies are based on the observation

that *H. rhodopensis* can thrive in both shady and sunny habitats. It was found that the adaptive mechanisms in plants during extreme drought vary depending on the light regime (publications B4-01, Г7-01, Г7-02, Г7-03, Г7-06). In plants from sunny habitats, a stronger suppression of CO_2 assimilation rate was recorded compared to shade plants, while light energy capture and transfer were less affected. Some protective mechanisms in sunny plants involve strong stimulation of non-photochemical quenching to limit photoinhibition. A series of experiments with both wild plants and those grown under laboratory conditions revealed adaptive changes occurring at the tissue, cellular, and molecular levels during drought and subsequent recovery (publications B4-02, B4-03, B4-04, Г7-04, Г7-05, Г7-07, Г7-08, Г7-09, Г7-10, Г7-14). Detailed analysis of changes in the photosynthetic apparatus showed finely regulated mechanisms for optimizing the use of captured light during extreme drought. These include increased sensitivity of Photosystem II compared to Photosystem I, altered balance in the trimeric and monomeric forms of LHCII, and migration of the complex between Photosystems II and I. A significant conclusion from these studies concerns the differences in light energy dissipation modes that are not used for photochemistry: dissipation from antenna complexes in sunny plants and from inactive reaction centers of Photosystem II in shade plants. Dr. Mihailova participated in studies that revealed effects on the stoichiometry and levels of key proteins in both photosystems and antenna complexes in thylakoid membranes of drought-stressed plants grown under low and high light intensities. The results provide a model for the sequential processes leading to the cessation of photosynthesis during extreme drought and the ability of the photosynthetic apparatus to resume functioning upon rehydration. In addition to limiting photoinhibition, very active antioxidant systems were found in dehydrated *H. rhodopensis* plants, which suppress the negative effects on cellular metabolism caused by reactive oxygen species (ROS). Sunny plants showed an enhanced role of antioxidant enzymes (transcript and protein levels), while non-enzymatic antioxidants were predominant in shade plants. Besides enzymes neutralizing ROS, significant accumulation of dehydrins and heat-shock proteins (HSPs) was recorded during drought. Dr. Mihailova also contributed to studies on the profiles of various carotenoid classes and soluble sugars, noting a strong increase in zeaxanthin, which correlates with increased xanthophyll cycle activity for non-photochemical dissipation of light energy. Specific differences were observed between sunny and shade plants under drought in the dynamics of sucrose and raffinose levels. Other adaptation mechanisms involved regulation of electrolyte leakage across membranes and ultrastructural changes in mesophyll cells, affecting chloroplast

and vacuole size. Morphological changes in leaf blades were also observed, limiting excess light access to the mesophyll in drought-stressed sunny plants.

The second direction of Dr. Mihailova's research is dedicated to elucidating the molecular mechanisms of cold tolerance in *H. rhodopensis* (Г7-11, Г7-12, Г7-13, Г7-14, Г7-15). Experiments were conducted both outdoors and under controlled laboratory conditions. A significant conclusion from the analyses relates to the effect of prolonged exposure to low positive temperatures on the frost tolerance of *H. rhodopensis* at sub-zero temperatures (acclimation, priming). Freezing causes dehydration in the aerial parts of the plants, and many of the structural and functional changes observed in the photosynthetic apparatus at sub-zero temperatures resemble those seen in extreme drought conditions. Increased Photosystem I activity and suppression of Photosystem II and CO_2 assimilation enzymes were observed. Enhanced thermal dissipation of unused light energy is an important mechanism for limiting photoinhibition under low-temperature stress. The candidate participated in experiments analyzing the levels of key photosynthetic and stress-induced proteins. Characteristic changes in the composition of the oxygen-evolving complex and the appearance of new classes of dehydrins and early light-induced proteins (ELIPs) were recorded. Differences were found in the ratio between sucrose and hexose monomers during acclimation and freezing. Important adaptation changes were also observed at the ultrastructural level, including an increased share of unsaturated fatty acids in cell membranes and increased electrolyte leakage without disrupting membrane integrity. At the tissue level, new mechanisms for rapid water removal under low-temperature stress were identified, utilizing channels in the leaf epidermis. Comparative analysis between *H. rhodopensis* and other drought-tolerant plant species showed a similar response to low temperatures.

Relevance of the Candidate's Scientific Theme and Its Significance for Science and Society

The relevance of the scientific theme related to the study of the physiology and biochemistry of resurrection plants is evident in the context of efforts to better understand the mechanisms of plant adaptation to environmental stress factors. Plant species with tolerance to extreme climatic changes are a valuable source of information on the genetic and epigenetic foundations of higher plants' responses to a changing environment. Such research is also significant for developing future strategies to enhance economically important traits in cultivated plants and mitigate the adverse

effects of climate change on agriculture. The scientific significance of Dr. Mihailova's work is evidenced by the citations her publications have received from other research teams working on this topic.

Organizational and Educational Activities

In the documents submitted for this competition, Dr. Mihailova has provided evidence of her educational activities with students from Sofia University "St. Kliment Ohridski" and the New Bulgarian University. Under her supervision, a Bachelor's thesis was successfully completed and defended, and she conducted practical laboratory training for a total of three students. Dr. Mihailova's participation in a substantial number of funded projects with partners from Bulgaria and other European countries demonstrates her experience in conducting collaborative scientific research. She also possesses academic administrative experience as a secretary of the “Plant Physiology and Biochemistry” section at the Union of Scientists in Bulgaria and as a secretary of the General Assembly of IPPG-BAS.

Critical Remarks and Recommendations

I have no critical remarks regarding the candidate. Concerning the results obtained thus far in her research, I have several recommendations. I believe that, while investigating the mechanisms of cold tolerance, experiments with drought-tolerant plants should include measurements with drought-sensitive species as well. A comparative analysis will provide information on which molecular, physiological, and biochemical markers are specific to resurrection plants and which are part of the general stress response but with more pronounced quantitative changes in tolerant genotypes. In her future work, the candidate is encouraged to seek new scientific partnerships for conducting interdisciplinary research and to enhance the study of the stress transcriptome, proteome, and metabolome of resurrection plants at a systems level. The application of an untargeted approach could lead to the discovery of previously unknown regulators of the stress response, offering a much higher innovative potential than studying changes in well-known protein and non-protein markers. The recent sequencing of the *H. rhodopensis* genome offers a significant boost for such systemic studies. Additionally, I would recommend that the candidate include an analysis of the physiological and biochemical changes in the roots of *H. rhodopensis* in her future

research, as they play a crucial role in conditions of water deficit, but have not been studied by the research team till the moment. Regarding organizational and educational activities, I would suggest that Dr. Mihailova prioritize two areas: (1) the training of Master and Ph.D. students, and (2) leading and coordinating nationally or internationally funded research projects.

CONCLUSION

Based on the materials provided for review and additional checks performed, I believe that Dr. Gergana Mihailova, as the sole candidate in the current competition, fully meets the regulatory requirements for the academic position "Associate Professor" at IPPG-BAS. The candidate's long-standing research on the physiology and biochemistry of resurrection plants under abiotic stress conditions is relevant and has potential future biotechnological applications. The candidate's research interests are fully aligned with the topics investigated in the "Photosynthesis – Activity and Regulation" Laboratory at IPPG-BAS. For these reasons, **I recommend that the esteemed scientific jury propose to the IPPG-BAS Scientific Council the awarding of Dr. Gergana Kirilova Mihailova for the academic position "Associate Professor"** in the professional field 4.3. Biological Sciences, scientific specialty "Biochemistry."

November 11, 2024 Signature:
(Kiril Mishev)