REVIEW

Considering the competition for **the academic position "Associate Professor"** in the field of higher education 4. "Natural Sciences, Mathematics, and Informatics", professional direction 4.3. "Biological Sciences", scientific specialty "Biochemistry", for the needs of the Laboratory "Photosynthesis - activity and regulation" at the Institute of Plant Physiology and Genetics - Bulgarian Academy of Sciences, announced in "State Gazette", issue 62, published on July 26, 2024.

Reviewer: Prof. Emilia Lyubomirova Apostolova, PhD Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences, member of the Scientific Jury according to order No. РД-01-43/20.09.2024 of the Director of IPPG - BAS.

Candidate: Senior Assist. Prof. Gergana Kirilova Mihailova, PhD

For participation in the announced competition for **the academic position, "Associate Professor"** one candidate has submitted documents: Senior Assist. Prof. Dr. Gergana Kirilova Mihailova. The candidate has attached all the necessary documents in accordance with the requirements of the Act on Development of the Academic Staff in the Republic of Bulgaria as well as the Regulations for its application approved by BAS and IPPG-BAS. The materials presented by the candidate are precisely prepared and well arranged.

Education and career development

Dr. Gergana Mihailova graduated in 2004 with a bachelor's degree in "Molecular Biology" and in 2006 with a master's degree in "Biochemistry" at the Faculty of Biology of the Sofia University "St. Kliment Ohridski". She defended a dissertation and received the educational and scientific degree "doctor" in 2012. The title of her dissertation is "Drying of the resurrection plant *Haberlea rhodopensis* in conditions of high temperature and different light regimes" at the Institute of Plant Physiology and Genetics - BAS. Dr. Mihailova's scientific career began at the Institute of Plant Physiology and Genetics in 2009, and she was assistant and senior assistant professor. The candidate has many years of experience in scientific research (14 years and 9 months). Doctor Mihailova was on specializations in Spain on COST STSMs at the Instituto Valenciano de Investigaciones Agrarias in 2014. During the period 2007-2023 she worked on joint projects with Germany (Institute of Molecular Biosciences, Goethe University of Frankfurt), Italy (Institute of Biometeorology, CNR) and 3 institutes in Hungary (Institute of Botany and Ecophysiology, Szent Istvan University - Gödólo; Department of Plant Physiology and Molecular Plant Biology, Institute of Biology, Eötvös Loránd University - Budapest; Institute of Plant Biology, Biological Research Center – Szeged).

Research activity

Dr. Mihailova's scientific research focuses on the response of the photosynthetic apparatus to adverse environmental conditions. Studying the mechanisms of tolerance to abiotic stress, with the aim of increasing the resistance of plants to adverse environmental factors, underscores the relevance of Dr. Mihailova's research.

The candidate's total publication activity includes 43 publications with a total IF=96.097. The publications submitted for the competition are related to the study of the tolerance mechanisms of the resurrection plant *Haberlea rhodopensis* to drought under conditions of high light intensity or low temperatures. Dr. Mihailova participated in the competition for associate professor with 20 publications, of which 15 have an IF or SJR (Q1 – 12, Q2 – 2, Q3 – 1), one with SJR (Q3), one book chapter, and three publications without IF or SJR. The habilitation extended reference (group indicator B of the presented reference) includes 4 publications with IF (Q1). According to the report submitted by the candidate, the total number of points in scientometric indicators is 942 points (indicator A - 50, indicator B - 100, indicator G - 280, indicator D- 392, indicator E - 120) with a requirement of 540 points. Doctor Mihailova exceeds the minimum national requirements and the regulations for their application approved by BAS and IPPG-BAS. The candidate also presents 196 citations.

Dr. Mihailova has presented materials from her research as 41 posters and 5 reports at 32 scientific forums, including 20 abroad. The candidate participated in the development of 23 scientific research projects, one of which she was the supervisor.

The main part of the experimental work was conducted at IPPG-BAS. Some of the analyzes were carried out in laboratories in Hungary, Italy and Germany.

Scientific activity of the candidate

The scientific works with which Dr. Mihailova participated in the competition are divided into two main thematic areas:

1. Desiccation of the resurrection plant *Haberlea rhodopensis* under conditions of high light intensity:

    - Photosynthetic and biochemical characteristics of *Haberlea rhodopensis* plants growing under different light regimes in their natural habitats (publications: В4-01, Г7-01, Г7-02, Г7-03, Г7-06).
    - Comparison of defense mechanisms examining the drought response of sun and shade plants of *Haberlea rhodopensis* from the same habitat (publications: В4-02, В4-03, В4- 04, Г7-04, Г7-05, Г7-07, Г7-08, Г7-09, Г7-10, Г7-14).

2. Cold resistance of the resurrection plant *Haberlea rhodopensis*. Photosynthetic and biochemical mechanisms of plant adaptation to low positive and negative temperatures. Comparison of the response of emergent plants of the genus *Haberlea*

and the genus *Ramonda*, family Gesneriaceae, to low temperatures (publications: Г7-11, Г7-12, Г7-13, Г7-15).

The main contributions related to the first thematic direction are:

- For the first time, the effect of light intensity in the drying process of *Haberlea rhodopensis* is evaluated, by comparing photosynthetic and biochemical characteristics of plants growing under different light regimes in their natural habitats. Experimental evidence shows that desiccation at high light intensity inhibits the rate of photosynthesis to a greater extent than the activity of photochemical reactions, even in highly desiccated leaves. The protective role of non-photochemical quenching was established only in sun plants. Higher photosynthetic activity, reduced chlorophyll content, and increased dissipation of excitation energy during the desiccation of *Haberlea rhodopensis* under high light intensity are hypothesized to protect plants from photoinhibition.

- Comparison of the effect of drought on sun and shade *Haberlea rhodopensis* plants from one habitat reveals phenotypic differences between the two types of plants (sun and shade), but the ultrastructure of chloroplasts and the organization of thylakoid membranes were similar, both in control and desiccated plants. Experimental evidence shows that morphological and structural changes in *Haberlea rhodopensis* are important adaptation mechanisms to protect against the damaging effect of excess light, especially in sun plants. Changes in cell structure upon desiccation are associated with changes in membrane integrity and oxidative stress, with more pronounced effects in the sun plant *Haberlea rhodopensis*.

- Very high antioxidant and antiradical activity, especially at very low water content, was found in both sun and shade plants of *Haberlea rhodopensis*. Research studies also reveal that the amount of non-enzymatic antioxidants and the activity of antioxidant enzymes reach their maximum in fully dried sun and shade plants. The important role of the ascorbate-glutathione cycle in overcoming oxidative stress during drought and after rehydration of *Haberlea rhodopensis* has been demonstrated.

- Using immunoblot analysis, it is shown that the amount of the photosystem I (PSI) reaction center protein, PsaB, decreases to a smaller extent in the desiccation process compared to D1, which correlates with greater sensitivity of the photosystem II (PSII) compared to PSI. Desiccation of sun and shade plants of *Haberlea rhodopensis* was found to reduce the amount of both the antenna proteins of PSII, Lhcb1, CP29 and CP43, and of PSI, Lhca1, especially in shade plants.

- It has been shown that the inhibition of photochemical reactions during drought in sun and shade plants is associated with an increase in the proportion of non-photochemical reactions. It has been shown for the first time that the two

Haberlea rhodopensis ecotypes use different strategies to quench excess light energy. Shade plants dissipate the excess excitation energy from the photoinactivated PSII reaction centers, while in sun plants the thermal dissipation of the excess excitation energy is carried out mainly by the antenna complexes.

- It was found that drought induced by low temperature or water stress induced similar changes in the polypeptide profile of both ecotypes, but these changes were more significant in the plants dried up as a result of water stress alone. This result supports the idea that the two ecotypes use almost identical defense mechanisms that provide resistance to drought induced by low temperature and water stress.

- Newly synthesized proteins were found in the bands around 48 and 14 kDa in the dry leaves of *Haberlea rhodopensis*, which relate to carbohydrate/phenylpropanoid metabolism (enolase, UDP-Dapiose/UDP-D-xylose synthase 2, V-type proton ATPase (VHA) subunit H), the protective proteins (isomerase, ELIP, pectin methylesterase, galactose mutarotase) and some with unknown function.

- It was found that during drought, the expression of some genes related to sugar metabolism (SUS, UDP), redox regulation and cell detoxification (APX, SOD, MDAR, TRX), genes related to chloroplasts (PTL, ERD), as these changes starting earlier in sun plants.

The main contributions related to second thematic direction are:

- It has been established that during acclimatization to low temperatures, the redistribution of excitation energy between the two photosystems is affected, the repair cycle of D1 and non-photochemical defense mechanisms are activated. Experimental evidence is presented showing a decrease in the amount of photosynthetic proteins of the core complex of PSII (D1, D2, CP43), the oxygen evolving complex (PsbQ) and the Calvin cycle (RcbL, RsbS, RA), as well as an increased content of hexoses, proline and the enzyme SUS1 related to hexose metabolism.

- For the first time, a broad screening of the content of photosynthetic proteins in *Haberlea rhodopensis* is done by immunoblot analysis. Changes in the protein stoichiometry of the light-harvesting complexes is found, resulting in an increase in the content mainly of those related to thermal energy dissipation (Lhca4, Lhcb3, CP26, CP24). It has been suggested that the stability of the oxygen evolving complex depends on the stability of the small 16 kDa protein PsbQ rather than the major 33 kDa PsbO.

- It has been established that the main defense mechanisms of *Haberlea rhodopensis* in desiccation caused by negative low temperatures are: increased content of unsaturated fatty acids, accumulation of sugars, especially sucrose and raffinose, changes in pigment protein complexes and increased thermal energy dissipation of excitement.

- For the first time, the cold resistance of two species of the genus Ramonda *(Ramonda serbica* and *Ramonda nathaliae*) was proven. Comparing the response of the three resurrection plants, *Haberlea rhodopensis*, *Ramonda serbica* and *Ramonda nathaliae*, shows that increased non-photochemical quenching (NPQ) values and carotenoid content have an important protective role in the process of acclimatization to low positive temperatures. Experimental evidence is presented showing the important role of stress-induced proteins for resistance to low negative temperatures in both *Haberlea rhodopensis, Ramonda serbica* and *Ramonda nathaliae.*

Personal contribution of the candidate

The candidate's personal contribution to the research in the publications presented for the competition is related to the conduct of physiological, biochemical (stress markers, pigment contents, Western blot analyses, enzyme activity), molecular-biological (gene expression) studies, and microscopic studies. Doctor Mihailova participated in the processing of the obtained results, their discussion and visualization and the design of the publications, which shows the significant contribution of the candidate in all publications. She is first or corresponding author on 7 of the presented publications.

Administrative, organizational and training activity

Doctor Mihailova is the thesis supervisor of a student from the Faculty of Biology of the Sofia University of “St. Kliment Ohridski”. She was also the supervisor of 4 specialists from the Faculty of Biology of the Sofia University "St. Kliment Ohridski" and NBU.

The candidate has lectured at seminars at: Institute of Molecular Biosciences, Goethe University of Frankfurt, Germany and IPPG-BAN, Bulgaria.

Doctor Mihailova is the secretary of the "Physiology and Biochemistry of Plants" section at the SBU and the secretary of the General Assembly of IPPG-BAS. She was also secretary of the "Photosynthesis" section, IPPG-BAS from 2012-2017.

Critical notes and recommendations

I have no critical notes.

Future research

Directions for Dr. Mihailova's future research include expanding and deepening research on the response of the photosynthetic apparatus of higher plants under conditions of

abiotic stress and the protective mechanisms that allow them to overcome the adverse impact. Investigations on the influence of abiotic stress on PS1 will be deepened. Research will be conducted to elucidate the signaling pathways that chloroplasts use to communicate with the rest of the plant cell under physiological conditions and stress, to develop strategies for improving the plant tolerance.

Conclusion

The investigations of Dr. Mihailova provide new knowledge about the tolerance of the resurrection plant *Haberlea rhodopensis* to drought under conditions of high light intensity or low temperatures. From the submitted documents for the competition, it is clear that Dr. Mihailova's scientific output and scientometric indicators fully meet and even significantly exceed the recommended requirements for occupying the academic position **"Associate Professor"** according to the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for application of the law in BAS and the specific requirements of IPPG-BAS.

Based on the above, I allow myself to recommend to the members of the Scientific Jury to propose to the Scientific Council of IPPG - BAS to elect **Dr. Gergana Mihailova for the academic position "Associate Professor"** in professional direction 4.3. "Biological Sciences", scientific specialty "Biochemistry".

Data: 11.11.2024 г. Signature:

(Prof. Emilia Apostolova, PhD)