# R E P O R T

Considering the competition for the academic position "**Assoc. Professor**" in the area of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty "Biochemistry" announced in the State gazette issue 62 dated July 23, 2024, for the needs of the Institute of plant physiology and genetics, Bulgarian Academy of Sciences, Laboratory of "Photosynthesis – activity and regulation", Institute of Plant Physiology and Genetics - BAS.

with candidate in the competition **Res. Assistant Dr. Gergana Kirilova Mihailova**

**by Prof. DSc Stefka Germanova Taneva**, Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences

## *General presentation of the materials received*

The only candidate in the competition is Res. assistant Dr. Gergana Kirilova Mihailova from the "Photosynthesis - activity and regulation" laboratory, IPPG - BAS. All materials presented by the candidate have been prepared very precisely according to the Regulations for the Scientific Development of the Academic Staff of IPPG-BAS and the criteria for occupying the academic position "Associate Professor".

The total number of points on the scientometric indicators is 942 points (indicator A - 50, indicator B - 100, indicator D - 280, indicator D - 392 and indicator E - 120), which significantly exceeds the minimum national requirements and regulations of ZRAS - IPPG-BAS (540 points) for the academic position of "Assoc. Professor".

## *Education and career development*

Dr. Mihailova received her bachelor's degree in Molecular Biology in 2004 and a master's degree in Biochemistry in 2006 from the Faculty of Biology of the Sofia University "St. Kliment Ohridski". In October 2009 she was appointed as an Assistant and since August 2014 an Assistant Professor in the Laboratory of "Photosynthesis – activity and regulation", IPPG - BAS, where she works until now. In 2012 she defended a dissertation on the topic: "Drying of the resurrecting plant Haberlea rhodopensis in conditions of high temperature and different light regimes" with supervisor Prof. Dr. Katya Georgieva.

***Scientific indicators*** Dr. Gergana Mihailova has a total of 43 publications, of which 22 are with quartile Q1, 8 with Q2, 4 with Q3, 1 with Q4 and 8 without IF/Q. In the competition, she participates with **19** publications with total IF of 43.952 and **1 review**. **4** publications are included in the habilitation thesis, all of them are with quartile Q1; and non-habilitation 14 publications (8 with Q1, 2 with Q2, 2 with Q3, 1 publication with SJR, and 1 in a peer-reviewed journal not indexed in WoS

and Scopus) and **1 book chapter**. All published materials are related to the topic of the competition.
Dr. Mihailova is first and/or corresponding author in 7 of the publications.

The high influential international journals with a high IF in which some of the scientific works of the candidate have been published are impressive: Environmental and Experimental Botany, IF 9.257; Plants, IF 8; Plant Physiology and Biochemistry, IF 6.122; International Journal of Molecular Sciences, IF 5.6; Physiology and Molecular Biology of Plants, IF 3.023. The publications are cited 199 times according to reference (according to Scopus, November 11, 2024), h-index 8 (Scopus). This points out the high scientific level of the publications.

She participated in 32 international and national scientific forums, and in the development of 23 projects (financed by FNI, under EDB-BAS (with Hungary, Latvia, Germany, Italy), National programs, COST action and others).

It should be noted that Dr. Mihailova was a supervisor of a graduate student, a pre-graduate internship student, and students on the "Student Practice" program.

***Major Scientific Contributions***

The research activity of Res. Assistant Mihailova was focused on understanding the physiological and biochemical responses of the photosynthetic apparatus of plants to adverse environmental conditions. As a model system for elucidation the mechanisms of tolerance to drought in conditions of high light intensity or under the influence of low temperatures, the resurrection plant Haberlea rhodopensis was used.
Two groups of scientific contributions can be highlighted in the field of: (1) Desiccation of Haberlea rhodopensis in conditions of high light intensity, under different light regimes in their natural habitats, and the protective mechanisms during desiccation of sun and shade plants; (2) Cold resistance of Haberlea rhodopensis; photosynthetic activity and biochemical mechanisms of plant adaptation to low positive and negative temperatures and comparison the response of plants of Haberlea and Ramonda, two genera in the family Gesneriaceae, to low temperatures.

**(1)** For the first time, the effect of light intensity in the desiccation process of H. rhodopensis on photosynthetic activity and its dependence on the amount of stress markers was evaluated under different light modes in natural conditions. H. rhodopensis plants from four different habitats in the Rhodopes were used. It was found that at high light intensity, drought inhibits the rate of photosynthesis faster and more strongly, while their photochemical activity remains higher compared to shaded plants, and the process of $CO_2$ assimilation is more sensitive to stress compared to photochemical reactions. In highly desiccated sun plants, the non-photochemical quenching has a protective role, while in shade plants other mechanisms are suggested to be responsible for protecting plants from photoinhibition.
Increased electrolyte leakage in completely dry leaves was found to be a protective survival mechanism based on a reversible modification of the structure of the cell wall, plasma membrane and changes in the vacuolar system of the cells. For the first time, the kinetics of electrolyte efflux in resurrection plants has been determined using a mathematical model, and it has been shown that electrolyte

efflux occurs at a slower rate in shade plants than in sun plants, possibly as a result of their higher photosynthetic activity.

For the first time, H. rhodopensis sun and shade plants were studied in parallel and compared. Inhibition of photochemical reactions during drought in sun and shade plants is associated with an increase in the proportion of non-photochemical reactions.

It is shown for the first time that the two H. 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. This is also confirmed by changes in the structure and organization of pigment-protein complexes associated with the monomerization of LHC in shade plants and preservation of the amount of trimeric LHCII complexes in sun plants. Drought decreases β-carotene content to the greatest extent in sunny plants, while that of zeaxanthin increases several times, especially in shady ones.

It has been shown that $CO_2$ assimilation is the most sensitive photosynthetic process during drought. This is associated with decreased amounts of the 33 kDa protein (PsbO) from the oxygen evolving complex. The amount of photosystem 1 reaction center protein PsaB (PS1) was found to decrease to a lesser extent than the PSII core protein PsbA (D1) in the desiccation process.

The reduction of PSII complexes, stable PS1 levels and migration of LHCII to PS1, resulting in high PSI activity during drought are compensatory defense mechanisms. The protective role of non-photochemical quenching has been established in highly desiccated sun plants, while other mechanisms have been suggested to dissipate the excess energy in shade plants.

H. rhodopensis sun plants are subjected to additional stress during drought under high light intensity conditions, but nevertheless recover faster after rehydration compared to shade ones.

The important role of the ascorbate-glutathione cycle in overcoming oxidative stress during desiccation and after rehydration of H. rhodopensis has been demonstrated. It has been shown that non-enzymatic antioxidants have a leading role in the desiccation process of shade plants, while in sun plants such a role is played by enzymatic antioxidants and superoxide dismutase.

One of the most important defense mechanisms that resurrection plants use to reduce ROS produced as a consequence of inhibition of photosynthetic activity is to maintain an antioxidant system functioning during drought.

The desiccation process was characterized by an increased expression of all studied genes, except for LHC, which started earlier in sun plants. A new gene (ELIP) was annotated, showing the most strongly increased expression especially in sun plants.

**(2)** It was established that the maintenance of high photosynthetic activity, the changes in the distribution of excitation energy towards PSI, the reduced proportion of PSII supercomplexes and the increased proportion of PSI-LHCII, the activation of the D1-repair cycle and non-photochemical defense mechanisms, as well as an increased content of hexoses, proline and the SUS1 enzyme associated with hexose metabolism play a role in acclimation of plants to low temperatures.

A change in the stoichiometry of the LHC proteins was detected, resulting in an increase in the content mainly of those related to thermal energy dissipation (Lhca4, Lhcb3, CP26, CP24), and the stability of the oxygen evolving complex seems to depend on the stability of the small 16 kDa protein PsbQ.

Low positive and negative temperatures induce the appearance of new early light proteins ELIPs and different classes of dehydrins, the response of H. rhodopensis plants to low temperature and desiccation involves the accumulation of sugars and upregulation of dehydrins/ELIP protein expression.
Similarity was found in the drought and cold tolerance mechanisms that H. rhodopensis uses to survive under these adverse environmental factors.
For the first time, the cold resistance of two species of Ramonda, Ramonda serbica and Ramonda nathaliae, has been demonstrated. The maintenance of relatively high photosynthetic activity, increased non-photochemical quenching (NPQ) values and carotenoid content were shown to have an important protective role in the acclimation process of the three resurrection plants, H. rhodopensis, R. serbica and R. nathaliae, to low positive temperatures, and that the expression of stress-induced proteins plays an important role in their resistance to low negative temperatures. The $CO_2$ fixation was found to be more sensitive to low temperatures than the photochemical activity.

### *Prospects for future research*

The prospects and directions for the candidate's future scientific research are outlined, which are a continuation of her research work aimed at clarifying the process of photoinhibition of PS1, the mechanisms of plant tolerance to stress, the response of the photosynthetic apparatus of higher plants in conditions of abiotic stress and the defense mechanisms, elucidation of signaling pathways for communication of chloroplasts to the rest of the plant cell under normal and stress conditions. It is anticipated that these studies will lead to new strategies for improving crop plant resistance.

### *Critical notes and suggestions*

I have no critical comments on the documents that were precisely prepared, publications, list of citations presented in the competition. In my opinion the scientific contribution of the habilitation work should be summarized separately.

### *Personal contribution of the candidate*

Dr. Mihailova is the first and/or corresponding author of 7 of the publications, which implies a significant contribution of the candidate in the published works presented in the competition. She contributes to the experimental work, the processing of the results, their discussion and debate, and the publications writing.
The experimental work was carried out at IPPG-BAS, "Photosynthesis - Activity and Regulation" laboratory, some of the analyzes were carried out in collaboration with researchers from IPPG-BAS, as well as in laboratories in Hungary, Italy and Germany.

## CONCLUSION

The presented documents and materials from Assistant Professor Gergana Kirilova Mihailova, Ph.D., meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB in the Bulgarian Academy of Sciences and the specific requirements of IPPG-BAS for occupying the academic position of "Associate Professor". A significant volume of research data focused on elucidating the mechanisms of tolerance to drought in plants and their adaptation to low positive and negative temperatures has been presented, the scientific publications and the international recognition of the scientific results characterize the candidate as a competent and established researcher. The scientific metrics of Dr. Mihailova significantly exceed the recommended requirements for the scientific activity of candidates to occupy the academic position of "associate professor".

I strongly support the candidacy and recommend the members of the Scientific Jury and the Scientific Council of IPPG-BAS to award Dr. Gergana Kirilova Mihailova the academic position "Assoc. Professor" in the field of higher education 4. "Natural sciences, mathematics and informatics", professional direction 4.3. Biological Sciences, specialty "Biochemistry".

Sofia
11.11.2024 /Prof. DSc Stefka Germanova Taneva/