ИНСТИТУТ ПО БИОФИЗИКА И
БИОМЕДИЦИНСКО ИНЖЕНЕРСТВО - БАН

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REPORT

on the materials submitted for a competition for the academic position of "Associate professor" in the field of higher education 4. "Natural Sciences, Mathematics and Informatics", professional field 4.3 "Biological Sciences", scientific specialty "Biophysics", for the needs of the Department "Photoexcitable Membranes", Institute of Biophysics and Biomedical Engineering" - BAS, announced in SG No. 69/16.08.2024

by Prof. Dr. Maya Yaneva Velitchkova,
member of the scientific jury according to order No. 1352/14/10/2024 of the Director of IBBE-BAS

One candidate participated in the competition announced by the Institute of Biophysics and Biomedical Engineering – BAS (SG No. 69/16.08.2024) for the academic position of "Associate professor" for the needs of Department "Photoexcitable Membranes" –Sen. assistant professor Dr. Georgi Dimitrov Rashkov. In order to participate in the competition, all the required documents that meet the requirements of LDASRB and the Regulations on the terms and conditions for acquiring scientific degrees and occupying academic positions at IBBE - BAS have been submitted.

Professional and thematic development of the candidate

Senior assistant professor Dr. Georgi Rashkov graduated from the Faculty of Physics of the Sofia University "St. Kl. Ohridski" as a master's degree – physicist-engineer, specialty "Nuclear technology and energy" and second specialty "Metrology". Until 2005, he worked as a physicist at the Institute for Nuclear Research and Nuclear Energy, BAS. In 2006, he started working as a specialist physicist at the Institute of Biophysics, now IBBE. In 2014, he was enrolled as a doctoral student. In 2019, he successfully defended his dissertation on the topic: "Possibilities for the application of photosynthetic membranes as a bioreceptor for registration of pesticides" and acquired the educational and scientific degree "doctor" in the scientific specialty "Biophysics" (code 01.06.08). Since 2021, he is Sen. assistant professor in the same scientific specialty at the Institute of Biophysics and Biomedical Engineering. He specialized for 3 months in 2004 in Dubna, at Joint Institute for Nuclear Research, and in 2007 he specialized for one

month at the Biological Research Center at the National Academy of Sciences in Szeged, Hungary.

During the entire period of his professional development, Dr. Rashkov's scientific activity is in the field of the biophysics of photosynthesis with emphasis on the influence of various abiotic factors from the environment on the photosynthetic apparatus. This topic is directly related to the scientific direction of research in the Department "Photoexcitable membranes " of IBBE. Dr. Rashkov has co-authored 21 scientific publications in a number of renowned scientific journals presenting the results of his research, which have collected over 225 independent citations (excluding self-citations of all authors). A large part of the experimental results have been presented at international and national scientific forums as posters and reports.

Scientific data

The one presented by Dr. Rashkov's detailed report on the fulfillment of the minimum national requirements for holding the academic position "Associated professor" correctly reflects his scientific outputs and shows that these requirements have been met.

Dr. Rashkov participated in the competition with 16 scientific works, all of which are articles in scientific journals with an impact factor (total impact factor 52.4). The distribution of articles according to the quartiles of the journals in which the articles were published is as follows: 11 in Q1, 2 in Q2, 1 in Q3 and 2 in Q4. In two of the materials, Dr. Rashkov is the first author, and in nine articles he is the second author with more than 4-5 authors. A substantial part of the publications (11 issues), with which Dr. Rashkov has participated in the competition are from the last 5 years. To participate in the competition, the candidate submitted a list of 42 independent citations (excluding self-citations of all authors) of the articles submitted for the competition. This number of citations is expected given that these are articles published in 2022 and 2023. After excluding self-citations of all authors, Dr. Rashkov's h-index is 8 (Scopus). These scientometric data determine Dr. Rashkov as a scientist with active research activity.

The submitted report on the fulfillment of the minimum national requirements by the LDASRB and the Regulations for the specific conditions and procedure for occupying the academic position "Associate professor" at IBBE-BAN shows that the total number of points from the scientometric indicators with which Dr. Rashkov participated in the competition is 488 points, which fulfills and exceeds the required minimum of 430 points from the Regulations for the application of LDASRB in IBBE.

The distribution of points by indicators is as follows:

In group A (dissertation work for the award of ESD "doctor") - 50 points.

In group B.4, four articles are included, all in journals in quartile Q1, and the total number of points according to group B indicators is 100 points (the requirement is for a minimum of 100 points). The total impact factor of the articles in indicator B.4 is 19.86, with Dr. Rashkov being the first author in two of the publications.

Twelve publications are included in indicator group G, all of which are in indicator G7, distributed as follows: 7 are in journals with quartile Q1, 2 in Q2, 1 in Q3 and 2 in Q4, with a total impact factor of 32.54 and carry 254 points. The total number of points for indicator D is 250, with a required minimum of 220 points.

In group D (citations), the candidate has submitted a reference including 42 independent citations or 84 points with a requirement for a minimum of 60 points.

Dr. Rashkov has participated in the development of 14 scientific research projects, financed by the "Scientific Research" Fund and by budget subsidy from the BAS, and 4 of them are in international cooperation with India, Greece, Egypt and Slovakia. He has participated with posters at a number of scientific forums, reporting over 30 scientific communications.

Main directions in the scientific research work and scientific contributions of the candidate

Senior assisitant professor D. Rashkov's research focuses on the effects of abiotic factors, including drought, salinization, extreme temperatures, and light intensity, on photosynthetic activity and efficiency, which are crucial for cultivated plants. It also explores potential solutions to mitigate the negative effects of these factors. The application of highly sensitive and non-invasive methods based on chlorophyll a fluorescence allows to identify and analyze abiotic stress-induced changes in the energy interaction between chlorophyll-protein complexes in the photosynthetic apparatus and to understand the mechanisms that regulate the response of plants to stress conditions. Understanding the mechanisms by which plants perceive and respond to environmental stressors is important for developing strategies to increase plant resilience and ensure food security under changing climate conditions.

Senior assistant professor G. Rashkov's scientific research is unified in two main lines, as evidenced by the papers that have been submitted for review:

1. Study of the influence of abiotic stress factors on the photosynthetic apparatus and the mechanisms of its adaptation in different plant species, cyanobacteria and green algae. Chlorophyll a fluorescence methods (PAM and JIP test) for analysis of the photosynthetic apparatus.

2. Role of exogenously applied signaling molecules and nanoparticles under physiological conditions and under abiotic stress in different plant species.

In his scientific activity, Dr. Rashkov focuses on the influence of abiotic factors from the environment including salinization, UV radiation, drought on the physiological characteristics of plants, mainly economically important crops being studied. Changes in the Earth's climate, the presence of soil and air pollution and their impact on crops, to a large extent define the subject of Dr. Rashkov's scientific research as current and timely.

1. A large number of publications in the first direction are devoted to the effects of salt stress on the photosynthetic apparatus and its efficiency in several plants. Regarding salt stress, maize, sorghum, pea and two hybrid paulownia lines were also investigated. The main contributions can be summarized as follows:

- When comparing the influence of salt stress (0-200 mM NaCl) on a C3 plant (*Pisum sativum* L - pea) and a C4 plant (*Zea mays* L. - maize), a higher sensitivity of the photosynthetic apparatus of the pea was found compared to maize in terms of the size of the plastoquinone pool, the density of the QA-reducing reaction centers of PSII and the increase in the thermal dissipation of the excitation energy. It has been shown that the two plants also differ in the mechanisms for dissipation of the excess energy – while in maize this occurs through the regulated non-photochemical quenching which dependent on the xanthophyll cycle, in peas it is due to the unregulated non-photochemical quenching (B4). Based on the study on thylakoid membranes from pea and maize treated with higher concentrations of NaCl, it was shown that salt stress inhibits the activity of photosystem II (PSII) and the activity of the oxygen-evolving complex, and this negative effect is more pronounced in peas. It was found that grana situated centers of PSII ($PSII_{\alpha}$) compared to $PSII_{\beta}$) are affected to a greater extent. (B4, D12)
- It was found that with regard to treatment with high concentrations (150-200 mM) of NaCl, sorghum (*Sorghum bicolor* L. Shamal) showed a higher tolerance compared to maize (*Zea mays* L. Kerala), determined by main characteristics of PSII - more closed

centers of PSII and reduced efficiency of these centers, reduced quantum efficiency of PSII. This is associated with structural changes in photosynthetic membranes and activation of cyclic electron transport around photosystem I (PSI). In the study of two other varieties of maize and sorghum (*Sorghum bicolor* L. Albanus) and (*Zea mays* L. Mayflower), it was shown that when treated with higher concentrations (250 mM) of NaCl, the inhibition of photosynthesis, of linear electron transport and the photochemical activity of PSI is observed, and these parameters are more affected in sorghum (G1, G10).

• Cultivation of two lines of salinity-tolerant Paulownia species (*Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *elongata*) in saline soils was found to increase the photochemical quenching coefficient (qP) and linear electron transfer rate (ETR), while the maximum quantum yield of the primary photochemistry of PSII is not affected and an improvement in the photochemical energy conversion efficiency is observed (ΦPSII). Salinity leads to a delay in cyclic electron transport around FS1 in both studied lines, the effect being more pronounced in *P. tomentosa x fortunei* compared to *P. elongata x elongata*. The study of thylakoid membranes from paulownia plants treated in this way allowed conclusions to be drawn regarding the effects of salt stress on specific components and processes in the photosynthetic apparatus, and it was shown that after a ten-day treatment the activity of both photosystems was inhibited (as PSII was more affected), and the kinetic parameters of the oxygen scavenging complex are affected, with losses, double hits, and blocked centers increasing. With a longer treatment -25 days, a different adaptability of the two lines to high concentrations of NaCl in the soil was found, which is important in choosing a suitable paulownia line for cultivation in the respective soils. (G4, G5).

2. The influence of another environmental factor – drought has been studied on sorghum and maize and it has been shown that different levels of water deficit lead to a decrease in the efficiency of the primary processes of photosynthesis, affecting both photosystems. Sorghum was found to show higher sensitivity to drought and its inhibition started at lower drought levels. The established new information about the role of the regulated non-photochemical quenching and the transition between the states ("state transition") for

the protection of the photosynthetic apparatus during drought can serve to determine the tolerance of plants to water deficit. (G2).

3. When comparing the sensitivity of the cyanobacterium *Synechocystis salina* and the green alga *Chlorella vulgaris* to UV-B irradiation and to oxidative stress induced by exogenously applied sanosil, it was shown that in terms of the photochemical activity of PSII and oxygen evolution, the cyanobacterium is more sensitive to UV-B and hydrogen peroxide compared to green algae (G8, G11).

4. A higher sensitivity to high temperature of grana-located PSII ($PS2_{\alpha}$)compared to $PSII_{\beta}$ centers in pea plants (*Pisum sativum* L.) was found. The plants in which the content of carotenoids was affected by the application of an inhibitor of carotenoid biosynthesis fluridone, are more sensitive to temperature treatment (G7).

The second direction in the development of assistant professor Rashkov is dedicated to research on exogenously applied signaling molecules and nanoparticles and their effect on the photosynthetic apparatus under normal, physiological conditions and when abiotic stress is applied.

Several publications have reported the results of studies on the influence of sodium nitroprusside (SNP), which is an NO donor. Its effect on maize and sorghum under physiological conditions and under salt stress was compared. The contributions regarding the action of SNP can be summarized as follows:

1. The concentration dependence of the effect of SNP on the photosynthetic apparatus of sorghum (*Sorghum bicolor* L. Albanus) and maize (*Zea mays* L. Kerala) was established, and it was shown that low concentrations induced an increase in photochemical quenching (qp) and stimulated the flow of electrons to the acceptor side of PSI. A different effect of P700 was found in the two plants, suggesting species specificity of the SNP (B2).

2. Foliar application of SNP (50-150 mM) was found to have a protective effect on the photosynthetic apparatus in maize (*Zea mays* L. Kerala) against salt stress, and this protection occurs on the donor and acceptor side of PSII and leads to effective transfer of electrons along the entire electron-transport chain from the donor side of PSII to the final acceptors of PSI and affects positively the efficiency of photosystems. Foliar application of SNP to two salt-stressed sorghum cultivars

(*Sorghum bicolor* L. Albanus and *Sorghum bicolor* L. Shamal) was shown to alleviate NaCl toxicity on the photosynthetic apparatus and thylakoid membranes, and this protective effect was best expressed at concentrations up to 150 mM SNP and this effect was different in the two sorghum cultivars (B1, B3).

3. It was shown that the application to pea plants of zinc oxide nanoparticles - pure (ZnO NPs) and coated with a silicon shell (ZnO-Si NPs) - in concentrations up to 200 mg/L did not affect the activity of the two photosystems and the structure of stomata, but when applied to plants subjected to salt stress, reduced the negative effects of NaCl on the photochemistry of PSI and PSII, as well as on the content of pigments, stomatal closure and membrane integrity (G3).

Senior assistant professor Rashkov has correctly assessed and reflected his personal contribution to these developments and his expertise in relation to the biophysical methods used - chlorophyll a fluorescence - PAM and JIP test and polarographic determination of oxygen yields. The use of these methods and the sharing of information from them allows to make a comprehensive assessment of the photosynthetic activity in the norm and under stress, as well as the interrelationship and interaction of the individual components of the photosynthetic apparatus.

What has been presented so far clearly shows that Dr. Rashkov has outlined a scientific topic that is relevant both for enriching our knowledge about the response of plants to abiotic stress factors, but also has practical and socially significant dimensions.

Critical notes and questions to the candidate

The papers proposed for review contain much new and significant information on the influence of several abiotic factors on plants, and more specifically on the functionality of the photosynthetic apparatus in important crops. But in some of the contributions outlined in the habilitation report, Dr. Rashkov failed to synthesize and emphasize in the best way the significance and novelty of the research, which does not reduce the value of these contributions. Here is the question of the established different effect of foliar treatment with SNP on sorghum (*Sorghum bicolor* L. Albanus) and maize (*Zea mays* L. Kerala) under physiological conditions - whether this difference also occurs when treating the same plants subjected of on salt stress. Is the species specificity of SNPs manifested to the same extent in plants subjected to salt stress. Considering the large amount of data on chlorophyll a fluorescence parameters obtained with

PAM and JIP test from control and stressed plants, which are largely personal contributions of the applicant, I would recommend that he summarizes, analyzes and forms them into a publication, what will enable him to present a broad view of the mechanisms of response of the photosynthetic apparatus to abiotic stress and of overcoming the negative consequences for the efficiency of photosynthesis.

Prospects for future research

The outlined directions for future research are a logical continuation of the previous scientific work, updated with the modern achievements of science, and the emphasis on their potential for practical application should be noted. I would recommend the candidate to activate the activity in the future, above all, in the management of research scientific projects and in the training of masters and doctoral students.

Conclusion

The presented materials convincingly show that Dr. Georgi Rashkov is a scientist working in an up-to-date and significant field of modern science. The topic of his research is among the main directions in the goals and mission of IBBE. Much of the research is of an original nature and is a contribution to fundamental science, and many of them have serious potential for practical application. The scientific indicators of Dr. Rashkov fully cover and exceed both the national and IBBE criteria for occupying the academic position "Associated professor".

I positively assess the candidacy of senior assistant professor Dr. Georgi Rashkov for the academic position of "Associated professor" and I will vote FOR. I recommend the members of the esteemed Scientific Jury to propose to the members of the Scientific Council of IBBE to elect Dr. Rashkov to the academic position of "Associate Professor" in professional direction 4.3. "Biological Sciences", scientific specialty "Biophysics".

19. 11. 2024 Signature:

Sofia /Prof. Dr. M. Velitchkova/