REVIEW

on the competition for the academic position "Associate Professor" in the professional field 4.3 Biological Sciences, scientific specialty "Biophysics", announced in State Newspaper issue 69/16.08.2024 for the needs of the Department "Photoexcitable Membranes" at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences

Reviewer: Prof. Dr. Katya Marinova Georgieva, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, member of the Scientific Jury according to order №1352/14.10.2024 of the Director of IBPhBME-BAS

Assistant Professor Georgi Dimitrov Rashkov, PhD is the only candidate in the competition announced by IBPhBME - BAS for the academic position "Associate Professor" for the needs of Department "Photoexcitable Membranes". The materials presented have been prepared in accordance with the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation and the Regulations for the conditions and procedures for acquiring academic degrees and occupying academic positions in IBPhBME - BAS.

Scientific career

Georgi Rashkov completed his higher education at the Faculty of Physics of Sofia University "St. Kliment Ohridski" as a physicist engineer, majoring in "Nuclear Technology and Energy" in 2002. Until 2005, he worked as a physicist at the Institute of Nuclear Technology and Energy, BAS. In 2006 he started working at the Institute of Biophysics and Biomedical Engineering, BAS, as a specialist-physicist, after which he held the academic position of assistant, and from 2021 he is an assistant professor at the same institute. During the period 2014-2019, he developed and successfully defended a dissertation on the topic "Possibilities of application of photosynthetic membranes as a bioreceptor for registration of pesticides" and obtained a Ph.D. degree. Georgi Rashkov has 22 years of work experience in the specialty.

Research activity

Assist. Prof. Dr. Georgi Rashkov has a total of 21 scientific publications, which have been cited 225 times. In the competition for the academic position "Associate Professor" he submitted 16 publications with total impact factor 52.41. Their distribution by quartiles is as follows: 11 publications with Q1, 2 with Q2, 1 with Q3, and 2 publications with SJR without an impact factor.

The submitted report on the fulfillment of the minimum national requirements by the Regulations for the specific conditions and procedure for occupying the academic position "Associate Professor" at IBFBMI-BAN shows that the total number of points for scientometric indicators with which Dr. Rashkov participated in the competition is 488, which exceeds the minimum requirements of 430 points. The following indicators are presented:

Group A (Ph.D. Thesis) – **50** points

The total number of points according to **group B** indicators is **100** points (required minimum 100 points) – 4 publications Q1are included with a total JCR IF 19.866.

Group G includes 12 publications (Q1 - 7, Q2 - 2, Q3 - 1, with SJR without IF - 2) with a total number of points **254** (required minimum 220 points) and JCR IF 32.54.

Group D (citations) – **84** points (required minimum 60 points). A reference is presented for 42 citations of some of the publications participating in the competition in scientific journals referenced in Scopus or Web of Science.

Dr. Rashkov has participated in the implementation of 13 research projects, which shows his active scientific activity. He was a participant in 4 budget subsidy projects - BAS, 5 projects funded by the Scientific Research Fund, one of which was with Slovakia, as well as 4 bilateral projects (with India, with the Aristotle University of Thessaloniki, Greece, with the University of Cairo, Egypt, with the Hungarian Academy of Sciences). Georgi Rashkov has presented a list of 33 participations in 22 scientific forums, 6 of which abroad.

Analysis of scientific achievements

The scientific research of Assist. Prof. Dr. Georgi Rashkov is focused in two main directions: 1. Influence of abiotic stress factors on the photosynthetic apparatus and the mechanisms of its adaptation in different plant species, cyanobacteria and green algae; 2. Role of exogenously applied signaling molecules and nanoparticles under physiological conditions and under abiotic stress in different plant species.

Adverse environmental factors slow down the growth and development of plants and reduce their productivity. Elucidating the response of plants to stress and the adaptive mechanisms to overcome it is of great importance. A large part of the scientific publications presented for the competition are dedicated to the study of changes in the photosynthetic apparatus, which is particularly sensitive to adverse environmental conditions. The role of signaling molecules, nanoparticles and plant phytohormones to improve plant resistance in stress conditions has also been investigated. The studies were carried out on leaves and isolated thylakoid membranes using a number of biophysical and biochemical methods.

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 (publications B4, G1, G2, G4, G5, G7, G8, G10, G11, G12)

Original and significant results were obtained on the influence of salinization on the functional activity of the photosynthetic apparatus of various plant species (***publications B4, G1, G4, G5, G10 and G12***).

The study of the effect of treatment of C3 (*Pisum sativum* L.) and C4 (*Zea mays* L.) plants with different concentrations of NaCl (0–200 mM) on the photochemical activity of PS2 and PS1 showed the higher sensitivity of pea compared to the corn. Salt stress was found to cause a decrease in the relative size of the PQ pool and the quantum efficiency of PS2, as well as a decrease in electron transport to the terminal acceptors of PS1, which affected the activity of this photosystem. While treatment of maize plants with 200 mM NaCl caused an increase in non-photochemical quenching ΦNPQ, in pea plants the main part of the excitation energy that cannot be used for photochemistry is passively dissipated as heat and fluorescence, ΦNO (***publication B4***). The better resistance of maize compared to pea was also confirmed by studies with isolated thylakoid membranes, which gave additional information on the effect of salinity on the donor and acceptor sides of PS2 (***publication G12***). Salt stress has been shown to inhibit the photochemical activity of PS2, affecting energy transfer between PS2 pigment-protein complexes, Q_A reoxidation, and the function of the oxygen-evolving system, and these processes are more strongly affected in pea than in maize.

Comparing the salinity tolerance of maize and sorghum shows that it depends on the hybrid lines studied. The better resistance to salinization of sorghum (*Sorghum bicolor* L. Shamal) compared to maize (*Zea mays* L. Kerala) was found, which is expressed in a weaker inhibition of the photochemical activity of PS2, the stimulation of the cyclic electron flow around PS1 and more effective antioxidant defense (***publication G1***). On the other hand, the study of the hybrid lines *Zea mays* L. Mayflower and *Sorghum bicolor* L. Albanus concept showed better resistance of maize plants (***publication G10***).

It was established that the cultivation of two lines of the salinity-tolerant Paulownia species (*Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *elongata*) in saline soils led to an increase in the activity of PS2 and a delay in the cyclic electron transport around PS1 in both studied lines. Their resistance to salinization allows them to be used for phytoremediation of saline soils (***publication G5***). Studies with isolated chloroplasts show that 10-day NaCl treatment inhibits the photochemical activity of both photosystems, induces changes in energy transfer between pigment-protein complexes of thylakoid membranes and kinetic parameters of oxygen-evolving reactions. However, with long-term treatment, plants adapted to salinity, with *Paulownia tomentosa* x *fortunei* adapting to higher NaCl concentration compared to *Paulownia elongata* x *elongata* (***publication G4***).

The influence of other abiotic stress factors such as drought, high temperature stress, UV radiation on the photosynthetic apparatus was also investigated (***publications G2, G7,***

G8, G11). Drought was found to cause a decrease in the photochemical activity of PS2 and PS1, a decrease in pigment content and membrane damage, and it was stronger in sorghum compared to maize, suggesting its better drought tolerance. Stronger inhibition of photochemical reactions in sorghum is accompanied by an increase in the proportion of unregulated excitation energy quenching, while in maize the proportion of regulated non-photochemical quenching increases (***publication G2***).

The sensitivity of the photosynthetic apparatus of the cyanobacterium *Synechocystis salina* and the green alga *Chlorella vulgaris* to UV-B radiation was compared. UV-B irradiation induced changes in energy transfer between the two photosystems, lowering the maximum quantum activity of PS2 and inhibiting oxygen evolution, which was stronger in *S. salina.* (***publication G8***). A higher sensitivity of *S. salina* compared to *C. vulgaris* to sanosil-induced oxidative stress, which mainly affected the donor side of PS2, was also shown. The key role of light-harvesting antenna size in the sensitivity of green algae and cyanobacteria to oxidative stress has been suggested (***publication 11***).

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

In this scientific topic, Dr. Rashkov has presented 6 publications (**B1, B2, B3, G3, G6, G9**). Part of the research is aimed at clarifying the role of nitric oxide on photosynthetic activity under physiological conditions and salt stress. The effects of different concentrations (0–300 μM) of sodium nitroprusside (SNP) as an NO donor on the functions of the photosynthetic apparatus in sorghum (*Sorghum bicolor* L. Albanus) and maize (*Zea mays* L. Kerala) were investigated. Treatment of plants with the highest concentration of SNP was found to increase the level of oxidative stress markers and induce different changes in the photosynthetic apparatus of the two plant species studied (***publication B2***). The protective effect of SNP under salt stress conditions is shown. The obtained results show that the exogenous application of SNP in maize plants reduces the degree of NaCl-induced oxidative stress and changes in the fluidity of thylakoid membranes and the redistribution of energy between the two photosystems and improves the efficiency of PS2. Optimal protection under salt stress was achieved by foliar spraying with 50–150 μM SNP (***publication B1***). A similar result was obtained in foliar treatment with SNP of two sorghum varieties, which showed different resistance to salt stress. Nitric oxide has a more pronounced protective effect in the more salinity-tolerant variety. The results showed better protection of the photosynthetic apparatus in both sorghum cultivars studied at SNP concentration up to 150 μM (***publication B3***).

An important role in improving the growth and development of plants and increasing their resistance to abiotic stress is the application of plant hormones such as brassinosteroids, nanoparticles and microalgae. The optimal concentration of 24-epibrassinolide (EBR) that has the best effect on the functional characteristics of thylakoid

membranes isolated from pea plants was determined. The exogenous application of 0.1 mg/L EBR causes a structural reorganization of the pigment-protein complexes, which affects the kinetics of oxygen evolution, the distribution of energy between the two photosystems, increases the quantum efficiency of PS2 and the rate of electron transport. It has been suggested that EBR-induced structural changes in thylakoid membranes are necessary for plant adaptation to adverse environmental factors (***publication G6***).

The study of the effect of synthesized zinc oxide nanoparticles: pure (ZnO NPs) and coated with a silicon shell (ZnO-Si NPs) shows that they have a different effect on the photosynthetic activity of pea plants under physiological conditions and under salt stress. The impact of ZnO NPs strongly depends on the applied concentration, as 400 mg/L ZnO NPs induce oxidative stress, weak phytotoxic effects accompanied by stimulation of the cyclic electron flow around PS1 and an increase in non-photochemical quenching. Si-coated NPs (200 and 400 mg/L ZnO-Si NPs) did not affect the functions of the photosynthetic apparatus under physiological conditions, even a slight increase in photochemical activity was found at the higher concentration. Both types of nanoparticles (except 400 mg/L ZnO NPs) were shown to reduce the negative effects induced by NaCl on the activity of PS1 and PS2, as well as on pigment content and membrane integrity. The protective effect was better after treatment with ZnO-Si NPs compared to ZnO NPs (***publication G3***).

The study of the role of microalgae under conditions of cadmium stress shows that the presence of *Chlorella vulgaris* not only stimulates the growth and improves the functions of the photosynthetic apparatus under physiological conditions, but also reduces the toxic effect of Cd in rice. The data show that this is due to the sorption of the heavy metal by the microalgae, as well as the reduced accumulation of Cd in the roots and its transport from the roots to the stems (***publication G9***).

Critical Remarks and Recommendations

When presenting the contributions, it is good to summarize the obtained results presented in the publications that are thematically related. This will prevent the presentation of contrary conclusions, as in publications G1 and G10. The main results of each report need not be a separate contribution.

Applicant's personal contribution

Assistant Professor Georgi Rashkov is the first author of two and second author of nine of the submitted publications for the competition, which shows the significant contribution of the candidate. In the presented report, he has emphasized that his personal contribution is related to the assessment of the functional activity of the photosynthetic apparatus under physiological conditions and after the application of abiotic stress, by measuring and analyzing the parameters of chlorophyll fluorescence and the rate of oxygen evolution. These

studies show that chlorophyll fluorescence parameters can be used for rapid screening of plant resistance to various stressors.

Future research

Georgi Rashkov's future research is mainly related to: 1) Deepening the research on the mechanisms of resistance of photosynthesis to abiotic stress in different plant species; 2) Elucidation of the role of the degree of oligomerization of the light-harvesting complex of PS2 under abiotic stress using chlorophyll mutants of pea plants; 3) Investigation of the application of different nanoparticles on the functions of the photosynthetic apparatus under physiological conditions and under different stress effects.

CONCLUSION

The scientific research activity of Dr. Georgi Rashkov is extremely important for clarifying the response of the photosynthetic apparatus to stress impacts and the possible strategies for improving plant resistance. Scientific works contain significant fundamental and applied contributions that have received international recognition. The submitted documents for the competition show that Dr. Rashkov's scientific output and scientometric indicators meet all the requirements for occupying the academic position of "Associate Professor" according to the Law on the Development of the Academic Staff in the Republic of Bulgaria, as well as the specific requirements in the Regulations for its implementation in IBPhBME-BAS.

All this gives me reason to recommend to the respected members of the Scientific Jury and to the members of the Scientific Council of the Institute of Biophysics and Biomedical Engineering at the BAS to award Dr. Georgi Dimitrov Rashkov, the academic position of "Associate Professor" in professional direction 4.3. Biological Sciences, specialty "Biophysics".

November 19, 2024
Sofia

Signature:
/Prof. Katya Georgieva)