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 21/07.03.2023 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 №242/06.04.2023 of the Director of IBPhBME-BAS

Assistant Professor Martin Angelov Stefanov, 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.

Brief biographical data

Martin Stefanov graduated from the Faculty of Biology of Sofia University "St. Kliment Ohridski", with a master's degree in ""Plant Biotechnologies" in 2014. In 2019, he successfully defended his Ph.D. thesis on "Adaptive mechanisms of the photosynthetic apparatus to salinity and light stress in two *Paulownia* lines" and received the academic and educational degree "Doctor". Dr. Stefanov's scientific career began at IBPhBME - BAS as a specialist-biologist, then as an assistant, and since 2019 he has been an assistant professor at the same Institute. Martin Stefanov has 7 years of work experience in the specialty.

Research activity

Assist. Prof. Martin Stefanov has a total of 23 scientific publications, cited 133 times. For participation in the competition for the academic position "Associate Professor" he has presented 17 scientific publications with total impact factor 45.398, 8 of them have been published in journals with a rank of Q1, 3 in Q2, 4 in Q3 and 1 in Q4. The list of publications for the competition includes 1 book chapter.

The presented publications are distributed as follows: In the habilitation work (indicator B4) are included 5 publications - 4 of them in Q1 and 1 in Q2. The publications outside the habilitation work are 12: indicator G7 - 12, of which 4 with rank Q1, 2 with Q2, 4 with Q3 and 1 with Q4; according to indicator G8 – 1 book chapters.

The following table represents the total number of points of the applicant and the minimal required score according to the *Regulations for application of the Act on Development of the Academic Staff* at Institute of Biophysics and Biomedical Engineering – BAS.

Information on the fulfillment of the minimum requirements

Indicator group	Minimal score - IBPhBME	Score of Martin Stefanov
A	50	50
B	100	120
G	220	227
D	60	106
Total:	430	473

According to the report submitted by the applicant, the total number of points in indicators is 473 with the requirement of 430 points. Martin Stefanov is the first author in 9 of the publications. He has also presented a citation list of 2 of the most cited publications (Web of Science and Scopus) – a total of 53 (indicator D).

Evidence of his active research is also the participation in 13 research projects. He was a participant in 3 budget subsidy projects - BAS, 3 projects funded by the National Science Fund (NSF), one of which was with Slovakia, as well as 2 bilateral cooperation projects (with the Aristotle University of Thessaloniki, Greece and with the University of Cairo, Egypt). Dr. Stefanov has won two projects under the Program for the Support of Young Scientists at the Bulgarian Academy of Sciences and two projects under the National Scientific Program "Young Scientists and Postdoctoral Students". He is also a leader of a project funded by NSF. Assist. Prof. Martin Stefanov has presented a list of 39 participations in 26 scientific forums, 8 of which abroad.

The presented scientific production exceeds the minimum requirements for awarding the academic position "Associate Professor", according to the Regulations for the conditions and procedures for acquiring academic degrees and occupying academic positions in IBPhBME.

Relevance of research and scientific contributions

Plants are frequently exposed to the influence of unfavorable environmental stress factors, such as salinity, drought, heavy metals, high light, high temperature and others, which cause physiological and biochemical changes in plants, that inhibit plant growth and development and reduce the yield. The photosynthetic apparatus (PSA) is particularly sensitive to adverse environmental conditions and the maintenance of photosynthetic activity under stress is essential for plant survival. Therefore, elucidating the response of PSA to stress conditions and the mechanisms of plant acclimation are of great importance. Many of the scientific publications presented for the competition are devoted to the study of changes in the structure and functional activity of photosynthetic membranes as a result of the action of various adverse environmental factors such as salinity, drought, heavy metals, low temperatures and high light intensity. Special attention was paid to the mechanisms of PSA resistance and protection in different crop plants (maize, sorghum, pea, wheat, rice), as well as the effects of different signaling molecules (nitric oxide, salicylic acid), nanoparticles and herbicides on photosynthetic membranes under conditions of abiotic stress. The studies were carried out on leaves and isolated thylakoid membranes using a number of biophysical and biochemical methods. The results of the experimental work in the publications submitted for the competition are well described in the habilitation report and are summarized in 6 thematic areas.

The scientific contributions are presented in two groups:

Contributions related to publications from habilitation work (indicator B)

The habilitation work includes 6 publications and 3 main scientific contributions have been formulated. In all publications, Martin Stefanov is the first author.

It was found that changes in the primary photochemical reactions of photosystem 2 (PS2) induced by salt and water stress can be used as a criterion for the tolerance of maize and sorghum to these stress factors (Stefanov et al. 2020; Stefanov et al. 2021a; Stefanov et al. 2023c). Comparing the photosynthetic resistance of new hybrid lines of maize (*Zea mays* L. Kerala) and sorghum (Sorghum bicolor L. Shamal) to salinity shows the better resistance of sorghum, which is related to structural changes in photosynthetic membranes and the stimulation of cyclic electronic flow around PS1 at higher NaCl concentrations (Stefanov et al. 2021a). Studies on the effect of different concentrations of polyethylene glycol 6000 (PEG 6000) on the photosynthetic performance of maize (*Zea mays* L. Mayflower) and sorghum (*Sorghum bicolor* L. Foehn) showed a better drought tolerance of maize compared to sorghum. This study provides new information on the role of regulated energy loss and state transitions in the defense of the photosynthetic

apparatus under drought and can be used as a practical approach to determine plant tolerance to this stressor.

Furthermore, based on the established relationship between tolerance to salt stress and changes in the structure and function of PSA, the resistance to salinity of representatives with C3 (pea) and C4 (maize) type of photosynthesis was compared (Stefanov et al. 2022).

For the first time, the role of carotenoids, flavonoids and proline in the protection of PSA and the adaptation of *Paulownia* to high NaCl in nutrient solutions was evaluated. It has been found that NaCl-induced changes in energy transfer between pigment-protein complexes and kinetic parameters of oxygen evolution influenced the degree of inhibition of the photochemical activity of both photosystems (Stefanov et al. 2021b; Stefanov et al. 2023a).

Contributions of publications outside the habilitation work (indicator G)

An important part of Dr. Martin Stefanov's research is devoted to the influence of various signal molecules (nitric oxide, salicylic acid), as well as zinc oxide nanoparticles, microalgae and nitrogen supply on the functional activity of the photosynthetic apparatus in conditions of abiotic stress . Based on the results obtained from the publications outside the habilitation work, 5 scientific contributions were formulated, which can be summarized as follows:

For the first time, a more detailed analysis of the effects of sodium nitroprusside (SNP, an NO donor) on primary photosynthetic processes in two sorghum cultivars under salt stress was performed. Foliar application of SNP was found to attenuate the toxicity of NaCl on photosynthetic functions by increasing the number of active reaction centers of PS2 and increasing the photochemical activity of PS1. The obtained results indicate that the application of SNPs could be part of a reliable approach to improve the salt tolerance of sorghum cultivars by applying additional agronomic measures (Stefanov et al. 2023b).

The effect of foliar application of two types of zinc oxide nanoparticles, pure and coated with silica (ZnO (±Si) NPs), on the photosynthetic apparatus of pea plants under physiological conditions and salinity stress was investigated. Under control growth conditions, higher concentrations of ZnO NPs (400 mg/L) were found to be phytotoxic and induce oxidative damage in plant cells, while treatment with 400 mg/L ZnO-Si NPs had a stimulatory effect on the photochemical activity of the two photosystems. Both types of nanoparticles limit the negative effects induced by NaCl on the primary photochemical reactions of pea plants, and those containing Si have a more favorable effect (Elshoky et al. 2021).

The effects of salicylic acid (SA) and the microalgae *Chlorella vulgaris* on the PSA of rice plants were investigated, as well as the effect of nitrogen nutrition (NO_3^-) in wheat plants under conditions of increased Cd stress. Experimentally, 10 μM SA was found to be the optimal concentration for the growth and functional activity of the photosynthetic apparatus of rice plants under physiological conditions (Yotsova et al. 2018a). It was shown for the first time that SA, microalgae as well as nitrogen nutrition favorably affected the functional activity of PSA under Cd-induced stress by reducing the degree of oxidative stress, significantly improving plant growth, photochemical activities of both photosystems, pigment content and kinetic parameters of oxygen-evolving reactions (Yotsova et al. 2018b; Yotsova et al. 2022; Yotsova et al. 2020). The obtained experimental results provide strategies to improve plant growth and yield under cadmium stress conditions.

The involvement of the alternative cyclic electron transport around PS1 (the PGR5-dependent pathway and the PTOX-dependent pathway) in *Arabidopsis thaliana* - wild type (*wt*) and the carotenoid mutant (*lut2*) under simultaneous treatment with low temperature and high light intensity was evaluated. Two specific electron transport inhibitors, antimycin A (AntA) for PGR5 and octyl gallate (OG) for PTOX, were used. Addition of AntA causes inhibition of PS2 in the wild type, while addition of OG has a negative effect on the functional activity of PS2 in the *lut2*-mutant (Popova et al. 2022).

Antioxidant, cytotoxic and anticancer activity of hydroethanolic extract of the endemic plant *Sideritis scardica* on adenocarcinoma, Colon 26 was analyzed. Increased antioxidant activity and higher total phenolic and flavonoid content were found in the hydroethanolic extract of *S. scardica* from the Trigrad region in comparison with published data for other ecotypes of the same species, as well as its cytotoxic effect on colon cancer cells when the extract was at concentrations higher than 200 μg ml^{-1}. It has been suggested that extracts of *S. scardica* can be used for medicinal purposes against cancer formations (Dobrikova et al. 2023).

The contributions from the research activity of Dr. Stefanov have not only theoretical significance but also a certain practical orientation.

Future research

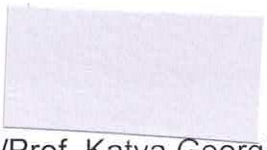
Martin Stefanov's future research is mainly related to the projects he is currently working on. In addition, he planned to expand and deepen the research on the resistance of photosynthetic apparatus to salt and drought stress using new plant species (legumes, cereals, medicinal plants), as well as suitable chemical agents (nanoparticles, acids, phytohormones), that could help plants to overcome the stress effects.

CONCLUSION

The research activities of Assist. Prof. Martin Stefanov are of significant importance for clarifying the response of photosynthetic apparatus to stress and the possible strategies for improving plant resistance. In addition, part of them have practical significance. The submitted documents for the competition confirm that Dr. Stefanov completely covers all requirements and even exceeds the minimum national requirements for acquiring the academic position of "Associate Professor", as well as the specific requirements of IBPhBME, BAS.

All of the above enables me to recommend to the respected members of the Scientific Jury to vote positively and to the members of the Scientific Council of the Institute of Biophysics and Biomedical Engineering, BAS to elect Assist. Prof. Dr. Martin Angelov Stefanov for the academic position "Associate Professor".

Date: 07.07.2023
Sofia

Signature: 

/Prof. Katya Georgieva, Ph.D./