REVIEW

Considering the competition for the academic position "**Assoc. Proffesor**" in the area of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty "Biophysics" announced in the State gazette issue 21 dated March 7, 2023, for the needs of the Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences, department of "Photoexcitable membranes".

with candidate in the competition **Res. Assistant Dr. Martin Angelov Stefanov**

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

General presentation of the materials received for review

The only candidate in the competition is Res. Assistant Dr. Martin Stefanov from the department of "Photoexcitable membranes" Institute of biophysics and biomedical engineering, Bulgarian Academy of Sciences. The materials submitted by the applicant are in accordance with the Regulations for Scientific Development of the Academic Staff of the IBPhBME-BAS and the criteria for occupying the academic position of Assoc. Professor.
The scientific publications for the announced competition have not been presented in previous for the candidate competitions for obtaining the educational and scientific degree "Doctor" and for the academic position "Research Assistant".

According to the information provided by the applicant, the total number of points on scientometric indicators is 733 points (indicator A - 50, indicator B - 120, indicator G – 227, indicator D - 106 and indicator E - 230), while 430 points according to the minimum national requirement and to the regulations of ZAS - IBPhBME-BAS for occupying the academic position of "Professor".

Education and career development

Dr. Stefanov graduated in Faculty of Biology at Sofia University "St. Kliment Ohridski", he has a bachelor's degree in "Biotechnology" (2012) and a master's degree in "Plant Biotechnology" (2014). From January 2015 to December 2018 he was a PhD student, speciality Biophysics, in the department "Photoexcitable Membranes", IBFBMI - BAS. He defended his doctoral thesis on the topic "Adaptation mechanisms of the photosynthetic apparatus to salinity and light stress in two Paulownia lines" with scientific supervisor Prof. Dr. Emilia Apostolova. From 2019 to the present he worked as a research assistant in the department "Photoexcitable Membranes".

Scientific indicators

Dr. Stefanov participated in the competition with **16** publications, **8** publications are in journals with rank **Q1** (all with IF, 6 in MDPI special issues), **3** with rank **Q2**

(3 with IF), **4** with rank **Q3** (2 with IF and 1 with SJR), **1** with rank **Q4** (with SJR), and one book chapter (Handbook of Plant and Crop Stress). In my opinion the publication Dobrikova et al., Journal of Chemical Technology and Metallurgy, 58, 1, 2023, is related to evaluation of the content of flavonoids and polyphenols in the Sideritis scardica plant, and the antitumor and cytotoxic effect of hydroethanolic plant extract, and does not fit into the topic of the announced competition.

The total impact factor of publications is **43.6**. It is noteworthy that 12 of the scientific works were published during the period 2020-2023.

According to the reference submitted by the candidate, the scientific works have been cited 108 times, a reference in Scopus dated 06.07.2023 shows 129 citations and **h-index 7** (Scopus). The most cited publication is "Effects of salinity on the photosynthetic apparatus of two Paulownia lines" in Plant Physiology and Biochemistry, Stefanov et al. - 46 times.

Dr. Stefanov has 39 participations in international and national scientific forums.

He participated in 13 research projects (2 funded by FNI, 4 Academy Exchange Projects of Bulgarian Academy of Sciences, and 4 for young scientists, BAS), he coordinated 5 of the projects.

Major Scientific Contributions

The scientific works presented in the competition are focused on characterizing the function of photosynthetic membranes in conditions of abiotic stress, which is part of the scientific topic of the department "Photoexcitable Membranes" - IBPhBME-BAS, closely related to the study of the mechanisms of adaptation of the photosynthetic apparatus in response to stress factors. Studies of plants' adaptation and tolerance under stress conditions are important for identification of resistant varieties, genotypes and hybrids.

Mechanisms of resistance and protection of the photosynthetic apparatus (PSA) have been studied in crop plants with different genotypes (maize, sorghum, pea, wheat, rice).

A number of biophysical methods was applied to determine: the pulse-amplitude-modulated chlorophyll fluorescence (PAM) of leaves at room temperature and of isolated thylakoid membranes (TM) at low temperature (77K); the redox properties of P700 of whole leaves; the photochemical activity of PS2 and PS1 (by the addition of external artificial electron donors and acceptors); the oxygen yields under flash and continuous white light illumination of isolated TMs showing changes occurring in the oxygen-evolving complex (OEC) of PS2.

Analyzes were carried out to determine antioxidant and antiradical activity; the content of pigments, anthocyanins, total flavonoids, proline; markers of oxidative stress (MDA, H2O2); electrolyte leakage; degree of membrane damage and relative water content in leaves.

The habilitation work (indicator B) *summarizes results on the resistance and adaptation of higher plants to salt stress and drought.*

A higher sensitivity of the primary photosynthetic processes and ETC components to salt stress was demonstrated in pea (Pisum sativum L.) compared to maize (Zea mays L.). This effect is hypothesized to be related to larger relative size of the plastoquinone pool, lower structure density of the photosynthetic apparatus, and

higher electron transfer activity in maize compared to pea. The demonstrated greater resistance of maize to salinity than that of pea, corresponds to a higher sensitivity of the processes occurring in the PSA in plants with C3 type metabolism at different levels of salinity, and could be used to determine salt tolerance in plants.

Water deficit was found to a decrease the photochemical quenching, the ratio of photochemical to non-photochemical processes, the effective quantum yield of photochemical energy conversion to PC2, the linear electron transport rate and the performance indices PItotal and PIABS; the observed effects being stronger in sorghum than in maize and dependent on the drought level. The photochemistry of PS1 (P700 photooxidation) in maize was inhibited only when treated with higher concentrations of PEG, while in sorghum inhibition was observed after all investigated levels of desiccation.
The increase in the regulated energy loss, the induction of state transitions and the cyclic electron flow around PS1 provide better protection of the PSA; therefore, these processes can be used as indicators of plant drought tolerance.

For the first time, a relationship between the amounts of flavonoids and proline and the degree of salt tolerance of a Paulownia species (two Paulownia lines (TF and EE) was revealed. The role of the amounts of proline, flavonoids and carotenoids in reducing salt-induced changes in PSA, and for plant tolerance to high salt content was demonstrated. Higher flavonoid and proline contents were found to alleviate electron transfer from QA to the PQ pool and photochemical inhibition of both photosystems under short-term NaCl treatment. The adaptation of the TF Paulownia line upon prolonged treatment with high concentrations of NaCl suggests reversibility of the salt-induced changes in PSA.

Non-habilitation publications (indicator G)

Modified ZnO nanoparticles (ZnO-Si NPs) were found to induce a more pronounced protective effect against salt stress in pea plants compared to ZnO NPs, which was attributed to a change in the electrical charge of the NPs from the deposited Si layer on the surface of the NPs and the reduced accumulation of Zn in leaves.

A protective effect of sodium nitroprusside (SNP, donor of nitric oxide) against salt-induced damage was demonstrated in two sorghum types (Sorghum bicolor L. Albanus and Sorghum bicolor L. Shamal), the effect being species specific and dependent on SNP concentration. SNP concentrations up to 150 μM had a better protective effect in both studied sorghum types, corresponding to about a 50% increase in the endogenous NO content in leaves compared to control plants. Foliar application of SNP was found to increase the number of active reaction centers of PS2 and increased photochemical activity of PS1. The SNP-induced salt tolerance in sorghum could be applied to reduce plant sensitivity to salinity.

Simultaneous application of two stress factors (low temperature and high light intensity) was found to have an inhibitory effect on the photosynthetic efficiency, which appeared stronger and earlier in the lut2 mutant than in wt Arabidopsis thaliana. Addition of the specific inhibitor AntA, which blocks the PGR5-dependent alternative electron flow, resulted in an increased degree of inhibition of PS2 in wt A. thaliana, whereas no clear effect of the inhibitor OG of the PTOX-dependent pathway was observed. The contribution of PGR5-dependent electron transfer to the photosynthetic efficiency of the lut2 mutant is not as pronounced as the one of wt A. thaliana, but PTOX-mediated electron transfer to O2 plays more essential role in the response to the two stress factors.

The uptake and distribution of Cd ions in plant tissues, as well as the ability to protect against oxidative damage were found to depend on the genes in wheat. It is suggested that the better protection of the photosynthetic process is due to the Rht8 allele in this genetic wheat variety. The data also suggest that the Rht8 allele has a role in neutralizing the toxic effect of Cd ions.

Investigation of the effect of salicylic acid (SA) on PSA of rice plants under cadmium stress has showen that SA protects Mn-cluster from cadmium damage, reduces oxidative stress markers, increases chlorophyll content, photochemical activities of PS1 and PS2, and flash oxygen yields.

Critical notes and suggestions

I have no critical comments on the publications presented in the competition, but I think the Habilitation reference for the scientific contribution could have been written much better. A list of citations is missing in the documents, citations are included only in the CV. There are some inconsistencies between the designations of publications (e.g., *, a, b, etc.) in the Habilitation reference and the list of publications and the copies of the publications themselves, where these designations are missing.

Personal contribution of the candidate

Dr. Stefanov is the first author of 9 and second author of 3 of the peer-reviewed publications, which implies a very significant contribution of the candidate in all published works.

The research investigations have been carried out in the Institute of Biophysics and Biomedical Engineering - BAS, and in collaboration with some foreign research institutions on joint projects (Department of Ecochemistry and Radioecology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius in Trnava, Trnava, Slovak Republic, Nanotechnology and Advanced Materials Central Lab, Agricultural Research Center, Giza, Egypt и други).

CONCLUSION

The presented documents and materials from Dr. Martin Stefanov meet all the requirements for occupying the academic position of "Assoc. Professor" according to the Act for the Development of the Academic Staff in the Republic of Bulgaria (ADASRB), the Regulations for the Application of ADASRB in BAS and the specific requirements of IBPhBME-BAS. The scientific production and scientific indicators of Dr. Stefanov significantly exceed all recommended requirements for the scientific activity of candidates for the academic position of "Associate Professor". The original results obtained by the research activity of Dr. Stefanov have significant contribution to the area of biophysics of ptohosynthesis. I strongly support the application and also recommend to the Scientific Jury and to the Scientific Board of IBPhBME-BAS to award to Res. Assistant Dr. Martin Angelov Stefanov the academic position "Associate Professor" in professional field 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological Sciences, scientific specialty "Biophysics".

Sofia
07.07.2023

/Prof. DSc Stefka Germanova Taneva/