

REVIEW

on the competition for the occupation of the academic post “Professor”

in the sphere of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, specialty “Plant Physiology” according to the announcement in the SG№12 /09.02.2024 for the needs of the Laboratory "Regulators of Plant Growth and Development"

with the candidate Iskren Georgiev Sergiev, PhD, Associate Professor in the the Laboratory "Regulators of Plant Growth and Development" at the Institute of Plant Physiology and Genetics at BAS (IPPG-BAS)

by the reviewer Miroslava Konstantinova Zhiponova, PhD, Associate Professor in the Department of Plant Physiology, Faculty of Biology, Sofia University “St. Kliment Ohridski” (FB-SU)

1. General information on the career and thematic development of the applicant.

In 1991, Iskren Georgiev Sergiev received a master's degree at FB-SU in the specialty "Biochemistry and Microbiology" and specialization "Plant Physiology", after which he started work as a specialist biologist at IPPG-BAS. In 1995, he began full-time doctoral studies at the same institute under the supervision of academician Emanuil Karanov, and by the beginning of 2000, he obtained the educational and scientific degree "Doctor", scientific specialty "Plant Physiology". The candidate is conducting specializations in Italy and Belgium related to experiments and biochemical analyses, and GC-MS and LC-MS/MS analyses of phytohormone content. In 2006, Dr. Iskren Sergiev is habilitated according to the regulations in IPPG-BAS, and in 2010 the position was transformed into Associate Professor, and so far his work experience in the specialty is over 32 years.

The thematic focus of the candidate's scientific research includes interests and skills in the field of plant physiology and biochemistry, studying the role of phytohormones and synthetic growth regulators in the physiological response of plants to stressors. The candidate is also distinguished by good technological skills, including high computer literacy, work with research equipment – spectrophotometry, spectrofluorophotometry, biochemical analyses, HPLC, GC-MS, and LC-MS/MS. During the years of his scientific career, Assoc. Prof. Dr. Iskren Sergiev developed active research, scientific-organizational, and educational activities, and was a member of scientific organizations and editorial boards. My conclusion is that the candidate is a long-term staff member of IPPG-BAS who keeps on contributing to the development of Bulgarian science at the national and international level.

2. Evaluation of the submitted materials according to the requirements for occupying the academic position "Professor"

The candidate has submitted a detailed report on the fulfillment of the minimum national requirements from the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, as well as the Regulations on the Specific conditions and procedure for acquiring scientific degrees and holding academic positions at IPPG-BAS:

- According to indicator A (50 points out of the required 50 points), a dissertation is included for awarding the educational and scientific degree "Doctor" on the topic "*Cytokinin antagonists – structure-activity relationship and some physiological properties*" with scientific supervisor academician Emanuil Karanov.
- According to indicator B (107 points out of the required 100 points), 5 publications are included (3 with Q1; 1 with Q2; 1 with Q4), in three of which the candidate is the first or corresponding author, and the total JCR IF is 10.225 .
- According to indicator D (287 items out of the required 220 items), 13 publications are included (4 with Q1; 4 with Q2; 3 with Q3; 2 with Q4), in seven of which the candidate is the first or corresponding author (135 points), and the total JCR IF is 16.156. Also included are 3 book chapters.
- According to indicator D (2620 points out of the required 200 points), 1310 citations are included in Web of Science or Scopus for the last 7 years (without auto-citations and semi-auto-citations).
- According to indicator E (160 points out of the required 150 points), 8 participations in national scientific and educational projects are included, 1 participation in an international project, and 1 project coordination of a national project. The funds raised are BGN 200,000. According to indicator E-13, the candidate is a scientific adviser of a PhD student that successfully defended a doctoral degree. According to the regulations of IPPG-BAS, no points are awarded.

According to all indicators, Assoc. Prof. Dr. Iskren Sergiev meets and exceeds the points for the minimum requirements. His total number of scientific publications in peer-reviewed journals is 89, of which 59 are indexed in Scopus or WoS, the total impact factor is 75.666, and the h-index is 13. He is author of 5 book chapters and 4 review articles. For the present competition, a total of 21 publications were submitted, of which 18 scientific articles (7 with Q1; 5 with Q2; 3 with Q3; 3 with Q4) and 3 book chapters. The candidate is the first author on 5 of the publications and the corresponding author on 5. The JCR IF of all publications in the competition is 26.381, with the JCR IF of the publications in which the candidate is the first or corresponding author being 15.694. The publications are in authoritative international journals of high rank, which is an indicator of the importance of the scientific output: Pesticide Biochemistry and Physiology, Biologia Plantarum, Plant Growth Regulation, Compt. Rend. Acad. Bulg. Sci., Oxidation Communications, Protoplasma, Zemdirbyste-Agriculture, Scientia Horticulturae, Biologia, Plants, Agronomy. In conclusion, the scientometric indicators of Assoc. Prof. Dr. Sergiev's scientific output are extremely high, and confirm the importance of the research for the national and international scientific community, and their number and quality fully satisfy the requirements for acquiring the academic position of "Professor".

3. Major directions in the candidate's research work

The scientific research activity described in the publications submitted for the competition was mainly carried out in the Laboratory "Regulators of Plant Growth and Development" laboratory at IPPG-BAS. Part of the analyses were carried out in cooperation with researchers from IPPG-BAS or from other scientific institutions in Bulgaria. Part of the results in the publications were obtained through bilateral inter-academic collaborations or thanks to the financial support of the Bulgarian National Science Fund and the INCO-

COPERNICUS program to the EU. The scientific research activity of the candidate tentatively covers four main directions from the field of functional plant biology:

1) Physiological action of herbicides on some components of plant metabolism and the functional activity of photosynthesis under optimal and suboptimal plant growth conditions and when treated with growth regulators and natural metabolites [Publications № 1, 4, 6, 7, 11, 20, 21].

The herbicides cause oxidative stress in plants, and the candidate aims to investigate the possibility of reducing damage by studying the mechanism of action of herbicides (paraquat, atrazine, glyphosate, Zerate) on different crop species.

- The possibility of reducing pea damage caused by the contact herbicide paraquat by pretreatment with hydrogen peroxide has been investigated [4, 6, 7]. Higher survival of H₂O₂-pretreated plants was proven, which is due to: reduction of the strong inhibitory effect of paraquat on the intensity of photosynthesis; stabilization of the structural organization of the leaves; increased antioxidant defense correlating with improved membrane stability and composition, increased levels of chlorophyll and carotenoids, and induction of protective enzymes.

- Prolonged exposure of pea plants to low concentrations of atrazine has been found to cause growth arrest accompanied by damage to cell membranes and disturbances in redox homeostasis, inhibited catalase activity and increased glutathione content and increased glutathione-S-transferase activity [11]. These results point to a re-evaluation of the continuous use of herbicides in the same agricultural areas.

- It has been established that when the total herbicide glyphosate is applied to young maize plants, the phenylurea cytokinin 4PU-30 exhibits a protective effect [1]. This is expressed through a reduction in biomembrane damage, activation of the glutathione-S-transferase defense system and an increase in the glutathione pool.

- Functional aspects of the reaction of the photosynthetic apparatus after treatment of Bulgarian varieties of wheat and triticale with the selective herbicide Zerate (Syngenta product) and subsequent exposure of the plants to stressful conditions such as drought or waterlogging of the soil were investigated [20, 21]. The independent application of the herbicide leads to certain fluctuations in the content of leaf pigments and the intensity of the photosynthetic process in both cultures, without significantly affecting the parameters of chlorophyll fluorescence. The effect on the two studied crops is differential and is determined by their sensitivity/tolerance to the specific stress. The wheat, which exhibits good drought tolerance, recovers after drought but not completely after waterlogging. The data show that pretreatment with the herbicide Zerate does not have an additional negative effect on the photosynthesis of dried wheat plants [20]. In triticale, which showed relatively low drought tolerance, the opposite trend was observed, therefore it is assumed that pre-drought herbicide treatment further delays the normalization of the photosynthesis process [21].

Publications in this direction [4, 6, 7] are related to the dissertation work of the PhD student Irina Moskova, and the personal contribution of the candidate is expressed in the coordination of the research – from the idea of the scientific development, through the discussion of the results, to the formation of the conclusions, participation in some of the analysis, and in the writing of the publications. In the other publications, the candidate

participates in the experiments, processing the results and shaping them into a publication, including as a corresponding author [20, 21].

2) Modulating action of synthetic auxins in the plant physiological response formation to abiotic stress [Publications № 13, 14, 16, 17, 19].

As a continuation of the topic from the previous scientific direction, the candidate focuses on structural analogues of the synthetic auxin naphthylacetic acid, provided by the Natural Research Center at the Lithuanian Academy of Sciences. Physiological effects of auxin analogs on crops have been tested in combination with herbicides, and under conditions of stress factors such as drought and high temperature. The applied compounds have been shown to modulate the physiological responses of the plants and help to overcome the studied stress factors. Their protective action is expressed in maintaining the growth of stressed plants, reducing the levels of stress markers, and having a positive effect on the levels of non-enzymatic antioxidants and the activity of antioxidant enzymes.

- The modulatory effect of the synthetic auxin analogs 1-[2-chloroethoxycarbonyl-methyl]-4-naphthalenesulfonic acid dicalcium salt (TA-12) and 1-[2-dimethyl methoxycarbonyl methyl]naphthalene chloromethylate (TA-14) on the pea physiological responses was investigated compared to the herbicides Glean-75, Glyphosate and 2,4-D [16 and 17]. Pretreatment with both compounds was found to reduce the negative consequences of herbicide application, which consisted in lower content of the studied stress biomarkers compared to plants treated with herbicide alone. The two compounds also modulated the main components of the antioxidant defense system (enzymatic and non-enzymatic), bringing them to physiological levels in the pretreated plants, in contrast to those found after self-applied herbicides. As a result of reducing oxidative stress, plant growth was improved. It has been hypothesized that the prior application of both auxins modulates in a specific way the biochemical response of plants regarding the enzymes related to the detoxification of xenobiotics depending on the mechanism of action of the specific herbicide.

- The protective effect of pre-application of auxin analogues was found in pea subjected to high temperature [13] or polyethylene glycol treatment of wheat and maize [19] and pea [14].

The candidate's personal participation is related to performing biochemical analyzes of stress markers, determination of enzyme activities, processing and visualization of the results, and in writing the articles, as in [17] he is a corresponding author.

3) Induction of stress tolerance in plants by natural and synthetic growth regulators [Publications № 5, 9, 10, 12, 15, 18].

In this direction, the candidate studies the mechanisms and potential application of natural and synthetic growth regulators to increase plant resistance to environmental changes. For this purpose, the effects of prior application of growth regulators in forming the response of plants to stress factors such as low temperatures, drought, salinization, phytopathogens, etc. are monitored. Changes in the levels of stress biomarkers, antioxidant enzymatic and non-enzymatic defense systems, as well as in the endogenous content of growth regulators from the group of polyamines are analysed.

- Foliar application of abscisic acid and phenylurea cytokinin 4PU-30 has been shown to increase the adaptability of young wheat plants to drought, reducing the levels of the stress

biomarkers malondialdehyde and free proline, and at the same time changing the amount of polyamines as part of the endogenous defense system of plants [5].

- The role of endogenous polyamines in overcoming the adverse effects of environmental stressors, such as low and high temperature, drought, salinity, deficiency of macro- and microelements, heavy metals, UV radiation, herbicides, hypoxia and anoxia, fungal, bacterial and viral infections, etc. [12]. It is emphasized that exogenously applied polyamines play an important role in increasing the plant tolerance and adaptation capacity to various abiotic and biotic stress factors.

- The probable mechanism by which polyamines exert their protective action under salt stress conditions has been discussed [10]. Pretreatment with natural and synthetic aliphatic polyamines has been found to contribute to the improvement of wheat resistance to low temperature stress. The increased tolerance of polyamine-treated plants to subsequent exposure to negative temperature is expressed in the significant accumulation of free proline, in a reduced amount of released electrolytes, and in a higher percentage of plant survival. Among the studied polyamines, the effects of diethylenetriamine stand out as a potential means of reducing damage from low-temperature stress [9].

- The possibility of reducing the disease incidence in tomatoes and peppers caused by the TSWV virus (Tomato spotted wilt virus) was studied by pre-treating the plants with cytokinin (4PU-30), a retardant (MEIA) or a higher aliphatic alcohol (Triacontanol – C₃₀H₆₁OH) [15 and 18]. Pretreatment with the retardant MEIA (β -monomethyl ester of itaconic acid) and the phenylurea cytokinin 4PU-30 was found to limit the development of subsequent infection in two lines of tomato plants and reduce the negative consequences of oxidative stress, which was shown by reduced levels of stress biomarkers malondialdehyde and free proline, compared to untreated infected plants [15]. Significant elimination of virus infection by triacontanol application was observed in pepper plants. The effects of triacontanol treatment before TSWV infection were more pronounced compared to post-treatment [18]. In both crops, a lower amount of virus was confirmed in the infected plants after application of the growth regulators.

The candidate's personal contribution to these studies is related to the assessment of oxidative stress and its consequences – level of induction, negative metabolic changes caused by stress, and overcoming them by applying different growth regulators. In experimental developments, he offers the ideas of treatment with growth regulators in the appropriate concentrations. He participated in the plant treatment, sample analyses, processing of results, and writing the articles. He is the corresponding author to the publications related to biotic stress [15 and 18].

4) Interaction between natural and synthetic growth regulators in *Arabidopsis* under normal and stress conditions [Publications № 2, 3, 8].

Research in this direction is related to studying the effects of interactions between different phytohormones and growth regulators in plants grown in optimal and suboptimal conditions.

- *Arabidopsis* wild type and an ethylene-insensitive mutant (*eti5*) were used for the research. Changes in the amounts of stress biomarkers free proline, malondialdehyde and carbonyl groups show that plants of the *eti5* mutant genotype are affected to a lesser extent by temperature stress compared to the wild type [8]. The higher tolerance of plants from the

ethylene-insensitive mutant *eti5* to high- and low-temperature stress correlates with higher constitutive levels of polyamines compared to those in the wild type [3]. These results prove that the physiological role of polyamines and ethylene in regulating the processes of growth and development of plants is interconnected and determines to a certain extent the sensitivity/tolerance of a given plant species to stress. Comparative analysis also shows that high-temperature stress has a greater effect on changes in polyamine content than low-temperature stress [3].

- Anticytokinins, structural analogs of cytokinins BA and 4PU-30, were found to reduce the senescence-retarding action of cytokinins in leaves of wild-type and ethylene-insensitive mutant (*eti5*) *Arabidopsis*, with effects correlating with changes in the total ribonuclease activity [2].

The candidate's personal participation in these studies is related to developing the idea, performing part of the biochemical analyses, processing and interpreting the results, and writing the publications.

The main contributions from the research activity of Assoc. Prof. Dr. Sergiev provide clear and well-systematized information about the performed scientific work. I believe that these contributions are significant and open opportunities for continuing research on the topic, as well as for applying the accumulated experience in new fundamental and scientific-applied research together with the team of the Laboratory "Regulators of Plant Growth and Development" of the IPPG, as well as and with other scientific groups.

4. Relevance of the scientific topic.

The candidate studies and applies phytohormones and growth regulators, which play a key role in the main stages of plant growth and development and have essential functions in determining the response of plants to environmental changes. Comparative studies of natural and synthetic effectors in control conditions and under stress conditions are aimed at finding strategies to increase the efficiency of basic physiological processes in economically important crops, such as peas, corn, wheat, triticale, tomatoes, pepper.

Fundamental research has been done on how the herbicides used to control weeds in modern agriculture work on crop plants. In practice, however, due to the accumulation of various environmental factors, it is not always possible to predict the action of herbicides under suboptimal growing conditions. In such cases, their physiological action should be studied experimentally. The candidate investigated the herbicides paraquat, atrazine, glyphosate, Zerate, Gleen-75, 2,4-D, and on the other hand he took into account the combined or individual influence of abiotic and biotic factors (drought, waterlogging, salinization, high and low temperature, viruses). To overcome the adverse effects on plants, the candidate is testing environmentally friendly natural and synthetic growth regulators as potential protective agents against oxidative stress. These are derivatives of auxins, cytokinins, abscisic acid, ethylene, polyamines, retardants, aliphatic alcohols.

A scientific-applied aspect of research is the search for opportunities to increase the resistance of plants to constantly changing environmental conditions with the help of growth regulators. The phytohormonal system carries out integral regulation of all processes of growth and development throughout the life cycle of plants. This is accomplished by fine-tuning hormone levels, by interacting with hormonal signaling pathways, and also by balancing the

effects of endogenous and exogenous plant growth regulators. Due to the continuous research of growth regulators of different chemical nature and their testing on various plant species and under different environmental conditions, the relevance of the research works of Assoc. Prof. Dr. Iskren Sergiev are of essential importance for science and their subsequent application in the project to achieve effective plant productivity for the needs of society.

5. Organizational and training activity.

The total number of participations of Assoc. Prof. Dr. Iskren Sergiev in projects that are included in the reference for fulfilling the minimum national requirements for occupying the academic position "professor" is 10 (9 national and 1 international). Assoc. Dr. Sergiev is the head of 1 ongoing project and a participant in another, financed by the Bulgarian National Science Fund of the Ministry of Education and Science. This active participation in projects (37 in total) demonstrates Assoc. Prof. Dr. Sergiev's ability to secure funding for research activities. The candidate also participates in the organization of scientific forums, which testifies to additional skills that contribute to effective communication of the international scientific community.

The candidate's participation in a project for "Support for the construction and development of young competitive scientific potential in the field of physiology, phytochemistry, genomics, proteomics and biodiversity of eukaryotic organisms" is impressive, which shows commitment to training activities of personnel. Assoc. Prof. Dr. Iskren Sergiev participates in the training of doctoral students in the course "Natural and synthetic growth regulators" at the BAS Training Center. In addition to this teaching activity, he was a scientific adviser to a successfully defended PhD student.

6. Critical notes and recommendations.

I have no comments on the presented documentation. I strongly recommend Assoc. Prof. Dr. Sergiev to continue to attract young and motivated scientists to whom he can pass on his rich research experience.

7. Conclusion.

The thorough review of the scientific papers submitted for review, their fundamental contributions and applied scientific potential, their international relevance, as well as the project activity, outline the candidate as an established plant physiology researcher. My conclusion is that the scientific production of Assoc. Prof. Dr. Iskren Sergiev completely satisfies the recommended criteria of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, as well as the Regulations on the Specific conditions and procedure for acquiring scientific degrees and holding academic positions at IPPG-BAS. Based on this review, I strongly recommend to the Honorable Scientific Jury and the Scientific Council of IPPG-BAS to vote in favor of awarding the academic position of "Professor" to Assoc. Prof. Dr. Iskren Georgiev Sergiev.

20.05.2024

Reviewer:

/Assoc. Prof. Miroslava Zhiponova, PhD/