R E V I E W

on a competition for the academic position “Professor” in a professional field 4.3. Biological Sciences, scientific specialty ”Plant Physiology”, announced in SG № 12/09.02.2024, for the Laboratory "Regulators of Plant Growth and Development", Institute of Plant Physiology and Genetics – Bulgarian Academy of Sciences (IPPG-BAS)

Candidate: Iskren Georgiev Sergiev, PhD, Associate Professor in the Laboratory "Regulators of Plant Growth and Development", IPPG-BAS
Reviewer: Professor Katya Marinova Georgieva, PhD, Laboratory “Photosynthesis – Activity and Regulation”, IPPG-BAS

Associate Professor Dr. Iskren Sergiev is the only candidate for the announced competition. The presented documents for participation in the procedure are prepared according to the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria and are in line with the Regulations for acquiring scientific degrees and holding academic positions at IPPG-BAS.

Scientific career

Iskren Sergiev completed his higher education in 1991 at the Faculty of Biology of Sofia University "St. Kliment Ohridski" and graduated with a master's degree in "Biochemistry and Microbiology", specialization in "Plant Physiology". In the same year, he was appointed as a specialist-biologist at the Institute of Plant Physiology "Acad. M. Popov" (currently Institute of Plant Physiology and Genetics). In the period 1995–1998, he developed and successfully defended a dissertation on the topic "Cytokinin antagonists - structure-activity relationship and some physiological properties" under the supervision of Acad. Emanuil Karanov and obtained a Ph.D. Degree. After completing his Ph.D., Iskren Sergiev continued to work at the Institute of Plant Physiology as an Assistant Professor. He obtained his habilitation degree in 2006 in the specialty "Plant Physiology" and until now works in the laboratory "Regulators of Plant Growth and Development" in the research area "Plant Ecophysiology". During the period 1996-2005, Assoc. Prof. Sergiev was a visiting scientist several times at the Istituto biosintesi vegetali – CNR, Milan, Italy and the University of Antwerpen – Belgium, which gave

him the opportunity to enrich his knowledge and learn new methods in the field of plant physiology and biochemistry.

The scientific research of Dr. Iskren Sergiev is mainly aimed at investigating the role of plant growth regulators and phytohormones in overcoming the adverse impact of various environmental stress factors.

General research metrics

Assoc. Prof. Dr. Iskren Sergiev has a total of 89 scientific publications (total impact factor 75.67), which have been cited 2774 times. In the competition for "Professor" he presents 21 articles, of which 16 are published in journals with an impact factor (total JCR IF 26.381). Their distribution by quartiles is as follows: 7 publications have Q1, 5 are with Q2, 2 with Q3, and 2 with Q4. The list of publications for the competition includes 3 book chapters and 2 publications in international journals without an impact factor.

The candidate participates in the competition with a total amount of 3224 points, which significantly exceeds the required minimum score of 720. The presented indicators for the fulfillment of the minimum national requirements for occupying the academic position "Professor" at IPPG-BAS are:

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

The total number of points according to **group B** indicators is **107** points (required minimum 100 points) – 5 publications are included (Q1 - 3, Q2 - 1, Q4 - 1) with a total JCR IF 10.225. Iskren Sergiev is listed as lead author in 3 of the articles.

Group C includes 16 publications (Q1 - 4, Q2 - 4, Q3 - 3, Q4 - 2 and 3 book chapters) with a total number of points **287** (required minimum 220 points) and JCR IF 16.156. Dr. Sergiev is first and/or corresponding author in 7 of these publications (**135** points).

Group D (citations) – **2620** points (required minimum 200 points). A reference is presented for 1310 citations in Web of Science or Scopus for the last 7 years.

G**roup E – 160** points (required minimum 150 points) – Assoc. Prof. Sergiev was a scientific consultant of a successfully defended doctoral student. He has participated in 10 research projects and is a leader of a project funded by the Scientific Research Fund and has received significant financial support for his investigations (200 000 BGN).

Dr. Sergiev has participated in a number of scientific meetings in the last 7 years (2018-2024): 6 scientific forums held abroad and 10 international/or national scientific

conferences held in Bulgaria, where 3 reports and 25 posters were presented. During this period, he prepared 33 reviews of scientific articles and 2 statements on competitions for the academic positions "Associate Professor" and "Professor".

The scientific production presented and the scientometric data achieved exceed the minimum requirements for occupying the academic position "Professor" at IPPG-BAS.

Analysis of the main directions in the candidate's research work and scientific achievements

The scientific research of Assoc. Prof. Iskren Sergiev covers 4 interrelated and complementary thematic areas, based on which scientific achievements, which are mostly fundamental, can be systematized. In addition, the use of plant growth regulators is a promising approach to increase plant resistance to abiotic and biotic stress and could find application in agricultural practice.

1. Physiological action of herbicides on plant metabolism under abiotic stress and when treated with plant growth regulators and natural metabolites

The use of herbicides for the selective destruction of weeds is widely used in agriculture. Their application is often accompanied by the action of various adverse environmental factors. Part of Dr. Sergiev's research is devoted to the physiological action of various herbicides (glyphosate, atrazine, paraquat, Zerate) under optimal and suboptimal conditions (drought, waterlogging) of growing different plant species (corn, peas, wheat, triticale) and when treated with growth regulators (4PU-30) and natural metabolites (H_2O_2) (***publications 1, 4, 6, 7, 11, 20, 21***).

Glyphosate and atrazine were found to increase lipid peroxidation, electrolyte leakage, proline and glutathione content, and guaiacol peroxidase and glutathione-S-transferase activities. Unlike glyphosate, which causes an increase in catalase activity, atrazine inhibits catalase activity and does not affect that of superoxide dismutase. Paraquat has been shown to induce morphological changes in pea plant leaves, increase lipid peroxidation and electrolyte leakage, as well as catalase and glutathione-S-transferase activities. In addition, it inhibits photosynthetic activity and decreases ascorbate peroxidase activity and the amount of ascorbate and reduced glutathione (***publications 4, 6, 7***). The obtained results show that

the studied herbicides induce oxidative stress (***publications 1, 4, 6, 7, 11***). For the first time, the effect of the selective herbicide Zerate (manufactured by Syngenta, Switzerland) on the photosynthetic activity of winter wheat (*Triticum aestivum* L.) and triticale was investigated and it was shown that it did not significantly affect the photosynthetic parameters studied (***publications 20, 21***).

New information was obtained on the application of Zerate in drought and waterlogging conditions that inhibit photosynthetic activity. Application of Zerate prior to exposure of wheat and triticale to drought or waterlogging was found to cause no additional changes in photosynthesis, i.e. reduced photosynthetic efficiency is a result of abiotic stress. Furthermore, while in wheat photosynthetic functions are almost completely restored in dehydrated plants, in triticale they are almost completely restored in waterlogged plants (***publications 20, 21***).

The protective role of cytokinins against oxidative stress induced by the action of herbicides has been established. Dr. Iskren Sergiev's research shows that the phenylurea cytokinin N1-(2-chloro-4-pyridyl)-N2-phenylurea (4PU-30) reduces the toxic effect of the herbicide glyphosate in corn plants by increasing the activity of antioxidant defenses (***publication 1***).

New information has been obtained on the protective role of exogenous application of low concentration hydrogen peroxide against the herbicide paraquat (***publications 4, 6, 7***). Treatment of pea plants with a low concentration of H_2O_2 (2.5 mM) has been shown to reduce herbicide-induced morphological damage, reduce lipid peroxidation, increase photosynthetic activity and antioxidant defense.

2. Modulating action of synthetic auxins in the physiological response of plants to abiotic stress

It was shown that pretreatment with the auxin analogues auxins 1-[2-chloroethoxycarbonyl-methyl]-4-naphthalenesulfonic acid calcium salt (TA-12) and 1-[2-dimethyla minoethoxycarbonylmethyl]naphthalene chlormethylate (TA-14) reduces the negative effect of various abiotic stress factors (***publications 13, 14, 16, 17, 19***).

It was found that TA-12 and TA-14 reduced the degree of oxidative stress induced by the exposure of pea plants to high temperature and water stress, which was expressed in decreased amount of malondialdehyde and non-enzymatic antioxidants and the activity of

the investigated antioxidant enzymes to the level of control untreated plants (***publications 13, 14***). It is hypothesized that TA pretreatment can increase the adaptive capacity of plants to stress by modulating physiological and metabolic processes, which will improve plant vigor and growth.

The ability of TA-12 and TA-14 to modulate the effects of water stress was also investigated in two economically important crops, wheat and maize, which differ in their drought tolerance, wheat being drought tolerant and maize sensitive to drought (***publication 19***). The obtained results show that the application of synthetic auxins before treatment with polyethylene glycol keeps the content of non-enzymatic antioxidants (proline, thiols of low molecular weight, phenols) and the activity of antioxidant enzymes (catalase and peroxidase) close to the control levels. This is more pronounced in maize than in wheat plants. Research shows that pre-application of TA-12 and TA-14 can reduce the adverse effects of moderate water deficit through crop-specific regulation of antioxidant defenses.

Pretreatment with the synthetic auxins TA-12 and TA-14 has been shown to reduce the herbicides glyphosate and glin-75-induced accumulation of reactive oxygen species and membrane damage by modulating the antioxidant defenses of treated pea plants (***publication 16***). Studies on the effect of exogenously applied auxin-type herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) on the growth and antioxidant defense of pea plants pretreated with TA-12 and TA-14 showed that the content of non-enzymatic antioxidants and the activity of the studied antioxidant enzymes decreased compared to those treated with 2,4-D alone (***publication 17***).

3. Induction of stress tolerance in plants by means of natural and synthetic growth regulators

Part of Iskren Sergiev's research is devoted to the study of the protective role of growth regulators to overcome the action of various stress factors (***publications 5, 9, 15, 18***). Two reviews have been devoted to the role of polyamines in increasing the tolerance of plants under abiotic and biotic stress (***publications 10, 12***).

The protective role of phenylurea cytokinin 4PU-30 and abscisic acid (ABA) against water stress was established. Pretreatment of young wheat plants with growth regulators increases plant resistance by increasing the endogenous content of the polyamines spermine,

spermidine and especially putrescine, which is accompanied by the reduced amount of stress markers (MDA and proline) (***publication 5***).

Treatment with polyamines - spermine, spermidine, putrescine, 1,3-diaminopropane (1,3-DAP) and diethylenetriamine (DETA) has been shown to increase the resistance of winter wheat to low temperature stress and may be a promising approach for agricultural application. Spraying with the investigated growth regulators before exposing the plants to low temperature stress (–12°C for 24 h) preserved membrane integrity and chlorophyll content, increased the amount of proline and the percentage of plant survival, the effect being most pronounced after the application of the synthetic polyamine DETA (***publication 9***).

The use of growth regulators is a promising approach to limit damage from important viral diseases such as *Tomato spotted wilt virus* (TSWV), which reduce quality and yield in tomato, pepper, tobacco and other crops. The study of the effect of the growth regulators 4PU-30 and β-monomethyl ester of itaconic acid (MEIA) on two tomato genotypes ("Keti" and VK1) infected with TSWV showed that they had an inhibitory effect on the infection in both tomato lines and reduce the negative consequences of oxidative stress. The protective effects of the investigated growth regulators are better expressed in the more sensitive to TSWV line "Keti" (***publication 15***). The growth regulator triacontanol (TRIA), applied before or after infection of young pepper plants with TSWV, was found to limit the virus development and the degree of oxidative stress, and the characteristic leaf disease symptoms (wilting, severe curling) were not observed (***publication 18***).

4. Interaction between natural and synthetic growth regulators in Arabidopsis under normal and stress conditions

Arabidopsis mutants have been widely used to elucidate changes in physiological and biochemical processes in plants under normal and stress conditions. In part of his research, Assoc. Prof. Sergiev used the *eti5* mutant of *Arabidopsis*, insensitive to ethylene and characterized by delayed senescence (***publications 2, 3, 8***).

Treatment of detached leaves of *Arabidopsis thaliana* wild-type and *eti5* mutant with the cytokinins N6-benzyladenine (BA) and 4PU-30 showed that they reduced senescence by preserving chlorophyll content and reducing RNase activity. The obtained results show that treatment with their structural analogues with anticytokinin properties 2PU-3 (1-(4-chlorophenyl)-3-(pyridin-2ylmethyl)urea) and TP-5 (3-benzyl-7-(4-methylpiperazin- 1-yl)-

3H-[1,2,3]triazolo [4,5-d]pyrimidine) eliminated the effects of BA and 4PU-30 when applied in combination with the cytokinins. (***publication 2***)

Comparative analysis of changes in the endogenous content of free and conjugated putrescine, spermidine and spermine in *Arabidopsis thaliana* wild type and *eti5* mutant as a result of treatment with low (4°C) or high temperature (38°C) shows that high temperature induces more significant changes in polyamine levels than low temperature. Furthermore, *eti5* showed a better ability to recover after temperature treatments compared to the wild type, partly as a consequence of changes in polyamine content. It has been suggested that the changes in polyamine levels in the wild type during recovery may result from the conversion of the bound to the free form and vice versa, whereas in the *eti5* treated plants *de novo* synthesis of spermidine and spermine is possible (***publication 3***). Better temperature resistance of *eti5* and ability to recover after low or high temperature treatment compared to wild-type *Arabidopsis thaliana* was confirmed by changes in the level of stress markers and non-enzymatic antioxidants, and antioxidant enzyme activity (***publication 8***).

In the presented documents, Iskren Sergiev clearly describes his own contribution to the publications. In general, it is expressed in coordinating the research, participating in the analyses, processing the results and in writing the publications. He is the first or corresponding author of 10 publications (***publications 1, 2, 13, 14, 15, 16, 17, 18, 20, 21***).

Analysis of scientific topic and significance for science and society

In my opinion, the scientific research of Dr. Iskren Sergiev is focused on a very important scientific field. The numerous citations of his publications prove the significance of his investigations. The obtained results contribute to elucidating the mechanisms of action of herbicides and different stress agents and some aspects of the relationships between different classes of phytohormones, show the protective role of growth regulators with different chemical nature and mechanism of action under different stress conditions and contribute to expanding the possibilities for their practical application. They are a very good basis for his future research in all four scientific directions, which are clearly described in the attached author reference.

Organizational and training activities

Dr. Iskren Sergiev's participation as a member of the Organizing Committee of a number of international scientific forums shows his good organizational skills. He leads the doctoral course "Natural and Synthetic Growth Regulators" at the Training Center of BAS and has trained 5 Ph.D. students. He was a scientific consultant of a successfully defended doctoral student. In addition, two students are involved in his ongoing project funded by the Scientific Research Fund, who will be trained in different research methods and approaches.

CONCLUSION

The research activity of Associate Professor Iskren Sergiev is important for clarifying the role of phytohormones and synthetic growth regulators in overcoming the adverse effects of various stress factors. He is an established scientist with proven contributions in the field of plant physiology and biochemistry, with opportunities to train young scientists and lead scientific projects. His participation in an impressive number of projects and the high citation rate of his publications characterize him as a distinguished and internationally recognized scientist. All this gives me reason to recommend to the respected members of the Scientific Jury and the Scientific Council of IPPG-BAS to award Dr. Iskren Georgiev Sergiev the academic position of "Professor" in "Plant Physiology".

22.05.2024 г. Reviewer:
Sofia /Prof. Katya Georgieva/