STATEMENT

by Prof. Dr. Elena Tomova Iakimova, Agricultural Academy, pensioner, appointed as a member of the Scientific Jury according to Order No. 04/27.03.2024 of the Director of IPPG -BAS

regarding the competition for "Professor" announced in State Gazette no. 12/09/02/2024, field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological sciences, scientific specialty Plant Physiology, in laboratory "Regulation of plant growth and development" at the Institute of Plant Physiology and Genetics (IPPG)-BAS

A single candidate in the competition: Associate Professor Dr. Iskren Georgiev Sergiev

Career and thematic development of the candidate: Assoc. Prof. Sergiev graduated in 1991 in the Faculty of Biology, Sofia University "St. Kliment Ohridski", MSc degree "Biochemistry and Microbiology" and specialization in "Plant Physiology". His professional development in the field of plant biology began immediately after the completion of his higher education with research activity entirely oriented to the area of plant physiology and biochemistry with a focus on the role of phytohormones and synthetic growth regulators in the physiological response of plants to stress factors.

The educational and scientific degree "Doctor" in "Plant Physiology" Iskren Sergiev acquired in 1999 as a full time PhD student at the Institute of Plant Physiology "Acad. M. Popov" - BAS (currently Institute of Plant Physiology and Genetics, IPPG), under the supervision of Academician Emanuil Karanov.

Dr. Sergiev's employment is accomplished without breaks in IPPG, Laboratory "Regulation of plant growth and development", where he successively held the positions of Research Associate, Assistant Professor, and Associate Professor (from year 2006 till present).

A significant contribution to increasing and expanding the qualification of Assoc. Prof. Sergiev in theoretical aspect, acquisition of additional experimental skills and for deeper integration into the international scientific community are also the several *specializations* carried out in prestigious European scientific centers such as Istituto biosintesi vegetali - CNR, Milan, Italy and the University of Antwerp, Belgium.

Assoc. Prof. Sergiev's *publication activity* in the nomenclature specialty covers a total of 89 scientific publications in peer-reviewed journals, 59 publications indexed in Scopus or Web of Science and 9 review articles and book chapters. The total impact factor of the works is 75.666, Hirsch index 13.

The publications not a subject to analysis in the current competition are 5 (no. 1, 2, 4, 6 and 8 from the basic list), that are related to the doctoral dissertation for acquisition of PhD and 22 related to the competition for the position of Associate Professor (no. 3, 5, 7, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 27, 28, 29 and 30 from the basic list, including year 2005).

After being appointed as Assoc. Prof., Dr. Sergiev has published 59 scientific papers.

In the competition for "Professor" he participates with 21 publications (no. 31, 32, 33, 34, 35, 36, 37, 38, 39, 42, 43, 47, 61, 62, 65, 66, 74, 77, 79, 83 and 87 in the basic list), all indexed in WoS or Scopus.

Of the publications presented in the competition, 7 are in quartile Q1; 5 in quartile Q2; 3 in quartile Q3 and 3 in quartile Q4 (Scopus). Without quartiles are 3 book chapters. Overall IF 26. 381 (WoS JCR).

The candidate is a *corresponding or first author* of 10 publications, which brings 130 points and is more than the required minimum of 110 points for the criteria in group D.

A total of 2774 *citations* (without self-citations and semi-self-citations) of 69 out of 89 publications are noticed; all citing sources are indexed in Scopus or WoS. For the last 7 years, the number of citations (without self-citations and semi-self-citations) in WoS or Scopus is 1310 of 51 out of 89 papers.

The candidate has participated with *posters and talks* in 10 national (with an international attendance) and 5 held abroad (Turkey, France, Russia, Lithuania, Bosnia and Herzegovina) scientific forums.

As a team member he participated in 8 national and 1 international research *projects*, funded by programs of the Ministry of Education and Science and later by the National Science Fund of Bulgaria (NSF). Dr. Sergiev is a leader of 1 ongoing project in IPPG, financed by NSF with a budget of BGN 200,000 leva.

The *organizational activity* of Assoc. Prof. Dr. Sergiev includes 6 participations as a member of the Organizing Committee (OC) and 2 as Chairman of the OC in international conferences, workshops and symposiums.

Expert activity: Assoc. Prof. Sergiev was a consultant of 1 successful PhD student; a member of scientific juries for election of Assoc. Prof. and Professor; a reviewer of at least 33 articles in prestigious international journals.

The *total number of points* for indicators A to E from the table with criteria for the academic title «professor» is 3224, which exceeds by 2504 points the minimum mandatory number of points in the field of higher education 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological sciences, according to the Law on Academic Staff Development in the Republic of Bulgaria and the specific requirements of the Regulations for its application in IPPG.

Significance of the scientific topic for science and society: The subject of Assoc. Prof. Sergiev's studies is extremely relevant and essential for the understanding and clarification of fundamental issues related to hormonal control and the effect of exogenous compounds with growthregulating activity on the metabolic processes and functional characteristics of plants in conditions of abiotic stress. In addition to the contribution of these studies to plant biology in general, is their importance in ecological aspect and the prospect of application in the selection and creation of genotypes with increased resilience to abiotic and some biotic stress factors, with improved tolerance to adverse effects of herbicides and optimized adaptive potential under the changing climate conditions. The importance of the achievements is proven by the below mentioned findings.

The discoveries, outlined in the publications for the competition can be conditionally summarized in 4 main topics. In their substantial part *the results offer novel information and are valuable contribution to the knowledge of plant growth and development regulation*.

Topic 1: Physiological effect of herbicides on some components of plant metabolism and the functional activity of photosynthesis

With original value are the achievement obtained from the testing of biologically active substances, incl. growth regulators as potential protective agents against the damaging effect of herbicides under suboptimal plant growing conditions.

The effects of the herbicide atrazine are studied in pea plants. Prolonged exposure of plants to low concentrations of atrazine is found to cause growth arrest accompanied by damage of cell membranes and disturbances of redox homeostasis, inhibition of catalase activity, elevated glutathione content and increased glutathione-S-transferase activity. Sublethal concentrations of this herbicide, similar to those that can be found as residues in soil, suppress growth and impair photosynthesis for long term, prompting a re-evaluation of the continued use of herbicides in the same agricultural areas. In the same crop, the possibility of reducing damage from the herbicide paraquat by pre-treatment with hydrogen peroxide (H_2O_2) is tested. Demonstrated is higher survival rate of H_2O_2 pre-treated plants which is associated with reduction of herbicide-induced oxidative stress by increasing the capacity of the antioxidant defense system, suppressing the inhibitory effect of paraquat on the intensity of photosynthesis and stabilizing the structural organization of the leaves. In young maize plants, the phenylurea cytokinin 4PU-30 is observed to exhibit a protective effect against the total herbicide glyphosate (a blocker of the shikimate metabolic pathway), by reducing biomembrane damage, activating the glutathione-S-transferase defense system, and increasing the glutathione pool, reminiscent to of the effects of some herbicide antidotes. The information is important in finding substances diminishing the damaging effects of herbicides on agricultural crops.

Some functional aspects of the reaction of the photosynthetic apparatus after treatment of Bulgarian wheat and triticale varieties with the selective herbicide Zerate and subsequent exposure of the plants to stress conditions during drought or soil waterlogging is studied. These experiments showed that treatment with Zerate leads to fluctuations in the content of leaf pigments and the intensity of the photosynthetic process, without significantly affecting the parameters of chlorophyll fluorescence. For both studied crops, it is concluded that the response of plants in relation to the dynamics of photosynthesis intensity, when exposed to the applied abiotic stress factors, largely depends on their sensitivity/tolerance to the specific stress. It is observed that the indices of plasticity (the capacity of plants to restore the usual levels for the relevant parameters) in wheat, which shows good drought resistance, are positive after drought, but remain negative after watterlogging. The data indicate that pre-treatment with the herbicide Zerate does not exert additional negative effect on the photosynthesis of wheat plants exposed to drought. An opposite trend is found in triticale, which expresses relatively low drought tolerance. Based on the calculated index of plasticity of the individual parameters in this crop, it is assumed that the herbicide treatment before drought further delays the normalization of the photosynthesis process. These data provide information on the photosynthetic status of plants in conditions of combined abiotic stress of herbicides, drought and waterlogging.

Topic 2: Modulating action of synthetic auxins on shaping plant physiological response to abiotic stress

Pioneering research on the growth regulating activity of new compounds with structural analogy of phytohormones is undertaken.

For the first time, the modulating action of the structural analogues of naphthylacetic acid: 1-[2-chloroethoxycarbonyl-methyl]-4-naphthalene sulfonic acid dicalcium salt (TA-12) and 1-[2-dimethyl methoxycarbonyl methyl]naphthalene chloromethylate (TA-14) on the physiological response of pea to herbicides Gleen-75, Glyphosate and 2,4-D is tested. It is found that pretreatment of plants with TA-12 and TA-14 suppresses the negative consequences of herbicide application, which is expressed in a lower content of stress biomarkers compared to plants treated with herbicide alone. It is also established that the tested synthetic auxin analogues modulate the main components (enzymatic and non-enzymatic) of the antioxidant defense system, bringing them to the physiological levels in pre-treated plants. The suppression of oxidative stress correlated with improved growth performance. Observed is that the activity of glutathione-S-transferase decreases at the combined application of glyphosate and 2,4-D and increases at treatment with Glean-75, while the activity of glutathione reductase is not affected by auxin pre-treatment and remains high in herbicide-treated plants. It is suggested that the prior application of both synthetic auxins modulates in a specific way the biochemical response of plants regarding the enzymes related to detoxification of xenobiotics and the defense processes are dependent on the mechanism of action of the certain herbicide.

The protective effect of prior application of the auxin analogues in pea subjected to high temperature or at the treatment of wheat and corn with polyethylene glycol is also investigated. Both compounds are shown to modulate plant physiological responses and help to overcome the negative stress impacts.

Topic 3: Induction of stress tolerance by natural and synthetic growth regulators

Part of the investigations in this topic is aimed at clarifying the influence of prior application of growth regulators with different chemical structure and mechanism of action on changes of biochemical stress markers, in antioxidant defense systems and the level of endogenous polyamines, as components of the plant response to stress factors such as low temperature, drought, salinization and others.

Foliar application of abscisic acid and phenylurea cytokinin 4PU-30 is observed to increase the adaptive capacity of young wheat plants to drought, by reducing the level of the stress markers malondialdehyde and free proline, and simultaneously altering the amount of polyamines as part of the endogenous defense system.

It is established that pre-treatment with natural and synthetic aliphatic polyamines contributes to improving the resistance of wheat to low temperature stress. The increased tolerance of polyamine-treated plants to subsequent exposure

to negative temperature involves significant accumulation of free proline, reduced amount of released electrolytes and higher rate of plant survival. Among the studied polyamines, the effects of diethylenetriamine are highlighted as a potential agent for reducing the damage from low temperature stress.

In review publications (book chapters), the role of endogenous polyamines in overcoming the adverse environmental effects such as low and high temperature, drought, salinization, deficiency of macro- and microelements, heavy metals, UV-radiation, herbicides, hypoxia, anoxia, fungal, bacterial and viral infections is summarized. The role of exogenously applied polyamines in increasing the tolerance and adaptive capacity of plants to various abiotic and biotic stress factors is discussed. The probable mechanism by which polyamines exert their protective action under conditions of salt stress is commented.

The possibility for reducing the disease severity in tomato and pepper plants, caused by *Tomato spotted wilt virus* (TSWV) by pre-treating the plants with the phenylurea cytokinin 4PU-30, the retardant MEIA (β-monomethyl ester of itaconic acid) or with higher aliphatic alcohol triacontanol ($C_{30}H_{61}OH$) is explored. Pre-treatment with MEIA or 4PU-30 is shown to limit the development of subsequent infection in two lines of tomato plants and to reduce the negative consequences of oxidative stress, which is expressed in reduced levels of the stress markers malondialdehyde and free proline. Significant suppression of virus infection by triacontanol is observed in both tomato and pepper plants. The effects of triacontanol treatment before TSWV challenge have been better pronounced compared to post-treatment. DAS-ELISA analysis confirmed lower amount of virus in the infected plants after application of the growth regulators.

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

The research in this direction is related to exploring the effects of interactions between different phytohormones and synthetic growth regulators in plants grown in optimal and suboptimal conditions. The studies are performed with ethylene-insensitive mutant (eti5) Arabidopsis (*Arabidopsis thaliana*) and wild type Columbia. Changes in the amounts of free proline, malondialdehyde and carbonyl groups indicated that plants of eti5 mutant genotype are less affected by temperature stress than the wild type. It is found that the improved tolerance of ethylene-insensitive mutant eti5 to high and low temperature stress correlates with higher constitutive levels of polyamines compared to those in the wild type. The comparative analyses have shown that high-temperature stress affects the changes in polyamine content to a greater extent than low-temperature stress. The obtained results are in line with the understanding that the physiological action of polyamines and ethylene in regulating the processes of plant growth and development is interconnected and to a certain extent determines the sensitivity/tolerance of a given plant species to stress.

Anticytokinins, structural analogs of cytokinins BA and 4PU-30, are found to reduce the senescence-retarding action of cytokinins in leaves of wild type and ethylene-insensitive Arabidopsis mutant, with effects consistent with changes in total ribonuclease activity. It is shown that in Arabidopsis the structural analogy between the two types of regulators (adenine and phenylurea cytokinin) is not a necessary condition for the elimination of cytokinin effects.

Assoc. Prof. Sergiev has correctly described his *personal participation* in the research in each of the topics, indicating that his overall contribution is related mainly to elaboration of the idea, performing a significant part of the biochemical analyses, data processing, interpretation and discussion of the results, writing and editing the publications.

Good impression makes the *candidate's clear vision* to continue and further expand his studies with a focus on the described research topics, in model systems of various and economically important wild, cultivated and mutant lines of plant species.

From the broad and well established professional competence in plant physiology and biochemistry, the significant scientific contribution of the discoveries, the publications in highly internationally ranked issues, the international recognition of the scientific works (citations), the participation, including in the organization of scientific forums, participation in national and international scientific projects and the whole scientific activity, it is evident that the career profile of the candidate in the current competition is characterized by purposefulness, consistency, systematic increase and enrichment of the qualification with firmly established interest in plant physiology and biochemistry, addressing primarily studies on the hormonal regulation of plant behavior at normal and stressful circumstances, elucidation of mechanisms for adaptation, as well as the possibilities for modulation of plant resistance to stress factors by exogenous application of substances with growth regulating and other biological activity.

Critical remarks and recommendations: I have no critical remarks. Only would recommend, in his future research, Dr. Sergiev to additionally extend his studies on the hormonal regulation of plant growth and development toward the level of molecular physiology.

Conclusion

From the analysis of the publication record, the research achievements, the overall contribution of Assoc. Prof. Dr. Sergiev to the relevant field of plant science, the perspective for his further realization in the thematic of plant physiology and biochemistry, I conclude that he is well established, highly qualified, ambitious and precise scientist. This gives me full assurance to vote positively. I recommend the honorable Scientific Jury also a positive vote and to propose to the Scientific Council of IPPG to elect **Assoc. Prof. Dr. Iskren Georgiev Sergiev** for the academic position "**Professor**" for the needs of laboratory "Regulation of plant growth and development" at the institute.

Date:17.05.2024 Signature:

Sofia Prof. Dr. Elena Tomova Iakimova