REFEREE REPORT

on the competition for filling the academic position of **Associate Professor** in the *Professional field* 4.3 Biological Sciences, *Scientific specialty* Biochemistry, announced on behalf of the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences (IPPG-BAS) for the needs of the laboratory "Regulation of gene expression", Department “Molecular Biology and Genetics”

Candidate: **Dr. Kiril Mishev, Assistant Professor** in the laboratory "Regulation of gene expression", IPPG-BAS

Referee: **Dr. Stoyno Stoynov, Associate Professor** in the "Laboratory of Genomic Stability", Institute of Molecular Biology - BAS, appointed as a member of the Scientific Jury by order RD 10-02/23.04.2021 of the Director of IPPG-BAS.

Dr. Kiril Mishev, Assistant Professor at the laboratory "Regulation of gene expression", IPPG-BAS, is the only candidate in the announced competition. All documents submitted by Dr. Kiril Mishev are prepared according to the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria Act (DASRB), the Regulations for its Implementation, and the Regulations of the specific conditions and procedure for acquiring scientific degrees and holding academic positions in IPPG-BAS.

1. Career development and research profile of the candidate

Dr. Kiril Mishev graduated with a MSc degree from the Faculty of Biology at Sofia University "St. Kliment Ohridski” in 2004. In 2009, he obtained a PhD degree defending a dissertation entitled “Functional state of the photosynthetic apparatus and gene expression in chloroplasts in dark-induced and natural aging” at IPPG-BAS. Since 2008, he has been working as an Assistant Professor at IPPG-BAS conducting research in the field of plant physiology, cell biology and biochemistry, mainly focusing on intracellular membrane traffic, phytohormonal signaling pathways, leaf aging, regulation of chloroplast gene function and the functional organisation of the photosynthetic apparatus. He has specialized in renowned foreign institutions, such as the University of Hohenheim, Stuttgart, Germany, the Flemish Institute of Biotechnology, Ghent, Belgium, and the Institute of Plant Physiology at Martin-Luther University of Halle-Wittenberg, Halle, Germany.

2. General overview of the submitted documentation

Dr. Kiril Mishev has submitted all required documents, including copies of his research papers. The research production of the candidate is impressive and fully covers the profile of the announced competition.

The total number of the research papers of Dr. Kiril Mishev is 24, as 20 of them are published in impact factor journals. The total impact factor of these articles, calculated according to Thomson JCR is 104.4. This high quality scientific production is an excellent proof of the serious and consistent research activity of the candidate. The publications have been cited a total of 285 times, which exceeds many times the requirements for the competition, and is an indicator of the excellent acceptance of the research of Dr. Kiril Mishev.

3. Publications for participation in current competition

In current competition, Dr. Kiril Mishev participates with 16 research papers that are not included in his doctoral thesis. All articles have been published in impact factor journals,

including some of the most prestigious international journals, such as Nature Chemical Biology, Nature Communications, Plant Cell, PNAS USA and others. The total impact factor of the publications is 98.9. The candidate has an H-index of 8. Dr. Kiril Mishev has reported his research results at international scientific forums with 7 oral and 16 poster presentations.

4. Major basic and applied research contributions

The research conducted by Dr. Mishev clearly shows that he has established himself as an internationally recognized molecular biologist studying intracellular membrane traffic, the mechanisms of hormonal regulation, regulation of gene expression, DNA methylation, which fully cover the topic of the announced competition. The main basic and applied research contributions can be summarized as follows:

1. New biologically active substances affecting intracellular membrane traffic have been discovered and their biochemical and molecular mechanisms of action have been studied. (**publications В4_1**, **В4_2**, **Г7_3**, **В4_4** и **Г7_7**).
   - The growth inhibitor Secdin, which causes the accumulation of plasma membrane proteins in the late endosomes, has been identified by chemical library screening. It has been shown that Secdin interacts with ARF-GEF proteins involved in vesicular transport.
   - An inhibitor of the cellular transport, called ES4, has been found to cause disruption of ARF-GEF-dependent intracellular membrane trafficking pathways.
   - A new inhibitor of clathrin-dependent endocytosis in plant cells, named ES9, has been discovered. In addition to inhibiting endocytosis, ES9 treatment has side effects, such as a drastic drop in cell ATP levels. The chemical modification of ES9 has produced the daughter molecule ES9-17, which does not affect ATP levels, but has the preserved ability to block endocytosis. ES9 and ES9-17 have been shown to directly interact with the heavy chain clathrin protein to inhibit endocytosis.
2. Study of the mechanisms of hormonal regulation in plants (**publications Г7_1, В4_3, Г7_5, Г7_6 и Г7_8**)
   - New aspects of the regulation of auxin biosynthesis and polar auxin transport by ethylene signals under salt stress have been identified.
   - The role of receptor-dependent endocytosis in the activity of the brassinosteroid receptor BRI1 has been discovered. Using a biologically active fluorescent-labeled brassinosteroid, it has been shown that the internalization of the BRI1-AFCS complex is mediated by clathrin-dependent endocytosis involving ARF-GEF protein regulators of intracellular traffic.
   - Using double *pub12pub13* mutants, it has been found that the U-box E3 ubiquitin ligases PUB12 and PUB13 directly ubiquitinate the BRI1 receptor kinase, which is of crucial importance for the endocytosis and degradation of the brassinosteroid receptor.
3. Study of the changes in the photosynthetic apparatus under stress (publications **Г7_9, Г7_10, Г7_11 и Г7_12**)
   - It has been found that, in contrast to the cotyledons, a stronger decrease in the transcription of the *psaB* and *rbcL* genes was observed in the true leaves during individual darkening, compared to the darkening of whole plants.

- It has been shown that the sensitivity of plastid RNA polymerases PEP and NEP to dark stress in zucchini cotyledons differs in the molecular mechanisms of aging from Arabidopsis cotyledons.
- It has been demonstrated that the individual darkening of cotyledons or true zucchini leaves leads to a change in the aging processes of the adjacent normally lit leaves.

4. Structural and functional organization of ribosomal DNA in Hordeum (**publications Г7_2 и Г7_4**)
   - Unmethylated CCGG regions in a small fraction of the rDNA repeats of common barley (*Hordeum vulgare*) have been detected.
   - The nucleotide sequence and structural elements in the 25S-18S rDNA of *Hordeum bulbosum* have been determined.

5. Participation of the candidate in research projects

Dr. Kiril Mishev has participated in 14 successfully completed Bulgarian and international projects as follows:

- ✓ Supported by the Bulgarian National Science Fund, Ministry of Education and Science and Framework Programs of the European Union: 12 projects
- ✓ Flemish Agency for Innovation through Science and Technology (IWT): 1 project
- ✓ BASF SE: 1 project
- ✓ Unfunded projects under bilateral agreements for scientific cooperation and exchange of the Bulgarian Academy of Sciences (EBR): 4 projects

In 4 of the projects, Dr. Mishev is a project leader / coordinator from IPPG, and the granted funding amounts to a total of BGN 68 550. This fact clearly shows that Dr. Mishev is a well-established scientist, able to obtain funding for his own research by winning projects on a competitive basis and, more importantly, to ensure their successful implementation through effective leadership and research activity.

6. Conclusions

The original scientific contributions in some of the most renowned scientific journals (IF 98.9) and the high citation rate (285 citations) lead to the conclusion that the candidate fulfills and in many aspects exceeds the minimum national requirements of ZRASRB and the Regulations for the specific conditions of academic position "Associate Professor" at IFRG-BAS for holding the academic position "Associate Professor" as follows:

Group of indicators A: the minimum points required is 50 - the candidate collects 50 points.

Group of indicators B: the minimum points required is 100 - the candidate collects 100 points.

Group of indicators Г: the minimum points required is 220 - the candidate collects 274 points.

The points collected from research publications in D, where the candidate is a corresponding and/or first author: the minimum points required is 70 - the candidate collects 87 points.

Group of indicators E: the minimum points required is 100 - the candidate collects 570 points.

According to the Regulations of the specific conditions and procedure for acquiring scientific degrees and holding academic positions in IPPG-BAS, the candidate for the academic position "Associate Professor" should achieve H-index at least 8 (without self-citations) - the candidate meets this requirement because according to the Scopus database, his H-index is 8.

Based on the overview of the presented research papers, their international recognition, the original basic and applied research contributions, I strongly recommend to the esteemed Scientific Jury to award Dr. Kiril Mishev the academic position of "**Associate Professor**" in the *Professional field* 4.3 Biological Sciences, *Scientific specialty* "Biochemistry".

June 22, 2021 Reviewer:

Sofia (Assoc. Prof. Stoyno Stoynov)