REFEREE REPORT

by Prof. Elena Ivanova Georgieva, PhD

on conducting a competition for the academic position of "Associate Professor",
announced for the needs of the laboratory "Regulation of gene expression"
at the Institute of Plant Physiology and Genetics (IFRG) - BAS;
appointed a member of the scientific jury by order RD 10 - 02 / 23.04.2021

1. Common part

The competition for "Associate Professor" in the specialty "Biochemistry", field of higher education 4. Natural sciences, mathematics and informatics, professional area 4.3. Biological Sciences is announced in the Official National Gazette no. 17 / 26.02.2021 for the needs of the Institute of Plant Physiology and Genetics (IFRG), laboratory "Regulation of gene expression" at the same Institute. According to the announced competition, there is one candidate – Assistant Professor Dr. Kiril Mihailov Mishev, appointed to the position of "Biochemist", and performing research in biochemistry, cell biology and plant physiology, in the specified Laboratory. The procedure for opening and announcing the competition has been followed. The documents are precisely prepared and fully comply with the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria, its amendments and the Regulations on the specific conditions and procedures for obtaining scientific degrees and holding academic positions at IFRG-BAS. The presented documents, as well as the copies of the scientific works meet the requirements for the academic position of “associate professor” and certify that the announced one can be given a course.

2. Brief biographical data and career development of the candidate.

Dr. Kiril Mihailov Mishev was born in 1981 in Sofia. In 1999 he was accepted as a student at the Faculty of Biology of Sofia University "St. Kliment Ohridski”, where in 2003 he obtained the Bachelor's degree in Molecular Biology, and in 2004 he graduated with a Master's degree in Plant Physiology. After graduating, Dr. Mishev started working at the Institute of Plant Physiology - BAS (currently the Institute of Plant Physiology and Genetics, IFRG - BAS), where he began his professional career. In the period 2005-2009 he was a doctoral student at the same institute, and in 2010, on the basis of a successfully defended doctoral thesis: "Functional state of the photosynthetic apparatus and gene expression in chloroplasts in dark-induced and natural aging", the Presidium of the Higher Attestation Commission awarded the educational and scientific degree "PhD" with the scientific specialty "Plant Physiology" (code 01.06.16; diploma issued by the Higher Attestation Commission on 01.02.2010). After winning a competition in the scientific specialty "Biochemistry" (code 01.06.10), Dr. Mishev was appointed assistant professor at the same Institute, where he still works as such. Since 2008 he has been on a permanent employment contract at IFRG-BAS, and so far he has 13 years of total work experience in the specialty. His direct scientific activity and research interests throughout his career are focused on basic research, entirely related to the topic of the competition. In the course of his career development, Dr. Kiril Mishev has made 2 short-term working visits to the Institute

of Experimental Botany at the Czech Academy of Sciences, Prague, and the University of Hohenheim, Stuttgart, Germany, to perform LC-MS / MS analyzes. In the period 2007-2015 has completed 3 long-term postdoctoral specializations at foreign universities: two at the Flemish Institute of Biotechnology, Ghent, Belgium, and one at the University of Halle-Wittenberg, Halle, Germany. The knowledge and experience gained within these international specializations contribute to the implementation of joint research projects, as well as to the establishment of Dr. Mishev as an internationally recognized scientist. Knowing the nature of working with plant organisms, I must rightly note that Dr. Mishev has achieved significant success and has established himself as an indisputable biochemist and molecular biologist in a very difficult field for experimental development. Using a number of modern tools and approaches, he has focused his research on solving important scientific challenges in plant biochemistry, namely - the study of many biochemical and physiological processes occurring in the plant cell, such as intracellular membrane traffic, phytohormonal signaling pathways, foliar aging, regulation of chloroplast gene expression, etc.

3. General data on the thematic development of the candidate

The scientific production of Dr. Mishev, including his doctoral dissertation and his publishing activity, covers the requirements for the scientometric indicators for this academic position and fully corresponds to the profile of the announced competition. The overall scientific activity of Dr. Mishev has so far been presented in 24 scientific papers with a total JCR Impact Factor 104,409. A scientific achievement is also information published in deposit bases: GenBank, Accession № HQ825319 related to partial sequencing of the 25S rRNA gene in Hordeum subsp. vulgar. For participation in the competition for the academic position "Associate Professor", Dr. Mishev presents 16 scientific articles. Five of the publications in the presented list with № 18, 19, 20, 21 and 22, as well as the attached copy of the abstract, are included in the dissertation of Dr. Mishev for the acquisition of ONS "PhD" 8 articles are not subject to review, but in formulating my final opinion, I will take into account its entire scientific output. Two of the 16 publications submitted for participation in the competition are scientific reviews. The publication of review articles requires a broad orientation and solid, in-depth scientific training, as evidenced by the high scientometric performance of the candidate. All scientific papers of Dr. Mishev, included in the competition, have been published in some of the most prestigious indexed and referenced international journals (Scopus and WoS) with a high Impact Index, namely: *Nature Communications IF 12.948; Nature Chemical Biology IF 12.124; Proc Natl Acad Sci U. S. A. IF 9.580; Plant Cell IF 8,631; Current Opinion in Plant Biology IF 7,848; Chemistry & Biology IF 6.586; Plant Physiology IF 6.456* et al.

Dr. Mishev is the first author in 4 publications and a co-author in 12. The total JCR Impact Factor of all competition publications is 98,964, and the total JCR IF of the competition publications in which the candidate is the first author is 17,963. With posters and oral presentations, some of the candidate's results by 2019 have been presented at 23 international and national conferences. Due to the imposed epidemic situation for 2020, all present scientific forums have been suspended so far. For the period 2004-2021, the citations observed in the WoS and Scopus databases were 285 without autocitation and semi-autocitation. Evidence of the high international response of the candidate's works are the total number of 338 citations observed in the Web of Science and Scopus, but also in other scientific journals and in deposit bases (excluding auto-citations and semi-auto-citations). Hirsch citation index ("h-index") is an

objective indicator of the scientific activity of the candidate, who is currently on Scopus is 8. A detailed reference of authors and publications that cited his scientific works is attached. According to the requirements for the fulfillment of the minimum national requirements by the Law on the Promotion of the Academic Position "Associate Professor" from Table 1 of the Law on Public Procurement, for participation in the competition, Dr. Mishev forms the following indicators: group A - 50 points; group B - 100 tons; according to indicator D - 274 points at a required minimum of 100 points, and according to indicator D (cited) collects 570 points at a minimum of 100 points. Thus, at a required minimum for "Associate Professor" of 420 points, according to PPZRASRB, Dr. Mishev forms 994 points, which is more than twice the minimum national requirements required for this academic position. The distribution of the candidate's scientific output by quartiles is remarkable - 14 articles with quartile Q1 and two with quartile Q4. All the above confirms the high quality of the scientific production of the candidate, as well as that it has found a serious response in the international scientific community, which is why it deserves high praise.

4. Evaluation of the scientific achievements in the research work of the candidate

The research activity and achievements of Dr. Mishev are aimed at solving scientific-applied and fundamental problems in the field of biochemistry, molecular genetics and plant physiology. The presented reference for his publishing activity reflects his wide and thorough preparation. In summary, the scientific contributions of the candidate are grouped in 4 areas, which I fully accept. In each of his contributions, the candidate has made an accurate assessment of his personal contribution, which makes a very good impression. The first scientific field related to the approaches of chemical genomics and proteomics Dr. Mishev has devoted to the study of the molecular mechanisms of regulation of intracellular membrane traffic (publications B4-1, B4-2, G7-3, B4-4 and G7-7). The published experimental results were realized with the significant participation of Dr. Mishev, which will be described in detail. In this scientific direction, the analyzes are devoted to the mechanisms of regulation of the processes of exocytosis and endocytosis in *Arabidopsis*, in which the cell transports large molecules encompassing highly dynamic biological processes that can regulate protein components of signal transduction chains. Despite the large number of published synthetic molecules and natural organic compounds with an effect on exo / endomembrane traffic, biochemical approaches to detect specific protein targets of the studied molecules are still poorly applied in plant genetics and do not fully understand the specificity and mechanisms of action. Following a large-scale screening of the DIVERSet (ChemBridge) chemical library, a growth inhibitor called Secdin was identified, causing aberrant accumulation of plasma membrane marker proteins in late endosomal compartments (G7-3). By affinity purification followed by LC-MS analysis, the target proteins of Secdin from the *Arabidopsis* proteome were identified. Thus, a potential inhibitor of the ARF-GEF family of inhibitors (guanine nucleotide exchange factors for small ARF GTPases) was found which play a central role in all intracellular vesicular trafficking pathways. With biochemical approaches, Secdin has been shown to interact with all ARF-GEF proteins from the *Arabidopsis* proteome. In collaboration with an international research group led by Prof. Jiří Friml, another low molecular weight inhibitor of intracellular membrane traffic, ES4, which also interacts with ARF-GEF proteins (B4-2), has been identified and characterized. ES4 treatment results in disruption of all ARF-GEF-dependent pathways of intracellular vesicular traffic. The effect of ES4 on ARF-GEF proteins alters the ratio between the membrane-bound and cytosolic fraction

of ARF1 GTFase, which is a substrate of ARF-GEF proteins. Attempts to detect new inhibitors of clathrin-dependent endocytosis in plant cells have identified a new low molecular weight compound ES9 with proven activity in other eukaryotic systems (B4-4). Additional studies have shown that treatment with ES9 leads to nonspecific effects, such as a drastic drop in ATP levels in the cell. After chemical modification of the initial structure of ES9, a daughter molecule ES9-17 was obtained with the preserved ability to block endocytosis (B4-1). The mechanism of action of ES9 and ES9-17, which is associated with direct interaction with heavy chain protein clathrin (CHC) ES9 treatment, has been shown to cause nonspecific effects, such as a drastic decrease in ATP levels in the cell as well as a decrease in cytoplasmic pH. A similar nonspecific effect was found in the most widely used endocytosis inhibitor TyrA23 in cell biology. It is concluded that due to the side effects of TyrA23, ES9-17 is the only specific inhibitor of clathrin-dependent endocytosis for use in plant cell biology. A review article (G7-7) is in this direction, in which a comparative analysis of the pathways for intracellular vesicular traffic in different eukaryotic systems is made and the screening studies published so far have been summarized, which led to the identification of new low molecular weight traffickers. The second direction includes publications G7-1, B4-3, G7-5, G7-6 and G7-8, related to the analysis of the mechanisms of hormonal regulation in plants and interaction between phytohormonal signaling pathways, in which the personal participation and the serious contribution of Dr. Mishev is in all publications. Plant tolerance to salt stress is defined by a number of stress-responsive genes controlled by different signaling pathways. A large number of biochemical analyzes have revealed new aspects of the regulation of auxin biosynthesis and polar auxin transport under action is of ethylene signals under conditions of salt stress (G7-1). For this purpose, Dr. Mishev received and studied two mutant model lines of *Arabidopsis* - ethylene-insensitive and one with an active ethylene signaling pathway. Differences in the effect of salt stress on auxin transport in the epidermal layer and in the conductive elements in the root core in the two studied genetic systems were identified.

The importance of receptor-dependent endocytosis for the signaling activity of the brassiosteroid receptor BRI1 (G7-8) has been elucidated. To visualize the ligandreceptor complexes, a biologically active fluorescent-labeled brassinosteroid (AFCS) was developed and it was found that the internalization of the BRI1-AFCS complex was performed by clathrin-dependent endocytosis involving ARF-GEF protein regulators. The role of U-box E3 ubiquitin ligases PUB12 and PUB13 in the brassinosteroid signaling pathway (B4-3) has been elucidated. Both enzymes have been shown to directly ubiquitinate the BRI1 receptor kinase. Using dual *pub12pub13* mutants, the two ubiquitinating enzymes studied were found to be crucial for endocytosis and degradation of the brassinosteroid receptor, and that impaired expression of *PUB12* and *PUB13* resulted in retention of BRI1 in the plasma membrane membrane in protein degradation. For the first time in functional plant biology, the induced protein aggregation approach was applied, as BIN2 kinase from the brassinosteroid signaling pathway (G7-5) was studied as a model protein. Genetic constructs with their encoding nucleotide sequences in common reading frame with the GFP reporter protein gene have been developed, used for temporary expression in tobacco leaves for BiFC assays and for transformation of *Arabidopsis* plants for phenotypic and expression assays. In *Arabidopsis* transgenic lines, a phenotype associated with a constitutive response to a brassinosteroid stimulus associated with attenuation of the kinase activity of BIN2, which is a negative regulator of the brassinosteroid signaling pathway, has been identified. Review article G7-6 summarizes information on the available low

molecular weight biologically active substances that can be used to study brassinosteroid activity. The approaches for creating new biologically active synthetic analogues of brassinosteroids are also commented. The third direction covers publications G7-9, G7-10, G7-11 and G7-12, related to structural and functional changes in the photosynthetic apparatus under stressful environmental conditions. The candidate's contribution in this group is related to the conduct and analysis of all experiments described in publications G7-11 and G7-10, with the analysis of mRNA levels of photosynthetic genes from cotyledon plastoma and true zucchini leaves under conditions of dark stress, with the isolation of thylakoid membranes from peas and preparation of samples with isolated thylakoids in buffers of different ionic strength. Depending on the application of dark stress - darkening of the whole plant or just a single leaf, differences were found in the mechanisms of photoprotection in the cotyledons and true leaves of *Arabidopsis* in terms of non-photochemical NPQ quenching and unregulated energy dissipation ΦNO (G7-11). The levels of the transcripts of the chloroplast genes *psaB* and *rbcL* were studied and it was found that their expression is strongly dependent on the degree of darkening in individual cotyledons and in cotyledons of whole plants. In the actual leaves, a stronger decrease in mRNA levels of the above two genes was observed with individual darkening compared to the darkening of whole plants. In contrast to previously published data, analyzes of chlorophyll fluorescence and mRNA levels of the chloroplast genes studied and the marker *SAG12* nuclear gene encoding leaf aging-associated cysteine protease *SAG12* in the recovery phase from dark stress showed the ability of photosensitivity to overcome the negative effects of blackout. Differential sensitivity of plastid RNA polymerases (PEP and NEP) to dark stress has been found in zucchini cotyledons, which differ in molecular aging mechanisms from *Arabidopsis* cotyledons. A detailed analysis showed that the decreased rate of total chloroplast transcription is due to a decrease in the activity of chloroplast-encoded PEP polymerase, while nuclear-encoded NEP is not significantly affected by stress and increases its relative share (G7-10). It was found that the eclipse-induced decrease in transcript levels of the plastidly encoded photosynthetic genes *psaB* and *rbcL* was due to a reduced transcription rate of these genes. Another important contribution is the proof that the individual darkening of cotyledons or true leaves of zucchini causes changes in the aging processes of the neighboring normally illuminated leaf organs (G7-9). Biophysical, biochemical and microscopic approaches have been combined to clarify the compensatory response to locally applied stress in non-obscured organs. New aspects of the mechanism of biological action of the amphiphilic peptide melittin from bee venom have been discovered. Thylacoid membranes of chloroplasts (G7-12) were used as a model system in these studies. In attempts to study the electrophoretic mobility and light-scattering properties of tilacoid with increasing concentrations of melittin, significant differences in the mechanism of action of the peptide were found depending on the salt composition of the medium. When thylakoids were irradiated with photosynthetically active radiation, a negative effect of high concentrations of melittin on the photochemical efficiency of FS II was registered. The in-depth preparation of the candidate in the field of molecular genetics is also demonstrated in the next fourth scientific field, focused on the structural and functional organization of rDNA in *Hordeum* (articles G7-2 and G7-4). Dr. Mishev's personal involvement is related to the isolation of genomic DNA from reconstructed barley lines and from *Hordeum bulbosum* from different geographical areas, with autoradiographic analyzes after molecular hybridization with genomic DNA and restriction with methylsensitive endonucleases, as well as PCR analyzes to determine of R128 the repeated portion of the intergenic spacer. Unmethylated CCGG regions were localized in a small fraction

of the rDNA repeats of common barley (*Hordeum vulgare*) near the start of transcription, in the outer transcriptional spacer, and in the rRNA coding sequences (G7-4). A mutant having only one nuclear organizer was used as a model system, but with increased activity, which is probably related to the open fraction of hypomethylated rDNA. The nucleotide sequence and structural elements in the 25S / 18S rDNA region of the genomic DNA of *Hordeum bulbosum* (G7-2), having only one nuclear organizer, were determined. When comparing the nucleotide sequence of *Hordeum bulbosum* obtained from sequencing with other representatives of cereals, it was found that the intergenic spacer contains two sections with subrepeats - R143 (2 repeats) and R128 (5 or 6 repeats). R143 repeats found elements common to R79 from *Hordeum vulgare*, and R128 repeats resembled R135 from common barley. R128 / R135 repeats are characterized by a conservative sequence of 31 bp, found in all studied cereal species. The different lengths of the repetitive regions of the intergenic spacer are likely to be related to the nuclear dominance observed in hybrids between *H. vulgare* and *H. bulbosum*. In the comparative analysis of the promoter regions of the two studied species of the genus *Hordeum*, a significant evolutionary divergence was found.

5. Participation in scientific projects and other activities of the candidate

Dr. Mishev's professional skills are reflected in his development and participation in 18 national and international projects, of which 4 are supervisors / coordinators; at 10 he is a member of the research team; in 3 he is a scholarship holder and in 1 he is a mentor in student internships. The funding organizations are: Research Fund at the Ministry of Education and Science; Operational programs of the European Union - 12 projects; 1 project by the Flemish Agency for Innovation through Science and Technology (IWT); BASF SE and 4 projects under bilateral agreements for scientific cooperation and exchange of the Bulgarian Academy of Sciences (EBRD). Dr. Mishev's scientific research is also reflected in finding funding to support research. The financial resources received from competitively won projects, of which Dr. Mishev is the manager, amount to approximately BGN 67,000, which is a decent financial resource to cover research decisions. Special attention should be paid to the grants and awards won by Dr. Mishev. For his short scientific experience, he was awarded a distinctive prize for high scientific achievements in the field of plant biology by the Rotary Club in Sofia. Several grants have been won: one from the Flanders Science Foundation (FWO), covering funding for two international conferences: the ENPER conference in Lecce, Italy (2014) and the EMBO in Brno, Czech Republic, as well as other student grants awarded from the Federation of European Society of Plant Biology (FESPB) for participation in two of the FESPB congresses in 2006 in Lyon, France and in 2008 in Tampere, Finland. Proven success and high appreciation for the scientific activity of the candidate is his membership in editorial boards and councils of specialized scientific publications. Dr. Mishev is currently an editor at Frontiers in Plant Science (JCR rank: Q1 and IF: 4,402) and an editor at Plants MDPI (JCR rank: Q1 and IF: 2,762). Membership in scientific organizations is also recognition of high academic activity. Dr. Mishev is a member of the USB, Plant Physiology and Biochemistry Section, and a member of the FESPB and the American Society of Plant Biology (ASPB). The reviews and opinions on procedures for educational level for scientific degrees and academic positions, prepared by Dr. Mishev as an assistant professor, are 6 in number. It is noteworthy that for the most part these reviews and opinions are at the invitation of international institutes - Spain and Belgium. The high competence of the candidate is assessed by the 30 reviews of articles and projects prepared by him, most of which are to

international scientific organizations and journals: German Research Foundation (DFG), Research Foundation - Flanders (FWO), Current Genomics, Plant Growth Regulation, The Plant Cell, Plants MDPI, South African Journal of Botany and others. These facts prove productive academic development with personal contribution and high scientific competence of the candidate.

6. Training of personnel and educational and pedagogical activity

Dr. Mishev's participation in teaching and training is significant. The information about his workload certifies that for the period 2013 - 2021, under a project of the Ministry of Education and Science "Student Internships", funded by OP NOIR, he is a mentor of 8 interns from the Faculty of Biology at Sofia University "St. Kliment Ohridski". For each trainee Dr. Mishev has conducted 240 training hours, or their total number under this program is 1860 hours. In addition, for each intern, he led a separate scientific topic related to biochemical, genetic and microscopic approaches to the study of the plant genome. He is a co-supervisor of a student's thesis in Ghent/Belgium.

7. Conclusion

The only candidate who applied to participate in the competition for "Associate Professor" is Assistant Professor Dr. Kiril Mihailov Mishev. The academic and professional history of the candidate's scientific production show that he is an established specialist with in-depth knowledge, an expert in the field of plant biochemistry, physiology and genomics, a sought after and desired partner for research and development. The entire scientific production of Dr. Mishev is reflected in highly indexed international journals with a total IF = 98.964, and is very well accepted by the international scientific community, for which their high citation rate can serve as a certificate - over 300. For the competition Mr. Mishev presents a large number of successfully implemented projects, high educational activity, participation in international scientific organizations, received prestigious awards and distinctions, etc. The scientometric indicators of the candidate and the accompanying materials of the competition exceed the recommended criteria of the Law on Academic Development in the Republic of Bulgaria, the Rules of its Application as well as of the Internal Rules and Regulations of the Institute of Plant Physiology and Genetics at the Bulgarian Academy of Sciences for the academic position "Associate Professor", therefore I strongly recommend the Scientific Council of IFRG-BAS to vote positively and to award Dr. Kiril Mihailov Mishev the academic position "Associate Professor".

June 5, 2021 Reviewer:

/Prof. Elena Georgieva, PhD/