EXPERT OPINION

By: Assoc. Prof. Dr. Zhenya Pektova Yordanova,
Faculty of Biology, Sofia University "St. Kliment Ohridski",
member of the scientific jury appointed by order No РД 01-16 / 22.04.2025 of the Director of Institute of Plant Physiology and Genetics, Bulgarian academy of Sciences, Prof. Dr. V. Vasileva

Regarding: Competition for the academic position of „Associate Professor" in the professional field 4.3. Biological Sciences, specialty „Genetics", at the Laboratory of „Regulators of Plant Growth and Development", Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences.

1. General presentation of the procedure and the candidate

1.1. Documents

The competition for the academic position of "Associate Professor" in the field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, specialty "Genetics", was announced for the needs of the Laboratory "Regulators of Plant Growth and Development" at the Institute of Plant Physiology and Genetics of the Bulgarian Academy of Sciences in State Gazette No. 16, dated February 25, 2025. Within the legally established deadline, the only applicant who submitted documents for the competition is Chief Assistant Dr. Maria Ivanova Petkova. She is currently employed on a permanent contract in the same laboratory.
The procedure for announcing and opening the competition has been duly followed, and the materials submitted by the candidate include all required documents in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria and its amendments, as well as the Regulations on the Specific Conditions and Procedures for Acquiring Academic Degrees and Holding Academic Positions at the Institute of Plant Physiology and Genetics – BAS. These documents meet the requirements for the academic position of "Associate Professor" and confirm that the procedure may proceed.

1.2. Biographical Data of the Candidate

Maria Petkova is a graduate of Sofia University "St. Kliment Ohridski," where she obtained a Master's degree in Biology and Chemistry with a specialization in Medicinal Plants in 2001. As a doctoral candidate in independent study at the Institute of Plant Physiology and Genetics – Bulgarian Academy of Sciences (BAS), she defended a dissertation titled "Biotechnological Approaches for Propagation and Biomass Production of Hairy Roots in Arnica montana L." in 2014 to earn a PhD degree in the scientific specialty "Genetics" (code 01.06.06). In 2003, she was appointed as a research associate III degree at the Institute of Genetics "Acad. D. Kostov," and since 2011 she has held the position of senior assistant (2011 – present) at the Institute of Plant Physiology and Genetics – BAS. Dr. Petkova's scientific interests are a natural continuation of her dissertation topic and are mainly related to the study of medicinal plants and biotechnological approaches for cultivation and synthesis of biologically active substances; cell and tissue cultures and genetic manipulations in plants.
A large part of her publications throughout her scientific career (47 out of 52) are related to the stated topics. Of these, 4 articles were used in the defense of her doctoral dissertation, and for participation in the competition, 21 articles were submitted – 17 experimental studies and 4 reviews. Dr. Petkova has participated in 15 national, international, or educational projects, acting as the leader of two of them and as a member of the scientific team in 13. She completed one specialization in Finland at the VTT Research Centre in 2010. She has presented her scientific

research at 32 national and international scientific forums, with documented participation including 40 posters and 2 oral presentations.

2. Evaluation of the submitted report on the minimum and specific requirements for the competition.

The report on the fulfillment of the minimum national requirements by the Law for the Development of Academic Staff in the Republic of Bulgaria for scientific field 4. Natural Sciences, Mathematics, and Informatics, professional direction: 4.3 Biological Sciences, aligned with the additional regulations adopted under the law in the Institute of Physiology of the Bulgarian Academy of Sciences (IPHR-BAS), certifies that Chief Assistant Dr. Maria Petkova has accumulated 928 points, out of the mandatory minimum of 540 points. These values not only meet but almost double the MNR for holding the academic position of "Associate Professor." The figures for the individual indicators are as follows:

Indicator group	Content	Minimum requirements	Points
A	Indicator 1	50	50
B	Indicator 2	-	-
C	Indicator 3 and 4	100	125
D	Sum of indicators 5 to 10	220	232
E	Sum of points in indicator 11	100	348
F	Sum of indicators 12 to end	70	173

The scientific indicators show that Dr. Petkova is an established scientist with exceptionally high professional qualifications in the field of plant biotechnology.

3. Analysis and Evaluation of the Candidate's Scientific Achievements in Research

The scientific work of Chief Assistant Dr. Maria Petkova is focused on the biotechnology of medicinal and aromatic plants, with emphasis on the application of biotechnological and genetic approaches for propagation and biomass production of medicinal plants -including economically important, rare, and endangered species that produce valuable biologically active substances. She investigates the potential for modulating secondary metabolite synthesis through targeted in vitro morphogenesis and the generation of high-yielding clones. She also develops in vitro systems for controlled cultivation of plant material with specific characteristics.

In her report on scientific contributions, Dr. Petkova defines two areas of research activity:

1. Development and optimization of highly efficient in vitro protocols for propagation, biomass production, and synthesis of biologically active substances from medicinal plants of the Asteraceae family.

2. Modulation of the synthesis of biologically active substances through gene transfer or the use of abiotic and biotic elicitors in medicinal plants of the Asteraceae family.

The first research area has been studied in depth and supported by significant scientific results published in prestigious high-ranking journals (B4-4, B4-5, B4-6, G7-3, G7-6, G7-9, G7-10, G7-12, G7-13, G7-14, G7-15). Protocols were developed and optimized for accelerated micropropagation of endangered plant species such as Arnica montana L. and Helichrysum arenarium (L.) Moench, as well as economically important species like Stevia rebaudiana Bertoni, Echinacea purpurea (L.) Moench, and Cichorium intybus L. Specific protocols were proposed for the production of plant material useful to the pharmaceutical industry, using nutrient media enriched with various plant growth regulators.

An optimized protocol for the rapid micropropagation of Arnica montana L. - rich in secondary metabolites - was developed using agar-solidified and liquid media in an automated temporary immersion system (RITA® type). A 100% in vitro rooting rate was achieved on media with half-strength macro salts and supplemented with IBA. Impressively, plants obtained through this environmentally friendly method, and acclimated in mountainous conditions (experimental field in the Beglika area), synthesized higher amounts of sesquiterpene lactones compared to conventionally propagated plants.

For the first time, an in vitro method for storing valuable genotypes of A. montana using sorbitol was developed, enabling six months of preservation while maintaining plant viability and multiplication rate. A novel study analyzed how the method of propagation affects the qualitative and quantitative composition of STLs in A. montana flowers. It was found that in vitro-propagated and acclimated plants contain higher levels of STLs - particularly 11,13-dihydrohelenalins compared to conventionally propagated plants. Although flavonoid and phenolic profiles showed no qualitative differences, in vivo grown plants had higher total phenolic content, possibly due to differences in altitude. Plant age and phenological stage also influenced lactone profiles: two-year-old plants had more 11,13-dihydrohelenalins, while three-year-olds had mainly helenalin esters. Seed origin was also significant - plants of Austrian origin accumulated nearly twice as many STLs as those from Ukraine.

These studies make an original scientific contribution by revealing new facts about the biological potential of A. montana, and the developed technology is both practically applicable and of innovative significance.

An economically viable protocol for the micropropagation and ex situ conservation of Helichrysum arenarium was developed using MS medium without growth regulators and with reduced salts and sucrose content. In vitro plants formed rhizomes that generated up to 55 well-developed and spontaneously rooted plants from a single explant. No ploidy-level deviations were found in in vitro plants, indicating genetically uniform material.

A protocol of economic importance for micropropagation of Echinacea purpurea was also developed, producing over 16 plants per explant using BAP and naphthaleneacetic acid. The entire process - from seed germination to ex vitro acclimatization - takes only 12 weeks. These in vitro plants showed significantly higher antioxidant activity and levels of both water- and fat-soluble antioxidants.

A practically valuable optimized in vitro protocol was also established for mass propagation of Stevia rebaudiana, ensuring 90% survival of ex vitro-acclimatized plants - an excellent biomass source for the food and pharmaceutical industries.

A novel achievement was the stimulation of micropropagation of Cichorium intybus using both the purine cytokinin BAP and the phenylurea cytokinin 4PU-30 combined with the auxin NAA, producing 7–9 new plants per explant. 4PU-30 was applied for the first time to this species and effectively induced regeneration while preventing hyperhydration of tissues.

In the second research area, original results were presented regarding Agrobacterium rhizogenes-transformed root cultures of Arnica montana, where for the first time the impact of various carbohydrate sources on growth and secondary metabolite accumulation was evaluated. Rapidly growing root cultures produced flavones, phenolic acids, organic acids, sugars, and other metabolites and are suitable for scale-up in bioreactors. Yeast extract was shown to stimulate the synthesis of bioactive compounds and antioxidant enzymes - unlike salicylic acid.

For the first time in A. montana, the stability of expression of nine candidate reference genes after treatment with methyl jasmonate, yeast extract, and salicylic acid was evaluated. Using four established algorithms, genes encoding the ATP synthase subunit and β-actin were found to be the most stable and suitable for RT-qPCR analysis, while PP2A and TUBb showed high variability. Validation was done by analyzing genes from the phenylpropanoid pathway, showing significant induction of PAL and HQT after methyl jasmonate treatment.

An innovative biotechnology approach was developed for the in vitro propagation of Stevia rebaudiana through direct organogenesis using nanofibers carrying silver particles. Low concentrations of peptidomimetic nanofibers stimulated growth and biosynthetic activity, while higher concentrations inhibited it, showing a hormetic effect. Treatment with nanomaterials increased antioxidant capacity and content of sweet glycosides and phenolic acids, as well as drought tolerance. Additionally, amino acids such as creatine and creatine-lysinate modulated physiological parameters in stevia and edelweiss. Several review articles summarized the effects of nanoparticles and elicitors on metabolic profiles and productivity of in vitro cultures from the Asteraceae family. These results enhance the understanding of physiological and biochemical processes and have practical value for sustainable biomass production.
All scientific publications by Chief Assistant Dr. M. Petkova are co-authored, with her individual contribution clearly and appropriately stated. The analysis of her scientific work shows that she plays a leading and essential role in the planning, methodological support, execution, and funding of the research, as well as in preparing and presenting the results. This reflects her high level of engagement and professionalism within the scientific team.

4. Relevance of the Scientific Topic and Its Importance to Science and Society
The research activities and interests of Dr. M. Petkova are highly relevant and aligned with current priorities in the fields of plant biotechnology and sustainable agriculture. Her studies focus on developing effective in vitro methods for the propagation and preservation of valuable medicinal and endemic plant species. Through the application of innovative approaches - including the use of nanofibers, elicitors, and molecular markers - she achieves optimization of the production of biologically active compounds, enhancement of antioxidant capacity, and increased tolerance to abiotic stress. This research area holds significant importance for fundamental science by contributing to a better understanding of the physiological, biochemical, and metabolic responses of plants under controlled conditions. At the same time, the developed protocols have high practical potential in the fields of phytopharmacy, cosmetics, and agriculture, supporting the sustainable conservation and use of plant resources.
The presented list of 17 peer reviews of scientific publications demonstrates that Dr. Petkova is an internationally recognized expert in her field and is sought after for her expert opinion on the quality of research conducted by other scientists in related disciplines.

5. Organizational and Educational Activities
Dr. Petkova is a leader and participant in national and international scientific research projects. She supervises one undergraduate and one master's degree thesis student at Sofia University “St. Kliment Ohridski”. She has also supervised practical training in in vitro cultivation of medicinal plants for postdoctoral researcher Magdalena Sozoniuk from the University of Life Sciences in Lublin, Poland, under the Erasmus scholarship program.

6. Critical Remarks and Recommendations
I have no critical remarks or recommendations. I was particularly impressed by Dr. Petkova’s critical self-assessment regarding her personal contribution to the published results, as well as her clear and well-founded perspective on future research, which I consider to be both promising and significant.

7. Conclusion
Following an analysis of the scientific activity of Chief Assistant Professor Dr. Maria Petkova, I express my positive opinion that she fully meets the requirements of the announced competition. Her scientific publications are of sufficient volume and high quality, and the points accumulated from her scientific output significantly exceed the requirements of the Act on the Development of

the Academic Staff in the Republic of Bulgaria and the Regulations for the Specific Conditions and Procedures for Holding the Academic Position of "Associate Professor" at the Institute of Plant Physiology and Genetics – BAS.

In addition to their high scientific value, Dr. Petkova's research has considerable potential for future development, which is well substantiated in her vision for post-habilitation professional growth. Based on the above, I confidently recommend to the esteemed members of the Scientific Jury and the Scientific Council of the Institute of Plant Physiology and Genetics – BAS, to award the academic position of **Associate Professor** to Chief Assistant Professor Dr. Maria Petkova in the professional field 4.3. *Biological Sciences*, specialty *Genetics*.

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Sofia

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