REVIEW REPORT

By Associate Professor Kalina Moneva Danova, PhD

Institute of Organic Chemistry with Centre of Phytochemistry

Bulgarian Academy of Sciences

Of the materials submitted for participation in the competition

For the academic position “Associate Professor”

At Laboratory “Regulators of Plant Growth and Development”

Institute of Plant Physiology and Genetics - Bulgarian Academy of Sciences (IPPG-BAS)

Professional field 4.3. “Biological sciences”, Scientific subject “Genetics”

In the competition for "Associate professor", announced in the State Gazette, issue No. 16/25.02.2025, the only candidate participating is Senior Assistant Professor Maria Ivanova Petkova, PhD from Laboratory “Regulators of Plant Growth and Development” IPPG-BAS

1. General presentation of the candidate's career and thematic development

The only candidate who submitted documents for participation in the announced competition was Senior Assistant Professor Dr. Maria Ivanova Petkova, Laboratory "Regulators of Plant Growth and Development" at the Institute of Plant Physiology and Genetics - BAS (IPPG - BAS), Doctor of Science in "Genetics" (code 01.06.06), obtained at the IPPG-BAS and Master of Science in Biology and Chemistry, Specialization Medicinal Plants, obtained at Sofia University "St. Kliment Ohridski", Faculty of Biology.

The candidate also completed an international three-month specialization in 2010 within the framework of the COST program, VTT Research Center, Finland on the topic of Production of Bovin β-lactoglobulin in plants and plant cell cultures.

Since 2011, Dr. Petkova has been working and developing her academic career at the Institute of Plant Physiology and Genetics - Bulgarian Academy of Sciences. The candidate has published a total of 52 scientific articles, with a total number of 425 citations (227 in Scopus). Her H - index (in Scopus) is 9. She has participated in Bulgarian and International scientific forums with 2 reports and 40 posters. The candidate is a reviewer of 39 scientific publications.

2. Assessment of the submitted report for compliance with the requirements of Art. 26, para. 1 of the Law on Academic Affairs and the specific requirements for holding the academic position of "Associate professor", reflected in Appendix 1 of the Regulations on the specific conditions and procedure for acquiring scientific degrees and for holding academic positions at the Institute of Plant Physiology and Genetics-BAS.

In the competition for "Associate professor", Dr. Petkova participates with a total of 21 scientific publications (4 of them - review articles). The distribution by quartiles is as follows - Q1 - 7, Q2 - 5, Q3 - 4 publications and Q4 - 3 publications. Two of the publications are in journals referenced by other databases without a quartile.

In 8 of the publications the candidate is the corresponding author, and in 8 - the first author (first and/or corresponding author in 10 of the publications). The JCR IF of all publications for participation in the competition is 36.511 (of which by indicator B - total JCR IF: 14.834). The JCR IF of publications for participation in the competition, in which Dr. Petkova is the first or corresponding author, is 12.188.

She has been a participant in 10 projects under the National Science Foundation (3 for young scientists), 2 - under the Ministry of Education and Science and the EU, 1 - Ministry of Environment and Water, 1 - international and 1 - EBR, of which she is the head of one Young Scientists' and one project for Fundamental Scientific Research under the National Science Foundation, in 11 of the projects she is a member of the scientific team and in 2 - a member of the target group.

The candidate exceeds the minimal national requirements of the Law on the Promotion of Higher Education and Research and the Regulations on the Specific Conditions and Procedures for Holding an Academic Position of Associate Professor at the Institute of Plant Physiology and Genetics-BAS, as follows: indicator A - 50 points (out of the minimum required 50), indicator B - 125 points (out of the minimum required 100), indicator Г - 232 (out of the minimum required 220), D - 348 (out of the minimum required 100), indicator E - 173 (out of the minimum required 70).

3. Analysis of the main areas and research work with the most important results and personal contribution of the candidate

Dr. Petkova's scientific interests are in the field of plant biotechnology, with the object of her research being medicinal and aromatic plants. Her professional expertise covers the application of biotechnological and genetic approaches to obtain plant biomass from economically important, rare and endangered species, a source of biologically active substances, influencing the accumulation of secondary metabolites through plant biotechnology methods. The candidate's scientific research activity is structured in two main directions:

- **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** (publications B4-4, B4-5, B4-6, Г7-3, Г7-6, Г7-9, Г7-10, Г7-12, Г7-13, Г7-14, Г7-15).

Protocols have been developed and optimized for rapid micropropagation of the endangered plant species *Arnica montana* L. and *Helichrysum arenarium* (L.) Moench, as well as economically important species such as *Stevia rebaudiana* Bertoni, *Echinacea purpurea* (L.) Moench and *Cichorium intybus* L..

By testing a large number of culture media supplemented with plant growth regulators, specific protocols have been developed to obtain prospective plant material for the needs of the pharmaceutical industry. Experiments have been made with modifying the composition of the growth regulators in solid agar media, as well as in a RITA ® bioreactor system. Of particular importance in this aspect is the development of a method for six-month maintenance of slow-growing cultures of *A. montana*, which is of special interest for the preservation and sustainable utilization of valuable highly productive lines, as well as in principle for the maintenance of an *in vitro* collection of this valuable plant species, which does not occur wild in our nature. The candidate's experimental work has also lead to specific practical developments regarding the successful micropropagation and *ex vitro* collections of valuable medicinal species established by her. In-depth studies have also been conducted regarding the potential of *in vitro* cultivated plants to accumulate secondary metabolites under various experimental conditions - regarding the production of sesquiterpene lactones in *A. montana*, as well as phenolic compounds in *E. purpurea* and *C. intybus*, as well as *S. rebaudiana*. Of particular practical importance is the high production

of target biologically active substances in *ex vitro* adapted and successfully grown plants in field conditions.

- Modulation of the production of biologically active compounds through gene transfer or application of elicitors of abiotic and biotic origin in medicinal plants of the Asteraceae family (B4-1, B4-2, B4-3, Г7-1, Г7-2, Г7-4, Г7-5, Г7-7, Г7-8, Г7-11).

In this direction, successful work has been done in terms of performing genetic transformation using *Agrobacterium rhizogenes* ATCC 15834 and working with genetically transformed "hairy roots" cultures. In this way, the production of monosaccharides and sugar alcohols, flavones, phenolic acids, organic acids, sugars and other metabolites has been successfully influenced in *A montana*.

The candidate has developed protocols for the successful elicitation of increased production of caffeoylquinic acids by treating *in vitro* cultures of *A. montana* with the biotic agent yeast extract and the abiotic agent salicylic acid. The enzymatic components of the antioxidant defense system in the species under the influence of the applied elicitors have also been studied. The stability of the expression of nine candidate reference genes after application of methyl jasmonate, yeast extract and salicylic acid for the purpose of induction of secondary metabolites of phenolic nature has also been studied.

The candidate is also conducting innovative research on micropropagation of *Stevia* through direct organogenesis using nanofibers formed from a derivative of the amino acid valine and nicotinic acid, as a carrier of the biologically active agent silver atoms/particles, as well as nanofibers obtained from silver salts of two types of derivatives of the amino acid L-asparagine with a monomeric and dimeric molecular structure.

In the research conducted and the publications of the results obtained, the candidate's personal contribution is clearly visible in terms of:

- Purposefulness in terms of planning the experimental design and bringing the experimental work to a successful result.

- Precise knowledge and application of the methods of plant biotechnology and genetic analysis.

- Analytical and critical approach in the interpretation of the obtained data and the presentation of the results in scientific publications.

- Approach and good synchronization view in the management of interdisciplinary projects with the ability to coordinate the implementation of the scientific tasks of the

working groups, which is clearly evident from the good presentation of the results in reputable scientific publications.

4. Relevance of the scientific topic and significance for science and society

The scientific topic developed by the candidate is undoubtedly of great importance in terms of the preservation of genetic plant material of valuable, rare and endangered medicinal plants, as well as species with high economic potential. On the one hand, Dr. Petkova possesses the necessary expertise in micropropagation and resource supply of selected planting material for reintroduction into nature or field cultivation, but also in the development of approaches for the controlled biotechnological production of biologically active substances under laboratory conditions.

It is of particular importance for the competition procedure to note that the scientific research was conducted mainly in the laboratory "Applied Genetics and Plant Biotechnology" at the former Institute of Genetics and in the Laboratory "Regulators of Plant Growth and Development" at the Institute of Plant Physiology and Genetics, which speaks of the role of the candidate in the scientific activity of the unit. The candidate also demonstrates skills for establishing productive scientific cooperation with other scientific institutes in Bulgaria and abroad - with the laboratory "Plant-Soil Interactions" of the Institute of Plant Physiology and Genetics-BAS, as well as other institutes of BAS and the Agriculture Academy and with the University of Natural Sciences, Lublin, Poland.

Of particular importance is Dr. Petkova's ability to raise funding for the conduct of her research activities - both as a participant and as a leader of scientific projects. This clearly outlines Dr. Petkova's scientific topic. All the prerequisites are available for its successful and sustainable implementation within the scientific Institution in which she works.

The candidate has clearly defined the immediate tasks that will guide her future work. The plans include developing various *in vitro* cultivation systems and increasing the production of target secondary metabolites by implementing new strategies to enhance the biosynthetic potential of plants, as well as testing a larger number of growth regulators and other elicitors.

Optimization of the synthesis of target secondary metabolites will be achieved through a deeper understanding of the molecular genetic, physiological and biochemical

characteristics of plants. Further studies on their biosynthetic genes of sesquiterpene lactones in the species *A. montana* are planned, analysis of elicitor-induced transcriptional changes in the genes FDS (farnesyl diphosphate synthase), GAO (germacrene-A synthase), and GAS (germacrene A oxidase), encoding key enzymes in the biosynthetic pathway of STLs, as well as other selected genes (HMGCR 3-hydroxy-3-methyl glutaryl coenzyme A reductase, IDI isopentenyl diphosphate isomerase, DXS 1-deoxy-D-xylulose 5-phosphate synthase and DXR 1-deoxy-D-xylulose 5-phosphate reductoisomerase), involved in the biosynthesis of terpene precursors, is planned. The obtained results are expected to provide valuable contributions to the development of metabolic engineering strategies for regulating the biosynthetic pathway of STLs through overexpression of biosynthetic genes in microorganisms or host plants.

It is foreseen that scientific developments will be continued by including other medicinal plants, with an emphasis on endangered and protected species of the Bulgarian flora, as well as those of economic interest. A prerequisite for achieving industrially significant yields of valuable metabolites is the expansion of the laboratory's instrumentation with the inclusion of effective cultivation systems based on the use of bioreactors.

5. Organizational and training activities

The candidate's ability to successfully conduct and coordinate scientific research, as well as her overall teamwork skills, is evident from the large number of projects in which she is a desired participant, as well as from the successful implementation of the work tasks of the projects of which she is the leader. Dr. Petkova is also the supervisor of two Diploma students from Sofia University, and has conducted practical training in *in vitro* cultivation of medicinal plants for postdoctoral student Magdalena Sozoniuk from the University of Life Sciences, Lublin, Poland under the Erasmus scholarship program.

6. Critical notes and recommendations

I have no critical remarks about the materials submitted for the competition by Dr. Maria Petkova. The author's reference for the contributions of the candidate's research activity is well structured, clearly presented and supported by the attached publications.

Regarding the methodology for obtaining genetically transformed "hairy roots" cultures, I have a question that is not directly addressed in the submitted materials. How is *A. rhizogenes* eliminated from the *in vitro* obtained lines after its inoculation for obtaining "hairy roots" cultures of *A. montata*?

7. Conclusion

The documents and materials presented by Senior Asst. Prof. Dr. Maria Petkova meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, as well as the Regulations on the Specific Conditions and Procedure for Acquiring Scientific Degrees and for Holding Academic Positions at the Institute of Plant Physiology and Genetics - BAS. The candidate in the competition has presented a significant number of scientific articles published after the materials used in the defense of her "doctor" degree. The works contain original scientific and applied contributions that have received international recognition by being published in journals published by international academic publishing houses. The theoretical developments have practical applicability.

After reviewing the materials and scientific papers presented in the competition, analyzing their significance and the scientific, applied and applied contributions contained therein, as well as my acquaintance with the overall characteristics of the candidate, I confidently give my positive assessment and recommend to the Scientific Jury to prepare a report-proposal to the Scientific Council of the Institute of Plant Physiology and Genetics - BAS - Sofia for the election of Dr. Maria Ivanova Petrova to the academic position of "Associate Professor" in the professional field 4.3. "Biological Sciences", scientific specialty "Genetics".

June 13th 2025 **review report by**: .....

Assoc. prof. Dr Kalina Danova