REVIEW

of the materials submitted for participation in a competition for the academic position of "Associate Professor" at the Institute of Plant Physiology and Genetics - BAS in the field of higher education: 4. "Natural Sciences, Mathematics and Informatics"; professional direction: 4.3. "Biological Sciences"; Scientific specialty "Genetics", announced in the State Gazette No. 16/25.02.2025 for the needs of the laboratory "Regulators of Plant Growth and Development" by Prof. Dr. Veneta Mihova Kapchina-Toteva, Faculty of Biology of Sofia University, retired, appointed as a member of the scientific jury, according to Order No. RD-01-16/22.04.2025 of the Director of the Institute of Plant Physiology and Genetics

I. Professional and career development of the candidate. For the competition "Associate Professor" only documents of Dr. Maria Ivanova Petkova, Chief Assistant Professor at the Institute of Plant Physiology and Genetics - BAS have been submitted. All documents for the competition are presented accurately and clearly according to the requirements set out in the Regulations on the terms and procedure for acquiring scientific degrees and occupying academic positions in the Institute of Plant Physiology and Genetics and fully meet the criteria for occupying the academic position of "associate professor". Maria Petkova graduated in 2001 from the Faculty of Biology of the Sofia University of St. Clement of Ohrid with a Master's degree in Biology and Chemistry, specialization Medicinal Plants. In the period 2011-2013, the candidate was a doctoral student in independent training at the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, where she defended her doctoral thesis on the topic "Biotechnological approaches for propagation, preservation and production of biomass from hairy roots of Arnica montana L" in the specialty "Genetics". Her scientific career began in 2003, holding successive positions: Research Associate III degree at the Institute of Genetics, Bulgarian Academy of Sciences (2003-2011), Chief Assistant at the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences (2011 - ongoing). Extremely important for establishing herself as a leading specialist and partner are the scientific researches, including optimization of the main phases of micropropagation; research and optimization of productivity and biosynthesis of valuable metabolites in medicinal and aromatic plants, an important part of Plant Biotechnology; specialization in 2010 under the COST action FA0804 program "Plants, producers of valuable proteins" at the VTT research center, Finland; teaching; active participation in scientific research projects and administrative engagement: reviews of scientific papers (39) in renowned international journals (regularly) such as *Plants*, *International Journal of Molecular Sciences*, *Horticulturae*, *Molecules*, *Biomolecules*, *International Journal of Plant Biology*, *Agronomy*, *Journal of Microbiology*, *Biotechnology*

and Food, International Journal of Plant Biology, Foods, proof of qualification in the field of Plant Biotechnology. Distinctive features of Dr. Petkova are her high work capacity, responsibility for the obligations undertaken, communication skills, teamwork skills. These qualities define her as a desirable partner in interdisciplinary research, and so far she has participated in 15 scientific projects with national and international funding from: FNI-10 (3 for young scientists); EBR-1; MOEW-1; Operational Program "Human Resources Development", MES – 2; International-1. Dr. Petkova's activity in the development and implementation of projects that have contributed significantly to its development and financially to the improvement of the scientific and research base is evident (leader of 2 projects, member of the scientific team in 11 and member of a target group in 2 projects). She has participated in 42 national and international forums, at which the results of scientific research have been presented with 40 poster presentations and 2 reports, of which Maria Petkova is the first co-author.

II. Teaching experience. Role of the candidate in the training of young scientific personnel. Dr. Petkova 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, 2023; she is the supervisor of two graduates (bachelor and master) from Sofia University.

III. Research and publication activity, citations. Fulfillment of the requirements for holding the academic position of "associate professor", personal contribution of the candidate. Senior Assistant Professor Dr. Maria Petkova is a co-author of 52 scientific papers published in renowned journals with IF/SJR such as: *Plants, BMC Plant Biology, Agriculturae Conspectus Scientificus, Central European Journal of Biology, Agronomy, Chemistry & Biodiversity, Acta Physiologiae Plantarum.* The publications submitted in the competition for "associate professor" and subject to review are 21 issues with a total IF - 36.511, of which 12.188 are publications for participation in the competition, in which M. Petkova is the first or corresponding author.

- Group A - 50 points (required 50 points): Dissertation on the topic "Biotechnological approaches to propagation, storage and production of biomass from hairy roots of Arnica montana L.", for obtaining the ONS "doctor", scientific specialty "Genetics".

- By indicator 4 in group B - 125 points (required 100 points): A total of 6 publications equivalent to a habilitation thesis are presented (3-Q1, 2-Q2 and 1-Q3 with SJR) with a total

JCR IF: 14.834. Number of points from publications in which M.Petkova is the first or corresponding author - 35 points with a total JCR IF- 4.0.

- By indicators in group G - 232 points (required 220 points). 15 publications outside the habilitation work with a total JCR IF are presented: 21,677 in publications referenced and indexed in world-renowned databases with IF and/or SJR (4-Q1, 3-Q2, 3-Q3, of which 1 in an edition with SJR without IF, 3-Q4, of which 2 in an edition with SJR without IF) and 2 in publications referenced by other databases without a quartile. Number of points from publications in which M. Petkova is the first or corresponding author - 110 points, total IF of these publications - 8,488. The personal participation of Dr. Maria Petkova in the indicated 21 scientific works is indisputably illustrated by the fact that in 10 of them she is the first or corresponding author.

- By indicators in group D - 348 points (required 100 points). A total of 227 citations were registered (h index: 9 by Scopus). The citations are in renowned journals with IF such as: In *Vitro Cellular & Developmental Biology-Plant, Journal of Plant Growth Regulation, Plants, Plant Cell, Tissue and Organ Culture, Acta Physiologiae Plantarum, Scientia Horticulturae, Frontiers in Plant Science* and others. Number of citations in world-renowned databases of scientific information (Web of Science and Scopus) for the last 5 years - 174 pcs.

- By indicators in group E - 173 points (70 points required). Dr. Maria Petkova has participated in 15 scientific projects: FNI-10 (3 for young scientists); EBR-1; MOEW-1; Operational Program "Human Resources Development", MES - 2; International-1. He is a member of the scientific team in 11 projects, a member of a target group in 2 projects and the leader of 2 projects: KP-06-H76/5 “Biotechnological approaches for targeted accumulation of secondary metabolites in the medicinal plant *Arnica montana* L. and study of the biosynthetic pathway of sesquiterpene lactones” (2023-ongoing); MU – B – 1409/04 “Establishment of biochemical markers for genetic identity of the medicinal plants *Gentiana lutea* L. and *Arnica montana* L. and studies on the possibilities for their in vitro propagation” (2004-2006). Dr. Petkova's activity in the development of projects, which also contributed financially (315,000 leva) to the improvement of the scientific and research base, is evident.

The total number of points from all indicators is 928, with which Dr. Maria Petkova exceeds the minimum requirements (540 points) for occupying the academic position of "associate professor" according to the Regulations of the Institute of Research and Development of the Bulgarian Academy of Sciences. My conclusion on this part of the analysis of the scientific,

pedagogical and expert activity of Senior Asst. Maria Petkova is that the procedure has been followed and the documentation has been prepared in accordance with the requirements of the Law on the Promotion of Research and Development of the Bulgarian Academy of Sciences and the Regulations for its application for occupying the academic position of "associate professor". The candidate's scientific production submitted for the competition does not contain works that go beyond the scope of the main nomenclature specialty. She participated in the competition with scientific works that fully correspond to the professional direction of the discipline in terms of volume, quality and clearly expressed personal participation. The candidate's personal contribution is related to the implementation of molecular (transformation, gene expression) and biotechnological methods. Dr. Petkova has actively participated in processing the obtained results, discussing them, and shaping the publications, which is a significant contribution of the candidate in all publications.

IV. Contributions. Chief Assistant Dr. Maria Petkova has a clearly expressed profile of a researcher, which fully corresponds to the wording of the announced competition. The result of the successful scientific profiling of Dr. Petkova in the two main areas developed by her are the significant scientific results obtained, most of which are of an original nature:

1. Development and optimization of highly effective in vitro protocols for propagation, biomass production and synthesis of biologically active substances (BAS) from medicinal plants of the Asteraceae family. (B4-4, B4-5, B4-6, G7-3, G7-6, G7-9, G7-10, G7-12, G7-13, G7-14, G7-15).

- New scientific data on the biological potential of arnica have been established, and the developed technology is ready for application in practice and creation of plantations of this endemic and endangered species for Europe (B4-4, G7-12, G7-15).

- The effectiveness of growth regulators on direct organogenesis in *Arnica montana* L. (B4-4 and G7-15) has been studied, accelerated propagation with RITA® (G7-12), 100% in vitro rooting on a nutrient medium with a concentration of reduced by half was obtained the main salts containing IBA and an increase in total phenols and antioxidant activity when reduced to a quarter of the salts (G7-10).

- Of original character (G7-9) is the higher amount of STLs found in the flowers of in vitro propagated and acclimatized in mountain conditions plants compared to in vivo plants propagated from seeds. In surface flavonoids, no qualitative differences were found between in vitro and in vivo propagated *A. montana* plants from seeds (G7-13), leaf extracts from ex vitro

and in vivo samples have a stronger antioxidant activity, measured by the DPPH method, than the corresponding flower extracts.

- Valuable for industrial application of micropropagation is the possibility of process automation, increased synthesis of sesquiterpene lactones (STLs) compared to plants from agar medium or static liquid culture (G7-12). Plants acclimatized in mountainous conditions (Beglika, Western Rhodopes) synthesize a higher amount of (STLs) than conventionally propagated ones (B4-4, G7-9). The total content of STLs is higher in the full flowering phase compared to the seed formation phase (G7-9). The origin of the seeds is also important for the accumulation of STLs in *A. montana* flowers, the most productive are those originating from Austria, followed by Germany and Ukraine (G7-9). - An optimized in vitro protocol for mass propagation of stevia has been developed, which guarantees the production of 90% ex vitro acclimatized viable plants, a source of large amounts of leaf biomass for the food and pharmaceutical industries (B4-5). In leaves of *A. montana* and *S. rebaudiana*, higher total phenols and flavonoids and higher antiradical activity (DPPH) were found in ex vitro adapted plants compared to in vitro propagated plants (B4-5, D7-13). The content of water-soluble and fat-soluble antioxidants (equivalents of ascorbate and α-tocopherol) and the amount of total phenols were higher in the leaves of in vitro propagated *S. rebaudiana* plants of Paraguay origin compared to that reported in leaf extracts of in vitro cultivated plants of USA origin (B4-5).

- In contrast to seed propagation of *Echinacea purpurea* (L.) Moench (echinacea), all in vitro propagated and acclimatized plants under field conditions are morphologically similar, with higher inflorescence yield and possess high antioxidant capacity (B4-6). 27% higher levels of antioxidant metabolites were reported in *in vitro* propagated *E. purpurea* plants compared to those propagated from seeds. The flavonoid content does not depend on the method of propagation (B4-6).

- The crucial importance of cytokinin for directing morphogenesis in in vitro cultivation of the protected species *Helichrysum arenarium* (L.) Moench (yellow smil) has been proven, without deviations in ploidy, established for the first time by determining the amount of nuclear DNA by flow cytometry (G7-6).

- The study of the influence of phenylurea cytokinin (4PU-30) on the in vitro propagation of *Cichorium intybus* L. is of original nature. No differences in the metabolic profile of in vitro and wild-growing plants have been established (G7-3).

The results of the above-presented studies are new information and complement the fundamental knowledge about growth regulators, physiological and biochemical response of in vitro cultures depending on the plant species. The influence of various factors on micropropagation, callusogenesis and genetic transformation of *A. montana* are reviewed and summarized in a review article, the potential of in vitro cultures for the production of plant material and obtaining BAV is analyzed (G7-14).

2. Modulation of the synthesis of biologically active substances by gene transfer or application of elicitors of abiotic and biotic origin in medicinal plants of the Asteraceae family (B4-1, B4-2, B4-3, G7-1, G7-2, G7-4, G7-5, G7-7, G7-8, G7-11).

- Transformed roots (hairy roots) are a promising alternative for the extraction of valuable metabolites, as they possess stable growth and biosynthetic characteristics. For the first time, the influence of different carbohydrate sources (sucrose, maltose and glucose) on the growth, development and metabolic profile of hairy roots of *A. montana*, which produce flavones, phenolic acids, organic acids, sugars and other metabolites and are suitable for large-scale cultivation in a bioreactor (G7-11), has been studied.

- The study of the influence of yeast extract on micropropagation, antioxidant potential and accumulation of caffeoylquinic acids in *A. montana* (B4-1) is of original nature. It has been proven that it stimulates the production of secondary metabolites and antioxidants in *A. montana*, and for the first time the enzymatic components of the antioxidant defense system in *A. montana* have been studied, and it has been established that treatment with yeast extract and salicylic acid increases the activity of superoxide enzymes dismutase, catalase and ascorbate peroxidase, while guaiacol peroxidase decreased its activity compared to the control (B4-1).

For the first time in *A. montana*, the stability of the expression of nine candidate reference genes after application of methyl jasmonate, yeast extract and salicylic acid was assessed using four widely used algorithms (geNorm, NormFinder, BestKeeper and ΔCt method) (B4-2). Analysis of the obtained results showed that the most stably expressed genes under elicitation conditions were a gene encoding a subunit of ATP synthase, as well as the ACT gene encoding β-actin. The obtained data provide useful information for future studies on the molecular processes regulating the biosynthesis of secondary metabolites and were obtained under a project led by Dr. Petkova.

-An innovative approach is the development of a biotechnological method for the production of multiple stevia plants in vitro by 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 (B4-3), 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 (G7-4). Treatment with nanofibers leads to stimulation of the antioxidant capacity, as well as the amount of sweet diterpene glycosides - stevioside and rebaudioside A in the leaves of *S. rebaudiana* (B4-3, G7-4), as well as mono- and dicaffeoylquinic acids (G7-4). Pretreatment with nanofibers of silver salts of aspartic acid derivatives increases the tolerance of ex vitro adapted plants to drought (G7-1) and is valuable information about the physiological mechanisms of stevia under water stress conditions.

-The independent addition of the amino acids creatine and the synthesized form creatine-lysinate affects the physiological status of in vitro plants *S. rebaudiana* and *Leontopdium alpinum* (edelweiss) by increasing the rate of multiplication, biomass accumulation and modulating the activity of antioxidant enzymes (G7-7).

- The influence of nanoparticles on plant growth and development (G7-8), as well as of various elicitors on the biosynthetic potential of S. rebaudiana (G7-5), are summarized in two literature reviews (G7-5, G7-8). The effect of abiotic and biotic elicitors on the productivity of in vitro cultures in plant species of the family. Asteraceae are reviewed and summarized in a review article (G7-2).

The research included in this direction enriches the fundamental knowledge about the physiological-biochemical and metabolic manifestations in the in vitro cultivation of mountain arnica and stevia. In addition, the created *in vitro* systems for controlled cultivation of plant material - cultures of roots "hairy roots" and those of above-ground parts "shoot culture", allow for precise monitoring of the metabolic profile of the obtained extracted plant biomass, and specific guidelines for application in practice are given. A prerequisite for achieving industrially significant yields of valuable metabolites in the future is the expansion of the laboratory's instrumentation with the inclusion of effective cultivation systems based on the use of bioreactors.

V. Personal impressions. I have known Senior Assistant Professor Dr. Maria Petkova since I was a student and I must note her positive development as a qualified, erudite and expeditious scientist, developing a modern, relevant topic with serious potential for application in practice.

CONCLUSION Based on the analysis of active scientific research, expert and pedagogical activity, volume of scientific production, interpretation of scientific data and contributions, their reflection in the international scientific literature, participation in scientific research projects,

presentation of results at international and national scientific forums, I firmly believe that Dr. Maria Petkova fully meets the requirements of the ZRASRB, PPZRASRB and the Regulations and recommended criteria for holding academic positions at the IFRG, BAS. All this gives me reason to evaluate POSITIVELY her overall activity. I would like to propose to the esteemed Scientific Jury to vote positively, and the Scientific Council of the IFRG to elect Chief Assistant Dr. Maria Petkova as an "Associate Professor" in professional field 4.3. Biological Sciences; Scientific specialty: Genetics.

Date: 23.05.2025 REVIEWER:

Sofia (Prof. Dr. Veneta Kapchina-Toteva)