REVIEW

By: Prof. Dr Reneta Toshkova, PhD, Institute of Experimental Morphology, Pathology and Anthropology with Museum–Bulgarian Academy of Sciences (IEMPAM-BAS); Member of the Scientific Jury determined by order No. RD-01-19/ 15.05.2025 by the Director of the Institute of Plant Physiology and Genetics (IFRG) – BAS

Regarding the competition for the occupation of the Academic position "Associate Professor" in the scientific specialty "Genetics" (01.06.23), in the Category of Higher Education 4. "Natural Sciences, Mathematics and Informatics, Professional field 4.3 "Biological sciences", for the needs of the laboratory "Regulators of Plant Growth and Development" at IFRG-BAS, announced in the State Gazette No. 24/21.03.2025.

The review complies with the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria (ZRASRB), the Regulations for its Implementation (PPZRASRB), and the Regulations on the Specific Conditions and Procedure for Acquiring Scientific Degrees and Holding Academic Positions (PSURPNSZAD) at the Institute of Plant Physiology and Genetics (IPG) - BAS. I declare that I have no conflict of interest with the candidate in this competition.

Materials on the procedure. The only candidate for the announced competition is Dr. Krasimira Nedyalkova Tasheva, Assistant professor in the Laboratory "Regulators of Plant Growth and Development," research direction "Plant Ecophysiology" at the Institute of Plant Physiology and Genetics - BAS.
I received the submitted documents in electronic format. They are precisely prepared and well systematized, containing all the applicable materials for the candidate. The documents are in accordance with the requirements of the Law on the Development of the Academic Staff, and fully meet all Institutional Regulations. The procedure for opening and announcing the competition has been followed.

Biographical data and career profile. Dr. Krasimira Tasheva was born in the city of Kardzhali, Republic of Bulgaria. In 2000, she graduated from the Faculty of Biology of the "St. Kliment Ohridski" University with a master's degree in "Molecular Biology" (specialization - genetics). Since 2001, she has worked at the Institute of Genetics, Bulgarian Academy of Sciences, (currently the Institute of Plant Physiology and Genetics, IFRG - BAS) as a biologist-specialist (2001-2008) and Research associate III-II degree (2008 - June 2011) and from June 2011 to the present, she has held the academic position of Assistant Professor in the Laboratory "Regulators of Plant Growth and Development" at IFRG-BAS. She has over 24 years of total work experience in the specialty. In 2011, she successfully defended her dissertation and received the educational and scientific degree "Doctor" in "Biology", in the scientific specialty "Genetics" (Code 01.06.06). Dr. Tasheva has completed 2 short-term international specializations: in 2010, at the International Centre for Genetic Engineering and Biotechnology, Trieste, Italy and in 2012, in University of Oslo, Department of Molecular Biology, Norway. In addition, she has completed 25 short national courses. All specializations have a very positive effect in her professional development. She is a member of scientific and professional organizations: the Union of Scientists in Bulgaria and the Federation of European Societies of Plant Biology (FESPB).

Description of the presented scientific publications. The total scientific production of Dr. Tasheva includes 42 titles, of which 38 are original scientific articles, 2 are review

papers and 2 are book chapters, with a total JCR IF of 60.357, a total number of citations of 374 (Sonix), 143 (Scopus) and an h-index of 6 (Scopus).

For the current competition for the academic position of Associate Professor, Dr. Tasheva participated with a PhD thesis abstract and 17 scientific publications (16 articles and 1 book chapter), in full text, which do not repeat those presented in previous procedures (PhD defense and the competition for Assistant Professor). All submitted works have been published in specialized international journals, that are refereed and indexed in the Web of Science and Scopus databases, and include authoritative scientific journals such as *Plants, Antioxidants, International Journal of Molecular Sciences, Journal of Alzheimer's Disease, Molecules, Biology, Applied Sciences*, etc. Articles could be grouped by journal quartile as follows: 9 articles with Q1, 1 with Q2, 5 with Q3, 1 with Q4, 2 publications in journals not classified by quartile but indexed with SJR, and 1 book chapter indexed in global databases. The total JCR IF of the presented articles is 39.218. All publications are co-authored, and Dr. Tasheva has clearly outlined her contributions to each paper. She significantly participates in planning, conducting, financing, systematizing, and publishing the research results. Her personal contribution is indisputable: she is listed as the first or corresponding author in 10 of the submitted publications. A list of 189 (Sonix) citations of 30 articles in the Web of Science and Scopus databases is presented after holding the position of “Assistant Professor”. In her research, Dr. Tasheva demonstrates extensive methodological experience, using classical and modern scientific approaches including biotechnological, genetic, bioinformatics, biochemical, statistical methods. The results of her research activities became available to the scientific community with 40 participations (reports and posters) at national and international conferences and congresses. Scientific achievements have been popularized with 4 public lectures and presentations.

Participation in scientific projects. Project funding is a strong point in the candidate's scientific research activity. She participates in a total of 18 scientific projects. Dr. Tasheva was the head of 2 (one current) and a participant in 7 projects financed by the National Fund for Scientific Research at the Ministry of Education and Science; 1 bilateral cooperation between BAS and India; 3 projects of BAS; 2 projects under the Operational Program "Human Resources Development", 2 projects under the COST actions program; and 1 project funded by the Ministry of Environment and Water

Teaching and expert activity. Dr. Tasheva actively participates in the training of 3 postgraduates from Institutes of the BAS (Institute of Neurobiology, IFRG, and IBEI), as well as the supervision of two graduate students (Master and Bachelor) from Sofia University. Her expert activity includes 27 anonymous peer reviews (Sonix) for international scientific journals (*Plants* - 2, *Agronomy* - 8, *Appl Sciences* -1, *Horticulturae* -13, and others.

Assessment of the compliance of the applicant's indicators with the quantitative criteria by groups of indicators. The candidate presents a certificate and evidence of meeting the minimum national requirements, as well as the specific institutional regulations of the IFRG-BAS for holding the academic position of "Associate Professor". The total number of points for each group of indicators is presented in detail. The data are summarized in the table below:

Table - Minimum required points by group of indicators in accordance with the Law on the Development of the Academic Staff in the Republic of Bulgaria and Regulations for its application in the country, BAS and IFRG-BAS.		
Indicator	Minimum points required	Number of points of the candidate, based on the evidence presented
A Indicator 1	50	**50** PhD Thesis
Б Indicator 2	-	-
B Indicators 3 and 4	100	**100**, obtained from 4 refereed publications (Scopus or WoS), Q1 (4 x 25=100)
G (Sum of indicators 5 to 10)	220	**237,** obtained from 14 refereed publications (Scopus or WoS): Q1 (5x25=125); Q2 (1x20=20); Q3 (3x15=45); Q4 (1x12=12); 2articles with SJR (2x10=20); 1 book chapter (1x15=15)
D (Sum of points in indicator 11)	100	**378,** obtained from 189 citations in foreign refereed publications (189x2=378)
E (Sum of indicators from 12 to the end)	70	**120** from participation in national (30t), international (20t) projects, management of a national (40t) project, attracted funds for projects (30t).
Minimum total number of points for indicators A, B, G, D and E	540	**887** total number of points of the candidate for indicators A, B, G, D and E.

The candidate's total number of points is 887 points, with a required minimum of 540 points. They are distributed as follows: indicator B - 100 points, obtained from 4 publications with quartiles Q1; indicator G - 237 points, obtained from 12 publications including Q1 - 5, Q2 - 1, Q3 - 3, Q4 - 1, 2 publications with SJR, and 1 book chapter; indicator D - 378 points, obtained from 189 citations in refereed (Scopus or WoS) journals; indicator E - 120 points, obtained from participation in national (30 points) and international (20 points) projects, management of a national (40 points) project, and attracted funds for projects (30 points). The analysis of the quantitative criteria shows that Dr. K. Tasheva meets the minimum requirements for each of the groups (A-E), exceeds them by indicators "G", "D", and “E”, and to the greatest extent for indicator E (citations), which is evidence of the high quality, international significance and recognition of the published scientific research. In summary, the candidate's quantitative criteria exceed the required minimum by 1.6 times and fully meet the national and institutional requirements for occupying the academic position of "Associate Professor".

Analysis of the candidate's research activities and contributions. The research activities of Dr. Krasimira Tasheva correspond to the profile of the competition. The

topic is relevant and is related to the development of innovative *in vitro* models uzing modern biotechnological, genetic, and bioinformatics methods for valuable and endangered plant species as well as their application in both fundamental and applied scientific research. The studies cover two interrelated and complementary areas: I - Biotechnological approaches for the development of *in vitro* cultures of medicinal plants for modulating the synthesis of secondary metabolites and for the protection of endangered species (B4-01, B4-02, G7-06, G7-08, G7-09, G7-10, G7-11, G7-12, G8-01) and II - Study of the effectiveness of extracts from medicinal plants as potential therapeutic agents in socially significant diseases (oncological diseases and Alzheimer's disease) (publications B4-01, B4-02, B4-03, B4-04, G7-01, G7-02, G7-03, G7-04, G7-05, G7-07).

The research has achieved original, confirmatory, and applied contributions, which are detailed in the "author's reference" attached to the competition documents. They are the result of in-depth, interdisciplinary, and innovative research by the candidate, which was performed in the Laboratory "Regulators of Plant Growth and Development", research area "Plant Ecophysiology" at the IFRG-BAS. Her work has been carried out in active collaboration with researchers from other Institutes of the Bulgarian Academy of Sciences (Institute of Neurobiology, IEMPAM, IOHCF), as well as with colleagues from the Faculty of Mathematics and Informatics at Sofia University.

The main scientific contributions of Dr. Krasimira Tasheva are related to the two main areas and can be summarized as follows:

Contributions under Direction I:

- An efficient micropropagation system was developed for the critically endangered Balkan endemic species *Sideritis scardica* (Mursal tea), to support its conservation and assess its potential for future medicinal use. The resulting *in vitro* plants were successfully adapted to the experimental field and showed higher polyphenol content and better antioxidant activity compared to plants cultivated *in situ* (B4-01).
- An effective protocol for micropropagation of *Clinopodium vulgare L.* (cat's foot) has been developed. For the first time, the phytochemical composition and antioxidant activity of extracts from individual anatomical parts of both *in vitro* cultivated and wild plants have been compared. Qualitative and quantitative differences in the phenolic composition were established: neochlorogenic acid predominated in flower extracts, rosmarinic acid in leaf extracts, and catechin is found only in cultivated plants. Aqueous flower extracts from *in vitro* cultivated plants showed the highest total polyphenol content and antioxidant activity (B4-02).
- The applied different *in vitro* and *ex vitro* cultivation systems for the endangered species *R. rosea* provide new opportunities for its conservation and for the accelerated synthesis of biologically active substances. It was found that three-year-old *in vitro* propagated plants, cultivated in mountain experimental areas produce, salidroside and rosavin (main components of the phenolic complex) in amounts exceeding the minimum pharmacopoeial standard (G7-06). No phenotypic differences were found in the regenerants cultivated *ex vitro* in an adaptation room, in a greenhouse and in the mountain. Cytological analysis shows a diploid chromosome set (2n = 22) of the *in vitro* produced plants in all examined samples and confirms the cytological identity with the wild type (G7-12).
- Another biotechnological approach involves the development of callus cultures as producers of secondary metabolites. The optimal combinations and concentrations of phytoregulators for successful callus culture induction and long-term cultivation were determined. A positive correlation was established between the composition of growth regulators in the culture medium and the effectiveness of callus induction and growth, suitable for long-term cultivation, biomass accumulation and synthesis of valuable secondary metabolites (G7-09). The influence of culture medium composition on the antioxidant activity in callus cultures was also proven. A good correlation was found

between total phenols, flavonoids, and antiradical activity. No relationship was established between the color and structure of the callus and the amounts of secondary metabolites (G7-10).
- Biotechnologcal methods were successfully combined with bioinformatics data analysis to determine the conditions for more efficient accumulation of biomass and secondary metabolite production in callus cultures. The analysis revealed a relationship between callus color and antioxidant production, which was not established in the biological experiment. The developed mathematical model provides new data on the role of phytohormones in the nutrient medium, contributing to the successful callus culture production (G7-12).
- A review on the role of biotechnology in stimulating the synthesis of valuable secondary metabolites in the *Rhodiola rosea* species, as well as the possibilities for its conservation is provided. The review also incorporates the author's own experimental findings (G7-11).
- A detailed discussion of biotechnological aspects in the study of endangered medicinal species of the genera *Rhodiola*, *Gentiana*, and *Leucojum* are presented. The techniques used for *in vitro* cultivation, the development of micropropagation systems, and the establishment of callus cultures are described in detail. Additionally, the possibilities for the application of metabolic engineering and biotransformation in these species are discussed (the chapter from the book (G8-01).

In direction II, 2 groups of contributions are highlighted:

In group II.A, **the contributions** are outlined using an assessment of the potential of medicinal plants extracts for therapy of oncological diseases. The anticancer effect of extracts obtained from *in vitro* micropropagated and cultivated medicinal plants *Clinopodium vulgare* (cat's foot), *Sideritis scardica* (mursal tea) and *Salvia aethiopis* (Mediterranean sage) was studied on permanent human tumor cell lines. A concentration- and time-dependent decrease in cell viability and proliferation of tumor cells was established (B1-01, B4-02, B4-04, G1-01).
- It was found that extracts from *in vitro* propagated and cultivated plants of the species *S. scardica* showed stronger antitumor activity in HeLa (cervical adenocarcinoma), HT-29 (colorectal adenocarcinoma), and MCF-7 (breast cancer), than those from the wild type. A significant, concentration-dependent cytotoxic effect on the HeLa cell line was reported. Samples from the plants cultured *ex vitro*, *in vitro*, and *in situ* most strongly suppressed the viability of MCF 7 tumor cells. In contrast, the control non-tumor cell line BALB/3T3 was substantially less affected, indicating selective cytotoxicity (B4-01).
- Aqueous extracts from different anatomical parts (flower, leaf, stem) of cultivated and wild *Clinopodium vulgare* plants showed significant *in vitro* antitumor activity against human tumor cell lines HeLa (cervical carcinoma), HT-29 (colorectal adenocarcinoma), and MCF 7 (breast carcinoma). It was found that leaf and flower extracts from *in vitro* cultivated plants exhibited the highest antiproliferative activity against cancer cell lines, combined with the lowest toxicity in the non-tumor human cells HaCaT (normal keratinocytes). Fluorescence microscopy, after fluorochrome labeling, revealed that flower extracts from *in vitro* cultivated *C. vulgare* induced considerable alterations in the cellular and nuclear morphology of HT-29 tumor cells (B4-02).
- The antitumor efficacy of total aqueous extracts from the aerial part of wild and *in vitro* cultivated *C. vulgare* plants was evaluated against a panel of human tumor cells: HeLa, Hep-G2, HT-29, MCF-7 with non-tumor controls BALB/3T3 mouse fibroblasts and HaCaT normal human keratinocytes. It was observed that the extracts exhibited a selective anticancer effect and significantly inhibited the proliferation and migration of cancer cells. The antitumor activity of the extracts from *in vitro* cultivated plants is similar to and even exceeds that of the wild plants. Fluorescence microscopy and flow cytometry analyses revealed that the observed antitumor effects of the *in vitro* plant extract are associated with both antiproliferative and proapoptotic mechanisms (B4-04).

- The phytochemical composition, antioxidant activity, and anticancer potential of a total aqueous extract of *in vitro* cultured *S. aethiopis* plants were evaluated. The extract showed a high content of total polyphenols and flavonoids and antioxidant activity. Using liquid chromatography-high-resolution mass spectrometry (LC-HRMS), 21 bioactive compounds were identified, including salvianic acid C, rosmarinic acid, and salvianolic acid K. Twelve (12) of these compounds were detected in the species for the first time. The extract exhibited low toxicity toward non-cancerous BALB/3T3 mouse fibroblasts, while showing selective cytotoxicity against hepatocellular carcinoma (HepG2), lung carcinoma (A549), and prostate cancer (PC-3) cell lines. Significant inhibition of cancer cell proliferation, suppression of migration, and G2/M cell cycle arrest were observed. The Flow cytometry analysis revealed a marked increase in apoptotic and necrotic cell populations after application of an extract of cultured *S. aethiopis* (G7-01).

The newly obtained data on the antitumor effects of extracts from *in vitro* micropropagated and cultivated medicinal plants identify the species *Clinopodium vulgare*, *Sideritis scardica* and *Salvia aethiopis* as promising sources of raw rich material in bioactive compounds with pronounced antioxidant and antitumor activity. These findings support further research into their potential therapeutic applications.

In group II.B, the contributions are related to the study of the neuroprotective effects of extracts from *in vitro* propagated and cultivated medicinal plants *Marrubium vulgare* (honeydew), *Clinopodium vulgare* (cat's foot) and Sideritis scardica (mursal tea) in an *in vivo* rodent experimental model of Alzheimer's disease (B4-03, Г7-02, Г7-03, Г7-04, Г7-05, Г7-07).

- It has been found that *M. vulgare* extract, standardized for marrubiin content, demonstrated a neuroprotective effect on memory and recognition abilities in both healthy and demented rats. The extract significantly alleviated scopolamine-induced memory impairment, showing a protective effect on cholinergic mediation in the hippocampus, increasing noradrenaline (NA) levels in the brain, restoring impaired expression of pCREB (cAMP response element-binding) in the cerebral cortex and reducing oxidative stress. In healthy animals, the extract upregulated the expression of memory and apoptosis-related genes (BDNF - Brain Derived Neurotrophic Factor, CREB - cAMP response element binding protein and Bcl2 - B-cell lymphoma 2) (B4-03). The aqueous extract of *M. vulgare* also exhibited neuromodulatory properties that may influence short-term memory functions. In rats with scopolamine-induced dementia, treatment with the extract led to significant improvements in working memory, decreased acetylcholinesterase (AChE) activity in the hippocampus, reduced oxidative stress (particularly in the cerebral cortex), and an enhanced in *in vivo* antioxidant potential (G7-03).
- It was established that extracts from *in vitro* obtained and cultivated plants *S. scardica* and *C. vulgare* administered alone or in combination, effectively reduced memory impairment in the scopolamine-induced roden model by improving recognition memory and maintaining monoaminergic function. *S. scardica* extract favorably affected the preservation of spatial working memory, while *C. vulgare* extract showed a better effect on recognition memory, by exhibiting strong antioxidant activity and inhibiting acetylcholinesterase. The extracts effectively counteracted the scopolamine-induced decrease in the levels of p-CREB/BDNF proteins (transcription and neurotrophic factor) in the cerebral cortex and hippocampus (G7-02).
- In healthy animals, the extract of *C. vulgare* and the combination of *S. scardica* and *C. vulgare* extracts stimulated dopaminergic signaling in the cortex. It was found that a single administration of an extract of cultivated *S. scardica* plants reduced the degree of memory impairment and anxiety-like behavior. The extract did not affect acetylcholinesterase activity, but it exhibited a normalizing effect on the reduced brain levels of noradrenaline (NA) and serotonin (Sero), and demonstrated moderate

antioxidant activity. In healthy mice, the aqueous extract of *S. scardica* showed neither suppression of acetylcholinesterase activity nor anxiolytic activity (G7-04).
- A novel multitarget combination of plant extracts from olive tree (*Olea europaea*) leaves and green tea (*Camellia sinensis*) has demonstrated a positive effect as an adjuvant therapy in an experimental model of Alzheimer's disease. The combination most effectively affects short-term, long-term, and spatial memory, which was associated with decreased acetylcholinesterase and lipid peroxidase activity, increased activity of superoxide dismutase in the cerebral cortex, increased activity of catalase and glutathione peroxidase, and increased levels of neurotrophic (BDNF) and transcriptional (pCREB) factors in the hippocampus. No histopathological changes or abnormalities in blood parameters were observed, indicating that this multitarget phytotherapeutic combination is both effective and safe candidate for potential use in Alzheimer's disease (G7-05).

New data have demonstrated the neuroprotective effects of extracts from *in vitro* propagated and cultivated plants *Marrubium vulgare*, *Clinopodium vulgare* and *Sideritis scardica* in an *in vivo* experimental model of scopolamine-induced Alzheimer's-type dementia in rodents (rats and mice). Aadministration of these extracts effectively ameliorated scopolamine-induced memory impairment, highlighting their potential as therapeutic agents for cognitive impairments associated with neurodegenerative diseases.
- Another significant contribution involves the use of plant extracts for wound treatment. It was found that application of plant extracts from *Plantago major (*broad-leaved plantain) and *Calendula officinalis* (marigold), alone and/or in combination with mucus from *Helix aspersa*, leads to more effective wound healing in an experimental rodent model. The best healing effect was observed with the combined extract treatment, suggesting a synergistic interaction among the three components in the experimental therapy (G7-07).

Personal impressions about the candidate. Dr. Tasheva is a hardworking and purposeful scientist with extensive expertise in plant genetics, cell biology, and biotechnology. She has strong theoretical and methodological knowledge, complemented by excellent organizational skills that facilitate effective teamwork and efficient project management.

Critical comments and recommendations. I don't have any critical notes. My recommendations fully coincide with the comprehensive information provided by Dr. Tasheva regarding the prospects for future scientific research. The recommendations include expanding and deepening research in the fields of biotechnology, plant genetics and physiology, as well as further exploring the biological effects of plant extracts, specifically their antitumor, oxidative and neuroprotective effects. Additionally, there should be an emphasis on expanding existing collaborations and establishing new contacts with working groups at the Bulgarian Academy of Sciences, in the country and abroad. I believe that Dr. Tasheva's academic growth will open up new horizons for her and will allow her to share knowledge and experience, and to unleash her full potential as an erudite researcher and lecturer, and contribute significantly to the training of doctoral students and the further development of plant biotechnology and genetics at the Institute.

Conclusion

The scientific research activity of Assistant Professor Dr. Krasimira Tasheva is entirely in the field of the announced competition. The analysis of the submitted materials shows that Dr. Krasimira Tasheva is a well-established scientist with in-depth knowledge and methodological skills in the field of plant genetics and biotechnology and a competent partner in joint scientific research projects. (Dr. Krasimira Tasheva is a reputable researcher with extensive expertise and methodological proficiency in the fields of plant genetics and biotechnology). The scientific output of the candidate has high

scientometric indicators—The research findings have been published in renowned international journals with impact factor and impact rank, and they have been cited many times, which proves the international authority and recognition (demonstrating the candidate's international authority and reputation). Original results have been obtained with an indisputable fundamental, methodological, and scientific-applied contribution character of importance to genetics, biotechnology, biochemistry, plant physiology, and ecology. The candidate's scientometric indicators fully cover all quantitative and qualitative criteria for awarding the scientific title of "associate professor" in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for the conditions and procedures for acquiring scientific degrees and holding academic positions at the Bulgarian Academy of Sciences and IFRG-BAS.

Based on the above, I give a positive and high assessment of the overall scientific and research activity of the candidate. I vote "Yes" and confidently suggest to the members of the Scientific Jury and the members of the honorable Scientific Council of the IFRG-BAS to choose (elect) Assistant Professor, Dr. Krasimira Tasheva in the academic position of "Associate Professor", in the Scientific specialty "Genetics" (01.06.23), in the Higher Education category 4. "Natural Sciences, Mathematics and Informatics", Professional field 4.3. "Biological Sciences" for the needs of the Laboratory "Regulators of Plant Growth and Development" at the Institute of Plant Physiology and Genetics (IFRG) - BAS.

30.06.2025г. Sign:
Sofia Prof. D-r Reneta Toshkova, PhD