REVIEW

on the competition for "Associate professor" in the field of higher education: 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences (Physiology of plants - symbiotic relationships in plants) in the BF of SU "St. Kliment Ohridski", announced in SG no. 32 of 04/09/2024

by Prof. Dr. Veneta Mihova Kapchina-Toteva, appointed as a member of the scientific jury, according to Order No. RD 38-304/10.06.2024 of the Rector of SU "St. Cl. Ohridski".

I. Professional and career development of the candidate.

For the "Associate professor" competition, documents have been received by Dr. Marieta Georgieva Hristozkova, assistant professor in the Department of Plant Physiology, BF at SU "St. Cl. Ohridski".All documents for the competition are submitted according to the requirements set out in the Regulations for the Terms and Conditions for the Acquisition of Scientific Degrees and the Occupancy of Academic Positions at SU. Marieta Hriztozkova graduated from the Science and Mathematics High School in the city of Vratsa in 1997 and from the Faculty of Biology at the University of St. Kliment Ohridski" in 2002 as a MSc in "Molecular Biology" with a specialization in Plant Physiology. In the period 2004-2007, the candidate was a doctoral student in the Mineral Nutrition and Water Regime section at the Institute of Plant Physiology and genetics, BAS. In 2007, he defended his doctoral thesis on the topic "Influence of molybdenum deficiency on nitrogen uptake in nitrogen-fixing plants, peas and alfalfa.Investigation of the general stress response in Sinorhizobium meliloti under conditions of nitrogen and carbon starvation" and received the scientific and educational degree "doctor". His scientific career began in October 2002, successively holding the positions of: specialist-biologist (2002-2004), assistant professor (2008-2018) in the mineral nutrition and water regime section at the Institute of Plant Physiology and genetics, BAS., from 2018 and until now he is the assistant professor in the Department of Plant Physiology at the BF of SU. Extremely important for the establishment and as a leading specialist and partner in scientific research in the field of mineral nutrition as an important part of Plant Physiology (studying the interaction and influence of Arbuscular mycorrhizae fungi on plant nutrient uptake and stress tolerance in the formation of symbiotic associations with most agricultural crops) are the specializations at renowned Universities (Georg August University, Göttingen, Germany; Laboratory of Plant-Microbial Interactions (LIPM) at UMR INRA /CNRS, Toulouse, France); teaching activity, including high classroom (403 hours) and total (640 hours) average employment for the last 5 years, active participation in research projects and administrative involvement: Technical editor in the journal "Genetics and Plant Physiology" (2012-2015); Guest editor of refereed journals: Agriculture IF=3.6 (MDPI), Special Issue "Arbuscular Mycorrhiza in Cropping Systems"(2024), Microorganisms IF=4.5 (MDPI) Special Issue "Research on Mycorrhizal Fungi" (2023-2024); Special Issue "Arbuscular Mycorrhiza and Its Influence on Crop Production" (2022-2023); Reviewer of scientific works for publication in international publications (regular).

II. Teaching experience.

All lectures, exercises and teaching practices developed or delivered by Dr. Marieta Hristozkova are in the field of Plant Physiology, her scientific research work with students in the bachelor's and master's graduation program is also active:

1. A new course of lectures (45 hours) on Plant Physiology has been developed for full-time study of bachelors from the BMUR specialty, full-time study, which are derived from ch. assistant professor M. Hristozkova during the last five academic years;
2. A new elective course (30 hours of lectures and 15 hours of exercises) "Superfoods of plant origin" has been developed for full-time study of bachelors from the BAE, BH, GB and MB majors, which is regularly chosen by students in the last two years after its admission .
3. Electronic learning resources on Plant Physiology have been developed for full-time and part-time study (for special BMUR, Biology, EOOS, BA, BH, BT, MB) on the Moodle university platform.
4. Assistant professor Hristozkova led exercises in Plant Physiology for all Bachelor's degree programs full-time and part-time; Head of summer study practice of MB students, two new developed practical classes have been introduced: “Comparing the antioxidant potential of pathogen-infected and uninfected
tree species inhabiting the urban environment" and "Physiology of plants in an urban environment. Comparison of pigment composition in leaves of park plants”.
5. Supervisor of 6 graduates who successfully defended their diplomas (3 of which in the July 2024 session); of 17 coursework and 5 courses from the MB specialty.
6. Participates in candidate student campaigns; in events for the promotion of science and presentation of the Plant Physiology Department (BioFest 2019, 2023; International "Fascination of Plants Day" 2019, 2022); Reviewer of diploma theses (5 for Bachelor degree and 5 for Master degree); Secretary of Scientific and Educational Activities, Secretary of the Plant Physiology Master's Program.

III. Scientific research and publication activity, citations.

In the competition for "Associate professor" Dr. Marieta Hristozkova participated with a total output of 35 papers, most of which were published in renowned journals with IF/SJR such as: Agriculture, Current Applied Science and Technology, Journal of Herbs, Spices & Medicinal Plants, Gesunde Pflanzen, Symbiosis, Medicinal Plants - International Journal of Phytomedicines and Related Industries, Applied Soil Ecology, Communications In Soil Science and Plant Analysis, Journal of Plant Protection Research, Turkish Journal of Biology, Journal of Experimental Botany, Plant Growth and Health Promoting Bacteria, Microbiology Monographs, Journal of Plant Nutrition, Biotechnology & Biotechnological Equipment, Acta Biologica Hungarica, International journal of agricultural&Biology, Ecology and Future.
The scientific works related to the doctoral dissertation (4, 7, 28, 30, 31, 34 and 35) are not subject to review. Publications presented in the competition for "Associate professor" and subject to review are 28 total IF-25,25 and SJR-2,072, of which:
- Group C: Publications in refereed and indexed editions in world-famous databases - 5 issues, all with IF - 115 points;
- Group D: Publications in scientific publications, referenced and indexed in the world-famous databases with scientific information - 18 items, of which 10 with IF and 8 with SJR - 264 points;
- Group D: Publications in non-refereed journals with scientific review or published in edited collective volumes - 4 issues, with 24 citations in Scopus;
- Chapter of a book – 1 issue;

The personal participation of Dr. Marieta Hristozkova in the mentioned 28 scientific works is illustrated undeniably by the fact that in 17 of them (60%) she is the first or second author, and in the rest she is the third or subsequent author.

- Group D: 393, and currently 554 citations (h index: 9 according to Scopus) of the scientific works were registered (542 points). Citations are in renowned IF journals such as: Plants, Gesunde Pflanzen, Environmental and Experimental Botany, Plant and Soil, Journal of Agricultural Science and Technology, Research, Society and Development, Journal of environmental management, Ecotoxicology and Environmental Safety, etc.
- Group E: Research is supported by the participation of Dr. Hristozkova in the development of three international and 5 projects financed by national organizations (120 points); Contract for scientific and applied research with the participation of companies (Trakia Synergy AD, Smartorganik); Participation in scientific networks: Program COST Action CA 19116 "Trace metal metabolism in plants - PLANTMETALS" (2020-2024) and Program COST Action CA 22142 "Beneficial root-associated microorganisms for sustainable agriculture (ROOT-BENEFIT)" (2023-2027) .The activity of Dr. Marieta Hristozkova in the development and implementation of projects that contributed significantly both to her development and financially to the improvement of the educational and research base is obvious.

Based on the regulated national requirements, which must be met by candidates for the academic position of "Associate professor" (400 points) and the analysis made, it is established that Dr. Marieta Hristozkova exceeds (1091 points) the minimum requirements, which is very a good certificate for her intensive scientific, research and organizational activity.

My conclusion on this part of the analysis of the pedagogical and scientific activity of the assistant principal. Marieta Hristozkova is, that the procedure has been followed and the documentation has been prepared according to the requirements of ZRASRB and the rules for its application for occupying the academic position of "associate professor". In the candidate's scientific production presented for the competition, there are no works that go beyond the main nomenclature specialty. She participated in the competition with scientific papers that fully corresponded to the professional direction of the discipline in terms of volume and quality.

IV. Contributions.

Assistant professor Dr. Marieta Hristozkova has a clear profile of a researcher that fully meets the wording of the announced competition. In recent years, rhizosphere microorganisms,such as mycorrhizal fungi and nitrogen-fixing bacteria, have gained great importance and applicability in organic farming. These microorganisms are a potential means of achieving sustainable agriculture. Multidisciplinary approaches are applied to elucidate the mechanisms of their adaptation in the rhizosphere, as well as the successful colonization of the root system. These are physiological approaches related to biological fertilization that induce intensive growth, resistance to diseases and troubles, and high productivity. The defining characteristic of the research activity is the undisputed relevance of the scientific areas in which she worked:Beneficial plant-microbial relationships in plants; Abiotic stress factors and influence of mycorrhizal fungi and nitrogen-fixing microorganisms on plant development; Quality and antioxidant activity of in vitro propagated, seed or wild medicinal and aromatic plants. In this regard, the publications participating in the competition are focused on studying the influence of mycorrhizal fungi on the development of exotic, medicinal and agricultural plants under different environmental conditions. The result of the successful scientific profiling of Dr.

Hristozkova are the obtained significant scientific results, most of which are of an original nature:

1.Beneficial plant-microbe relationships in plants:

● Cultivation of Physalis peruviana L. under drought conditions increases tolerance, which is expressed in the absence of visible symptoms and a significant reduction in the plant biome, mycorrhizal plants are more competitive and adaptable to drought stress compared to non-mycorrhizal ones . Adapted in vitro propagated Physalis peruviana L. plants show higher drought tolerance than seed plants. Mycorrhizal status was better in the roots of micropropagated plants inoculated with Claroideoglomus claroideum than in vivo propagated by seeds. Growth parameters increase (biomass of aerial parts and roots, number of fruits),content of plastid pigments, antioxidant activity and a weaker increase in the levels of oxidative markers in conditions of water deficit [2].

● The combination of arbuscular mycorrhizal fungi (AMG) and microalgae stimulates the development, the concentration of secondary metabolites (flavonols and anthocyanins), the nitrogen index and the activity of enzymes metabolizing nitrogen and carbon (glutamate synthase, aspartate aminotransferase and NADP-malic enzyme). Plants in symbiosis with AMG and green algae show the highest antioxidant potential, expressed by increasing the concentration of phenols, flavonoids, ascorbate, as well as the total esterase activity and that of superoxide dismutase (SOD) enzymesS-transferase (GST) and glutathione reductase [8, 12].

● A successful adaptation (survival rate 95%) of in vitro propagated garden thyme (Thymus vulgaris L.) was achieved as inoculation with arbuscular mycorrhizal fungi (Claroideoglomus claroideum, ref. EEZ 54) increased the antioxidant capacity by accumulating phenolic compounds (total phenols and flavonoids) and stimulating the activity of the enzymes superoxide dismutase (SOD) and guaiacol peroxidase (GPO) [9].

● The effect of LED light on plant development and mycorrhizal symbiosis was studied in Solanum lycopersicum L. Under the influence of white light, mycorrhizal fungi stimulate soil fertility, the accumulation of plant biomass and flavonoids in leaves, and in RB light (red 66% and blue 33 % lights) aboveground biomass increases and gas exchange parameters are positively affected in the inoculated variants. As a result of RG lighting (red 66% and green 33% light), it was proven to increase the urease activity of the soil, stimulate the development of mycorrhizal symbiosis and the nitrogen content in the leaves, in parallel with increasing the activity of nitrate reductase [11].

● Optimum conditions for growing papuda Vigna unguiculata (L.) Walp have been established. by varying the water content, the presence of symbiotic bacteria in the roots and the type of symbiotic association. The optimum soil moisture favorable for the formation of active symbiotrophic associations in papuda roots is about 60% water holding capacity (WHC). Under this condition, both Bradyrhizobium strains and AM fungi function successfully in terms of successful mycorrhization, the formation of tubers and nitrogen fixation, nitrogen and phosphorus uptake, and plant biomass production. At reduced water content, the symbiotic association between Br. japonicum-273 and Gl. intraradices камерата ви
was more successful [13].

● The application of synthetic nitrogen fertilizers, a regular practice in agriculture alone and in combination with mycorrhizal fungi in lettuce grown on different cultivars as a spring crop in

greenhouse conditions, is cultivar dependent, affecting the yield and quality of the produce [16].

In a large number of crops, the positive role of mycorrhizal fungi has been established for restoring and improving the quality of damaged soils, reducing the absorption of heavy metals, leading to a higher tolerance to this type of stress and accumulation of useful bioactive compounds depending on the strain used:

- The testing of four mycorrhizal isolates (Claroideoglomus claroideum (Cc1), Rhizophagus clarum, Claroideoglomus claroideum (Cc2)Funneliformis mosseae) from different habitats in symbiosis with Origanum majorana L. under conditions of heavy metal (Cd and Pb) pollution, showed the highest biomass of aerial parts correlated with the percentage of mycorrhization, relative mycorrhizal dependency, glomalin concentration and activity of acid phosphatase when inoculated with strains from natural metal-bearing deposits (C. claroideum (Cc2) and Funneliformis mosseae) [14].
- Mycorrhizal symbiosis helps to adapt Physalis peruviana to soil contaminated with heavy metals (Cd, Pb), having a positive effect on the number and quality of fruits, expressed in a significant reduction in the content of heavy metals and changing the composition and concentration of fatty acids in favor of unsaturated ones [15].
- Mycorrhizal fungi change the carotenoid profile of marigold flowers (Calendula officinalis L.) grown on heavy metal-contaminated soil and protect plants from their accumulation in usable parts, stimulating antioxidant capacity through the accumulation of important secondary metabolites (total phenolics, flavonoids, carotenoids). [17].
- An increase in antioxidant properties, changes in the composition of the essential oil of Origanum majorana L., and increased phenolic compounds were found in conditions of heavy metal pollution in the mycorrhizal symbiosis [20].

● The results for increased dry plant biomass, high activity of ascorbate peroxidase and superoxide dismutase and reduced level of antioxidant metabolites (ascorbate and reduced glutathione) after inoculation of Sideritis scardica Griseb are confirmatory. with Glomus intraradices [24]

1.1. Symbiotic nitrogen fixation in legumes:

Another aspect is the study of symbiotic nitrogen fixation, which is an important ecological process important for supplying nitrogen in a form available for uptake by plants. The formation and functioning of nitrogen-fixing symbiotic systems are particularly sensitive to changes in the environment. One of the unfavorable factors is related to the insufficient or increased level of mineral elements, both in free-living and in symbiotic forms of organisms (bacteria or plants). Under the influence of this type of stress, a certain genetic program is unlocked in free-living bacteria.At a later stage, this leads to changes in the establishment of symbiotic relationships and in nitrogen-fixing activity. This section outlines the following original contributions:

● Increased nitrogen fixation, higher concentration of amino acids and organic acids in alfalfa tubers under elevated CO2 concentration [21].

● Feeding with nitrogen foliar fertilizer Agroleaf® (0.3%) in peas and alfalfa leads to overcoming the negative impact of Mo starvation in terms of the efficiency of nitrogen fixation and nitrogen assimilation, as well as the accumulation of plant biomass.Foliar feeding in pea

in the absence of Mo has been shown to be more efficient in terms of nitrogen uptake than these processes in alfalfa. [22, 29, 33].

The greater sensitivity of alfalfa to Mo deficiency compared to pea plants has been demonstrated, which is determined by the significant decrease in Mo content in plant tissues, reduction of nitrogen-fixing activity and accumulation of the stress-induced amino acids alanine, γ-aminobutyric acid, threonine , proline and serine [22, 25].

● Class-wise comprehensive profiling of genes in Sinorhizobium meliloti affected by carbon and nitrogen starvation revealed the identification of 58 common overexpressed genes successfully involved in the general stress response. Wild type S. meliloti 1021 and two mutants - S. meliloti NitR and S. meliloti TspO - were studied. In conditions of limited nutrients in the environment, the most effective symbiotic system between alfalfa and Sinorhizobium meliloti TspO was found in terms of nitrogen-fixing capacity and plant biomass accumulation. [27]

● The lack of trace elements Mo and Cu in peas reduces the activity of enzymes involved in the initial stages of nitrate assimilation (nitrate reductase and glutamine synthetase), fresh weight and content of plastid pigments (total chlorophyll and carotenoids). Nitrate accumulation in plant tissues is enhanced, especially in Cu-free variants [32].

2. Influence of abiotic stress conditions on plant development:

● Sage (Salvia officinalis L.) grown on soil contaminated with heavy metals accumulates cadmium, lead and zinc, which leads to inhibition of plant biomass, but the yield and quality of the essential oil do not deteriorate as the neutralization of H2O2 is rather non-enzymatic , than an enzymatic process [23].

3. Methods for improving the quality and evaluation of antioxidant activity of medicinal and aromatic plants. Investigation of the antioxidant activity of in vitro propagated, compared to seed-grown or wild medicinal and aromatic plants.

● Adapted plants after in vitro propagation demonstrate higher drought resistance than plants grown from seeds. Growth parameters (biomass, fruit number), content of plastid pigments, antioxidant activity and levels of oxidative markers are increased [2].

● Variations were found in the content of metabolites with antioxidant potential in Origanum heracleoticum L., collected from different habitats in Bulgaria (two locations in the Kresna Gorge and two locations in the Rhodope Mountains). The obtained results showed differences in the content of metabolites with antioxidant potential in Greek oregano collected from the two locations from each of the two regions of Bulgaria. Higher contents of phenols and flavonoids were found in micropropagated plants as well as in wild plants, collected from two localities from the Kresna Gorge, compared to the wild types collected from the Eastern Rhodopes. Forty-five compounds were identified in the essential oil of O. heracleoticum collected from local populations and determined to belong to the carvacrol chemotype based on the composition of the essential oil and especially the content of carvacrol and thymol [3].

● An optimal protocol for micropropagation of Greek oregano (Origanum heracleoticum L.) with high adaptation and antioxidant potential, higher number of branches with zeatin addition and efficient rooting with indole-3-butyric acid was developed [5].

● The highest content of non-enzymatic low-molecular metabolites with antioxidant capacity, phenols and flavonoids in the flowers and leaves of in vitro cultivated Hyssopus officinalis compared to the methanolic extracts of plants propagated from seeds and from natural habitats

was demonstrated. However, the highest concentration of essential oil was noted in plants from natural habitats [6].

● An efficient and inexpensive protocol for large-scale propagation of garden thyme (Thymus vulgaris L.) has been developed. The maximum number of branches and roots were obtained when cultured on ½ MS medium supplemented with 0.1 mg L-1 IBA after 4 weeks of culture [9].

● An effective method for micropropagation of hyssop (Hyssopus officinalis L.) and successful ex vitro adaptation with 90% survival and influencing the antioxidant defense system of plants (superoxide dismutase, catalase, ascorbate peroxidase and guaiacol peroxidase) has been established [10].The antioxidant capacity of extracts from leaves and flowers of wild Sideritis scardica originating from Alibotush Mountain (Slavyanka Square, Southwestern Bulgaria) is much higher than samples collected from cultivated plants and more northern habitats. [18].

V. Personal impressions.

I know Assistant professor Dr. Marieta Hristozkova from a student and I must note her development as a qualified, erudite and expeditious scientist, developing a modern, topical subject with serious potential for application in agriculture.

CONCLUSION

Based on the analysis of the pedagogical work (high classroom occupancy in Bachelor and Master degree), active research activity, expert activity, volume of scientific production, interpretation of scientific data and contributions, their reflection in international scientific literature, participation in scientific research projects, presentation of the results at international and national scientific forums, I am convinced that Dr. Marieta Hristozkova fully meets the requirements of the ZRASRB, PPZRASRB and the Regulations and the recommended criteria for occupying academic positions at the Sofia University "St. Kliment Ohridski".All this gives me reason to positively evaluate the overall activity. I allow myself to propose to the honorable Scientific Jury to vote positively, and the Faculty Council of the Faculty of Biology at Sofia University "St. Kliment Ohridski" to elect chief assistant Dr. Marieta Hristozkova as "associate professor" in professional direction 4.3. Biological Sciences (Plant Physiology-Symbiotic Relationships in Plants).

Date: 22.07.2024 REVIEWER:

Sofia (Prof. Dr. Veneta Kapchina-Toteva)