#### SCIENTIFIC STATEMENT

**From:** Prof. Katya Marinova Georgieva, PhD, Laboratory "Photosynthesis – Activity and Regulation", Institute of Plant Physiology and Genetics – Bulgarian Academy of Sciences

**Regarding:** competition for the academic position "Associate Professor" in a professional field 4.3. Biological Sciences, scientific specialty "Plant Physiology", announced in SG Nº 32/09.04.2024, for the Department "Plant Physiology", Biological Faculty of Sofia University "St. Kliment Ohridski"

Assistant Professor Dr. Marieta Hristozkova is the only candidate for the announced competition. The presented documents for participation in the procedure are prepared according to the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria and are in line with the Regulations for acquiring scientific degrees and holding academic positions at Sofia University.

#### **Scientific career**

Marieta Hristozkova completed her higher education in 2002 at the Faculty of Biology of Sofia University "St. Kliment Ohridski" and graduated with a master's degree in "Molecular Biology", specialization in "Plant Physiology". In the same year, she was appointed as a specialist-biologist at the Institute of Plant Physiology "Acad. M. Popov" (currently Institute of Plant Physiology and Genetics). In the period 2004–2007 she developed and successfully defended a dissertation on the topic "Effect of molybdenum deficiency on nitrogen uptake in nitrogen-fixing plants pea and alfalfa. Investigation of the general stress response in *Sinorhizobium meliloti* under conditions of nitrogen and carbon starvation". After obtaining her Ph.D., Marieta Hristozkova continued her work at the Institute of Plant Physiology in the Laboratory "Plant-Soil Interactions" as an Assistant Professor. After 2018, she continued her scientific career in the Department of "Plant Physiology" at the Biological Faculty of Sofia University St. Kliment Ohridski, where she worked until now as an Assistant Professor.

In the course of her professional development, Dr. Hristozkova gained considerable experience, carrying out two specializations abroad. In 2005, she was a specialist in the SIGNAL program of the Marie Curie Foundation (the 6th framework program of the European Union) in the Laboratory of Plant and Microbial Relationships at the UMR INRA/CNRS, Toulouse, France for a period of 6 months. From June to September 2008, she conducted research at the Georg August University, Plant Nutrition Section, Göttingen, Germany under the DAAD-BG project at the Scientific Research Fund on the processes of CO<sub>2</sub> fixation in tubers of leguminous plants during their adaptation to low phosphorus concentrations in the medium.

#### Scientific publications and general research metrics

Assist. Prof. Dr. Marieta Hristozkova has a total of 35 scientific publications. In the competition for "Associate Professor" she submitted 28 articles. Their distribution by quartiles is as follows: 4 publications with Q1, 5 with Q2, 7 with Q3, 8 publications with SJR without impact factor, 3 publications in peer-reviewed journals not indexed in WoS and Scopus and 1 book chapter. The overall JCR IF of the publications for competition is 26.19, and in 14 of them she is the first author, which proves her significant personal contribution to these publications and her capacity for independent and thorough research work.

The submitted report on the fulfillment of the minimum national requirements by the Regulations for the specific conditions and procedure for occupying the academic position "Associate Professor" shows that the total number of points for scientometric indicators with which Dr. Hristozkova participated in the competition is 1130, which significantly exceeds the required minimum of 400 items. The following indicators are presented:

The candidate participates in the competition with a total amount of 1130 points, which significantly exceeds the required minimum score of 400. The presented indicators for the fulfillment of the minimum national requirements for occupying the academic position "Associate Professor" at Sofia University are:

#### Group A (Ph.D. Thesis) – 50 points

The total number of points according to **group B** indicators is **115** points (required minimum 100 points) – 5 publications are included (Q1 - 3, Q2 - 2).

**Group C** includes 23 publications (Q1 - 1, Q2 - 3, Q3 - 7, with SJR without IF – 8, in journals not indexed in WoS and Scopus - 3, 1 chapter of a book) with a total number of points **303** (minimum 200 points are required).

**Group D** (citations) – **542** points (required minimum 50 points). According to the provided reference, the articles for participation in the competition have been cited a total of 393 times, with 149 of them in scientific publications referenced in Scopus or WoS.

Group E – 120 points. The reference submitted by the candidate shows that she participated in the development of 6 national scientific projects (I acknowledge her participation in the PISA INI project 14/01.09.2005) and in 3 international ones (2 projects with Slovakia and one with Germany).

Dr. Hristozkova has participated in a number of scientific meetings where 19 posters were presented and she was the first author of 10 of them. She was the editor of 3 refereed publications, as well as a member of the editorial board of the journal "Genetics and Plant Physiology".

The presented scientific production and the achieved scientometric data are attestation of the good quality of the presented scientific production. They significantly exceed the minimum requirements for occupying the academic position of "Associate Professor" at Sofia University "St. Kliment Ohridski".

#### Analysis of scientific achievements

The scientific research of Assistant Professor Marieta Hristozkova are focused on three main topics: 1. Beneficial plant-microbial relationships in plants; 2. Influence of abiotic stress conditions on plant development; and 3. Methods for improving the quality and evaluating the 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. The contributions of Dr. Hristozkova's research activities have not only theoretical significance, but also a certain practical orientation.

I will consider the candidate's contributions, taking into account only the publications, submitted for participation in the competition with their numbering in the attached list.

# 1. Beneficial plant-microbial relationships

A significant part of Dr. Hristozkova's research work is related to the study of plantmicrobial relationships. In this scientific topic, she submits 20 publications for participation in the competition, which she divides into two subcategories.

### 1.1. Effects of arbuscular mycorrhizal fungi on plant development

Original and significant research results have been obtained on the effects of arbuscular mycorrhizal fungi on plants (*publications 1, 2, 6, 7, 9, 10, 11, 12, 13, 14, 15, 18, 22*). The importance of mycorrhizal fungi for the enhancement of plant nutrient uptake and the improvement of their quality and yield, as well as the role of these soil microorganisms in increasing the stress resistance of crops has been shown (*publication 1*). It has been emphasized that the addition of a suitable strain of mycorrhizal fungi to the root zone would significantly improve the growth and productivity of a number of crops, which has important economic significance.

The effectiveness of symbiosis between mycorrhizal fungi with important agricultural or herbaceous plants has been investigated, with some studies using double inoculation. Furthermore, the development of efficient protocols for *in vitro* propagation of a number of species has made it possible to compare the mycorrhizal symbiosis of seed-grown plants with that of *in vitro* propagated plants. Inoculation of *in vitro* propagated garden thyme plants with arbuscular mycorrhizal fungi was found to increase their antioxidant capacity (*publication 7*). Double inoculation with arbuscular mycorrhizal fungi and microalgae (*Scenedesmus incrassatulus* R83 and *Synechocystis* sp. R10) stimulates the mycorrhizal function, affects the metabolism and development of basil (*Ocimum basilicum*), which is expressed in an increase in the concentration of secondary metabolites, parameters of gas exchange, nitrogen index and the activity of nitrogen- and carbon-metabolizing enzymes and increases the antioxidant capacity of plants (*publications 6, 10*). The application of synthetic nitrogen fertilizers, alone and in combination with mycorrhizal fungi in the cultivation of

different varieties of lettuce, affects the yield and quality of the production and is variety dependent (*publication 14*).

The role of light quality and quantity as well as soil moisture on the effectiveness of plant symbiosis with mycorrhizal fungi was also evaluated. Under white light, mycorrhizal fungi were found to improve soil fertility, while a combination of 66% red and 33% blue light stimulated aboveground biomass production and gas exchange parameters in inoculated tomatoes (*publication 9*). Studies of the symbioses between papuda (*Vigna unguiculata*), mycorrhizal fungi (*Glomus intraradices*) and two strains of *Bradyrhizobium japonicum* indicate that the soil moisture optimum for the formation of active symbiotrophic associations is around 60% water holding capacity (*publication 11*).

Valuable information was obtained on the positive role of mycorrhizal fungi on plant tolerance to water stress and heavy metal pollution. An increase in drought resistance of *Physalis peruviana* plants inoculated with arbuscular mycorrhizal fungi was found compared to non-mycorrhizal plants (*publication 2*). Testing four mycorrhizal isolates from different habitats in symbiosis with Origanum majorana under conditions of heavy metal pollution showed the highest shoot biomass when inoculated with strains from natural metalliferous sites (*publication 12*). Furthermore, it was found that the mycorrhizal symbiosis helps for the adaptation of *Physalis peruviana* to soil contaminated with heavy metals. As a result of inoculation, a significant reduction of Cd and Pb was observed in fruits compared to noninoculated plants (*publication 13*). Mycorrhizal fungi stimulate the accumulation of important secondary metabolites and alter the carotenoid profile of flowers of marigold (Calendula officinalis), grown on heavy metal-contaminated soil and prevent their accumulation in the flowers (*publication 15*). Mycorrhizal symbiosis has been shown to alter the content of the main compounds of marjoram (Origanum majorana) essential oil under conditions of heavy metal pollution and to increase antioxidant activity in aerial parts due to increased levels of phenolic compounds (*publication 18*).

#### 1.2. Symbiotic nitrogen fixation in legumes

In this subcategory, Dr. Hristozkova presents 7 publications (19, 20, 23, 25, 26, 27, 28).

Since molybdenum (Mo) deficiency leads to a significant reduction in the activity of enzymes involved in the initial stages of nitrate assimilation and nitrogen fixation activity (*publication 27*), most research is directed at searching for opportunities to improve nitrogen assimilation under Mo deficiency. Foliar fertilization was found to increase nitrogen fixation and biomass accumulation, have a positive effect on the enzyme activities of glutamine synthetase, glutamate synthase and nitrate reductase, and reduce the inhibitory effect of Mo deficiency on root nodulation, dry plant biomass and protein content (*publications 20, 23, 26, 28*).

Comparing the responses of free living strains of *Sinorhizobium meliloti* (wild type 1021) and two mutants (NitR and TspO) to nitrogen and carbon limitation in the medium and their ability to form a symbiotic association with alfalfa (*Medicago sativa*) showed that the most

efficient symbiotic system in terms of nitrogen fixation capacity and plant biomass accumulation was found between alfalfa and TspO (*publication 25*). Furthermore, nitrogen fixation in alfalfa tubers has been shown experimentally to increase when exposed to elevated  $CO_2$  concentrations, leading to higher amino acid and organic acid concentrations in the tubers as well (*publication 19*).

#### 2. Influence of abiotic stress conditions on plant development

This topic includes two subcategories with one contribution each. The contribution to the first subcategory *2.1. Effect of salt stress and overcoming its negative consequences in plant development* is based on the research in publication 4 of the list of all scientific publications, which was not included in the list of publications submitted for participation in the competition.

# 2.2. Quality of production obtained from medicinal and aromatic plants grown on industrially contaminated soils with heavy metals and the possibilities of their use for phytoremediation

Sage (*Salvia officinalis*) grown on heavy metal contaminated soil was found to accumulate cadmium, lead, and zinc, resulting in inhibition of plant biomass, but yield and essential oil quality were not impaired (*publication 21*).

# 3. Methods for increasing the quality and evaluation of the 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.

A significant contribution in the experimental work of Marieta Hristozkova and the team she worked with was the development of effective protocols for *in vitro* propagation of a number of medicinal and aromatic plants, which has an important practical application. A protocol for micropropagation of Greek oregano (*Origanum heracleoticum*), thyme (*Thymus vulgaris*) and hyssop (*Hyssopus officinalis*) has been developed (*publications 4, 7, 8*).

The successful adaptation of *in vitro* propagated plants to *ex vitro* conditions has made it possible to compare their important characteristics as well as their resistance to abiotic stress with those of plants grown from seed or wild-growing. Adapted *Physalis peruviana* plants after *in vitro* propagation show higher drought resistance than seed-grown plants (*publication 2*). The essential oil composition and antioxidant properties of the leaves and flowers of *Origanum heracleoticum*, collected from four different natural populations in Bulgaria, were investigated and compared with those of micropropagated and field-adapted plants. The highest antioxidant potential was found in the flowers and leaves of the micropropagated plants. (*publication 3*). Comparison of biologically active compounds in the methanolic extracts of *Hyssopus officinalis* cultivated from seeds, propagated *in vitro* and from natural habitats showed the highest antioxidant potential in the *in vitro* propagated

plants, but the highest concentration of essential oil was found in plants from natural habitats (*publication 5*). Comparing the antioxidant capacity of *Sideritis scardica* leaf and flower extracts from different habitats shows that it is affected not only by the type of propagation but also by environmental conditions (*publication 16*).

# **Educational activity**

As an Assistant Professor in the Department of Plant Physiology" at the Biological Faculty Marieta Hristozkova is also engaged in active teaching. According to the report submitted for the period 2018-2023 she had a total of 3202 teaching hours (503 hours in 2018/2019, 647 hours in 2019/2020, 681 hours in 2020/2021, 831 hours in 2021/2022 and 540 hours in 2022/2023). Under her guidance, 3 graduates successfully defended their degrees.

## CONCLUSION

The analysis of the scientific achievements of Dr. Marieta Hristozkova clearly shows that she is an established and productive researcher in the field of plant physiology. Scientific publications contain significant fundamental and applied contributions that have received international recognition. The number of cited materials by foreign authors in prestigious scientific publications is significant. The evaluation of the scientific and teaching activity of Dr. Hristozkova shows that she meets all the requirements of the Law for Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its implementation, and are in line with the Regulations for acquiring scientific degrees and holding academic positions at Sofia University "St. Kl. Ohridski" to occupy the academic position of "Associate Professor".

All this gives me arguments to evaluate positively her overall activity and to recommend to the respected members of the Scientific Jury to vote positively and to the members of the Faculty Council of the Biological Faculty of Sofia University "St. Kliment Ohridski" to elect Assistant Professor Dr. Marieta Georgieva Hristozkova for the academic position "Associate Professor".

15.07.2024 г. Sofia Prepared the statement: /Prof. Katya Georgieva/