

R E V I E W

**ON THE COMPETITION FOR OBTAINING THE ACADEMIC POSITION
"Associate Professor"**

PROFESSIONAL FIELD 4.3. "BIOLOGICAL SCIENCES"

scientific specialty "Cell Biology", for the needs of the Department of "Biology, Medical Genetics and Microbiology" , Faculty of Medicine at the University "Prof. Dr. Assen Zlatarov"

Candidate: Dr. Vesselina Merhar

Reviewer: Assoc. Prof. Dr. Iliana Ivanova, BF at SU "St.K. Ohridski"

In the announced competition for the occupation of the academic position "Associate Professor" in **professional field 4.3. Biological sciences**, scientific specialty **"Cell Biology"**, for the needs of the **Department of "Biology, Medical Genetics and Microbiology" , Faculty of Medicine at the University "Prof. Dr. Assen Zlatarov"**, announced in the "State Gazette", no. 45 of 17th June, 2022, participated one candidate - Dr. Vesselina Stoyanova Merhar, assistant professor in the same department of the University. Dr. Merhar's documents submitted for participation in the competition show that the procedure for its disclosure and announcement was followed and they are in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its Implementation (LDASRBRI), as well as with the Regulations on the terms and conditions for acquiring scientific degrees and holding academic positions at the Universities in the Republic of Bulgaria and the the same at University "Prof. Dr. Assen Zlatarov".

1. General data on the candidate's career and thematic development

Vesselina Stoyanova Merhar obtained a Master's degree in "Biology", specialty "Molecular and Functional Biology" at the Faculty of Biology of Sofia University "St. Kl. Ohridski" in 1986. In the same year, she was appointed as a specialist biologist at the Institute of Physiology, Bulgarian Academy of Sciences (BAS), Sofia, Bulgaria, where she worked until 1989. In the period 1990-1993, she worked on her PhD thesis at the Institute of Plant Physiology of the Russian Academy of Sciences on "Soluble proteins in wheat germ in relation to seed viability". In 1994, after winning a competition, she was appointed to the academic position of "research associate II degree" at Institute of Plant Physiology - BAS. She has been working as a post-doctoral fellow at the University of Bloemfontein, Republic of South Africa since 1997. Since 2001, she has been appointed as an Assistant Professor at the University of KwaZulu-Natal, Durban, Republic of South Africa, where she has continued her professional practice for 17 years.

2. Scientific-metric indicators

Dr. Merhar's good scientific output and active research work during her career development makes a good impression. For the current competition, she submitted one

monograph and a list of 12 publications, as well as two book chapters published in collaboration with other authors. All of the publications participating in the contest bring points to the candidate, according to Art. 1a, par. 1 of the Regulations for the Application of LDASRBRI, being distributed in the following categories: 12 – in scientific publications, referred and indexed in world-famous databases with scientific information (SCOPUS and WEB of SCIENCE), 1 monograph that is not on the subject of the dissertation and is recognized as a habilitation thesis, 2 chapters from books or from collective monographs. Also included in the list of publications are 6 additional publications without IF or SJR that do not carry points but present significant results that are part of the candidate's scientific contributions, as well as 16 abstracts from international scientific forums and 4 from national scientific forums.

The publications are grouped according to the guidelines for meeting the minimum national requirements for holding the position of "Associate Professor" in Professional Direction 4.3 Biological Sciences, as follows:

The indicator from group "A" is fulfilled by the PhD thesis (50 points).

The 'B' indicator is not required for this post.

For group "B" indicator 3, a monograph "IMMUNOFLUORESCENCE METHODS FOR VISUALIZATION AND ANALYSIS OF THE CYTO- AND NUCLEOSKELETON OF THE CELL" is presented, equivalent to a habilitation work, (100 points)

Group "D" includes articles with quartiles Q1 (3 pcs), Q2 (2 pcs), Q3 (5 pcs) and Q4 (2 pcs), 2 book chapters, with which the total points reach 244, which exceeds the minimum required for this degree. Full-text publications in non-SJR non-IF conference proceedings are also attached. No points are awarded for this indicator. This for the scientists of Biological Sciences direction is a disadvantage, as certain points are given in other scientific directions. The total numerical value for publications in journals and books is 244 points, out of a required 200. Publications with quartiles 1, 2 and 3 predominate, which is proof of the importance of the author's research and of the international community high evaluation. According to indicator D11, citations of the candidate's articles are presented, collecting 120 points with a minimum requirement of 60, so this indicator also exceeds the necessary minimum requirements twice. According to indicator E, the candidate Dr. Merhar has four participations in international projects and one project of which she is the leader, again at the University of South Africa, so here too the points collected exceed the minimum requirements as per the LDASRBRI.

3. Main directions in the candidate's research work and most important scientific contributions

Dr. Merhar's scientific interests and contributions in her more than twenty-year professional career have been consistent and profound and are in the following areas: (1) visualization and characterization of various cellular components and (2) cellular response to stress, mostly induced by infections. Earlier studies were conducted on plant material and described changes in host cells as a result of plant-pathogen interactions. At later stage, the accumulated research experience and developed methods, such as immunohistochemistry and immunocytochemistry, combined with state-of-the-art microscopic techniques, were applied to placentas from HIV-infected preeclamptic patients. Complying with the requirements of the

legislation, the candidate has submitted a detailed habilitation report of the contributions in her scientific publications under indicator D. Included here are a total of 12 articles in journals with IF/SJR, the focus of which is the microscopic and biochemical study of plant and human cells under conditions of stress. The research has a fundamental character, and the scientific contributions of the publications can be grouped as follows:

A) For the first time, microscopic and biochemical analyzes were carried out on a selected wheat variety carrying a gene (Lr35) for resistance to leaf rust.

- The mechanisms and changes at the cellular and molecular level that lead to this resistance have been established. The studies included detailed histopathological analyzes of pathogen penetration in plant tissues of disease-susceptible plants (i.e., those lacking the Lr35 gene), as well as fluorescence microscopic studies of the hypersensitive response (HR) in tissues of resistant to disease plants (i.e. those carrying the Lr35 gene).

- For the first time, intercellular proteins with β -1,3-glucanase, chitinase and peroxidase activities associated with Lr35 gene-mediated resistance to leaf rust in wheat were investigated.

- The results of the mentioned studies have been published in three consecutive articles and are of great interest worldwide, which is visible from the 125 noticed citations in international journals with IF. Also, the results have been presented as papers and posters at a number of conferences in South Africa.

B) Of utmost importance are the candidate's studies on the so-called recalcitrant (sensitive) seeds. These are seeds that do not withstand drying, which leads to the impossibility of storing them for a long period of time. On the other hand, if stored fresh, these seeds are susceptible to infection due to the high water content of their tissues, which is a favorable environment for pathogens. Due to the impossibility of storing these seeds, many sensitive species, mostly tropical and subtropical, are threatened with extinction. Any research on recalcitrant seeds represents a scientific contribution as it relates to biodiversity conservation, which is a global issue of concern. The candidate's scientific contributions in this field can be systematized as follows:

- New data were obtained on the response of recalcitrant seeds of the mangrove species *Avicenia marina* to infection with the fungal strain *Fusarium moniliforme*, one of the most common species causing infection and destruction of many tropical species.

- For the first time, immunohistochemical TEM analyzes were performed on these seeds infected with *Fusarium moniliforme*. On this basis, a hypothesis explaining the high sensitivity of recalcitrant seeds to fungal infections is proposed. Dr. Merhar presented this hypothesis at the 8th International Workshop on Seeds in Brisbane, Australia, and the results were published as a chapter in the book by Steve Adkins, Sarah Ashmore and Sheldon Navy, *Seeds: Biology, Development and Ecology*.

- Using modern immunofluorescence methods and developing one of these methods, the candidate for the first time managed to visualize significant changes in the structure of the cyto- and nucleoskeleton of the embryos of *Trichilia dregeana* - another sensitive tree species distributed in South Africa, which is seriously threatened with extinction. The results are of particular importance, as establishing the reasons for the high sensitivity of recalcitrant seeds

to desiccation at the cellular and molecular level would lead to the development of methods to overcome this problem.

C) In recent years, Dr. Merhar has used her expertise in immunohistochemistry and modern microscopic techniques and softwares to evaluate the immunoexpression of the lymphatic markers LYVE-1 and PODOPLANIN in the placenta of HIV-infected women with preeclampsia. These studies were recently published and resulted in 7 citations in international journals. The results were reported at the International Symposium on Bioinformatics and Biomedicine, October 8-10, 2020, Burgas, where the paper was awarded with a certificate.

The candidate's monograph "Immunofluorescence methods for visualization and analysis of the cyto- and nucleoskeleton of the cell" makes a strong impression in the field of modern microscopic studies and immunohistochemical methods. Complying with the requirements of the legislation, Dr. Merhar has submitted the full text of the published monograph, as well as two reviews by respected scientists, which certify the contributions in her habilitation thesis, presented under indicator C. The main scientific contributions in the monograph are as follows:

- Description of immunohistochemical methods new to science for the study of the cytoskeleton and the skeleton of the nucleus (nucleoskeleton) on thin sections for light-microscopic analyses. This method for immunofluorescence visualization of the nucleoskeleton is a previously unpublished contribution of the author. The results of the application of the method on the model system - recalcitrant seeds, unequivocally show the presence of proteins inside the nuclei of the cells of these seeds, which immunohistochemically react with anti-lamin antibodies.

- The method developed by the candidate for direct visualization of the nucleoskeleton and the results obtained using this method constitute a significant contribution to studies of the internal nuclear skeleton, the existence of which is still disputed. This is the only study so far on the nucleoskeleton of recalcitrant seeds and is a significant contribution to the establishing of the reasons why these seeds do not tolerate dehydration. The results support the assumptions of some authors that the lack of structural organization in the nucleoskeletal network of dehydrated embryos is one of the main reasons for the inability of recalcitrant seeds to survive desiccation.

- The actin cytoskeleton and nuclear lamins are known to be universal to eukaryotic cells. For this reason, the methods described in the monograph, although tested on plant material, can also be applied to permanent preparations of human or animal tissues and used in medicine for the diagnosis of diseases associated with abnormalities in the cyto- and nucleoskeleton of cells of these tissues. Most of these diseases belong to the group of rare diseases for which there is currently no treatment, which reinforces the importance of the monograph as a reference point for the improvement of methods related to their early diagnosis. One of the dimensions affecting chromatin stability is nuclear architecture. It is unlikely that the molecular mechanisms of replication, transcription, RNA splicing, and transport through the nucleus function coordinately in a soluble matrix. This proves the hypothesis that the nucleoskeleton is the structure responsible for the topological organization of the components of the nucleus and the coordination of intranuclear processes.

- Apart from the justified defense of these productions, Dr. Merhar, as a good practitioner, dwells on other equally important points in her book, namely preparation of the material. A rule of thumb is that if the material is not well prepared, even the most expensive confocal laser scanning microscope cannot improve the quality of the images. Therefore, the choice of a correct method for processing the material and special care in its preparation for microscopy are particularly important for the result of the immunostaining, in order to avoid errors in the interpretation of the results. The author carefully lists all the factors affecting material preparation such as the choice of proper fixative, resins, appropriate antibodies and fluorochromes.

- The author examines the differences between conventional fluorescence and confocal laser scanning microscopy, dwells on the most modern and up-to-date methods in microscopic studies, and thoroughly substantiates the details in the preparation of the materials. The application of precise and specific instructions for the individual consecutive procedures, the extensive bibliography, as well as the two reviews by Prof. Minkov and Assoc. Prof. Ananiev, both doctors in medicine, also confirm the high quality of the habilitation work. I suggest that this detailed contribution to the development of microscopic techniques deserves to be published in English and to be the basis of future research.

- It makes an excellent impression that the colleague chooses appropriate methods in her studies that allow her to obtain reliable results. They are original, and majority of them represent new data for science. The analytical part in the publications submitted for review is thorough and comprehensive. The directions in which Dr. Merhar works are important, current and promising. Proof of the importance of the research conducted with her participation are the publications in renowned publications with an impact factor, as well as the many positive citations.

4. Notes and recommendations

IR and SJR calculation of of publications is incorrect. This minor inaccuracy do not negatively affects my impressions for the significance of the applicant's research, as well as the fulfillment of the national requirements for holding the position of Associate Professor.

5. Question

What are the candidate's future research plans when taking up the new academic position?

6. Conclusion

On the basis of the materials submitted for the competition by Dr. Vesselina Merhar, I am convinced that she fully meets, and in some indicators exceeds, the national criteria for holding the academic position "Associate Professor", defined by the Law on the Development of the Academic Staff in the Republic of Bulgaria and the Regulations for its application, as well as with the Regulations for the terms and conditions for acquiring scientific degrees and for holding academic positions at the Universities of the Republic of Bulgaria and the Regulations for the terms and conditions for acquiring scientific degrees and for holding

academic positions at the University "Prof.Dr. Assen Zlatarov". Dr. Merhar is a microscopist with a clearly defined scientific profile, a recognizable scientist at the world level, fully dedicated to her work and with proven scientific and scientifically-applied contributions. She is an excellent specialist at the world level, one of the few in Bulgaria, with enviable theoretical and applied skills, an incredible idealist, a fascinating researcher with her ideas and her incredible work ability, an extremely loyal colleague and a desirable partner for numerous national and international research and scientific projects.

The direction in which she works is the basis of basic branches of microscopy, with great scientific and applied potential. Contributions related to the conservation of plant diversity in South Africa are also enviable.

On the basis of the above, I strongly recommend the members of the Scientific Jury to support the election of Dr. Vseselina Stoyanova Angelova-Merhar for the academic position "Associate Professor" in professional direction 4.3. Biological Sciences, scientific specialty "Cell Biology", for the needs of the Department of "Biology, Medical Genetics and Microbiology" of the Faculty of Medicine at the University "Prof. Dr. Assen Zlatarov".

16.11.2022

Sofia

Reviewer:

(Assoc. Prof. Dr. I. Ivanova)