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REVIEW

on a dissertation for the **Doctor of Science** degree by: field of higher education 4. Natural Sciences, Mathematics and Informatics, Professional Field 4.3. Biological Sciences, scientific specialty Genetics.

Author of the dissertation: Вотіn Махмоv Bojinov, doctoral student in independent training at the Department of Plant Physiology, Biochemistry and Genetics at the Agricultural University, Plovdiv

Topic of the dissertation: Use of molecular markers in genetic diversity studies and for DNA profiling

Reviewer: acad., DSc. Atanas Ivanov Atanasov, SGC, Sofia University "Kl. Ohridski", BAS, Higher Education Area 4. Natural Sciences, Mathematics and Informatics, Professional Field 4.3. Biological Sciences, scientific specialty Genetics appointed as a member of the scientific jury by order No. RD -16-801 / 24.06. 2025 by the Rector of the Agricultural University of Plovdiv.

1. Brief presentation of the candidate.

Prof. Bojinov was born on 30.11.1965. He graduated in 1990 from the then Higher Agricultural Institute "V. Kolarov" (now Agricultural University-Plovdiv). The same year he began his scientific career at the Cotton and Durum Wheat Research Institute in Chirpan as a cotton breeder with a profile in Plant Biotechnology, and over a period of 5 years he grew from a 3rd level junior researcher to a 1st level junior researcher. During this period he also completed a 15-month specialization in the field of plant biotechnology at the Catholic University of Leuven, Belgium. From 1995 to the present he has been working at the Agricultural University-Plovdiv, going through all levels of academic development - assistant professor, senior assistant professor, chief assistant professor, associate professor and professor. Meanwhile, he conducted two long-term specializations in the field of plant biotechnology (9 months at the Laboratory of Cell Biology in Versailles, France) and genetics (12-month Marie Curie fellowship to work at CIRAD, Montpellier, France), as well as several short-term (1-3 months) specializations in the field of genetics, educational technologies and leadership in France and the USA. Author and co-author of over 90 scientific publications and 5 plant varieties. Leader of a number of scientific projects funded by the Agricultural University of Plovdiv, IAEA and the National Scientific Research Fund, as well as over 20 graduate students and 3 doctoral students. He gained administrative experience during his two terms as Head of

Department and two terms as Dean of the Faculty of Agricultural University of Plovdiv. He implemented new teaching methods at the Agricultural University of Plovdiv as the creator and head of the National Demonstration Center for New Technologies in Agriculture, funded by the America for Bulgaria Foundation.

2. Relevance of the problem.

Since the 1990s, Omics technologies based on genomics, transcriptomics, proteomics and metabolomics have gradually and widely entered genetic and breeding research in plants. Genomics is based mainly on DNA molecular markers, the efficiency of which has been continuously improved to reach its routine application in various wild and cultivated plants and animals, as well as in studying the nature and diversity of microorganisms. Their ability to facilitate both conventional breeding and efforts aimed at the accelerated creation of new varieties of organisms that better respond to rapidly changing environmental conditions and market requirements has been identified and well documented. As a result, a number of marker systems have been used for years to accelerate the breeding process, guarantee the genetic identity of breeding materials and protect their intellectual property in large and smaller breeding companies.

In Bulgaria, the good example in this area of genetic and breeding research during the mentioned period was successfully set by the Agrobioinstitute at the Bulgarian Academy of Sciences in the grapevine, rose, soft and durum wheat, barley, corn and sunflower, which contributed significantly to the continuous improvement of their genetic maps. Of the agricultural universities, the author of the present doctoral thesis was the first to show the relevance and perspective of the application of DNA-based markers in a number of plants and animals, and most clearly expressed in cotton, where the originality of this type of research is best demonstrated. Due to the high initial cost of application, the use of these marker systems by breeding teams relying on public funding in our country was limited until recently. With the development of DNA sequencing and amplification technologies, the cost of their use has decreased significantly and currently this toolkit is easily accessible both from a financial and technological point of view. It is surprising, however, that in Bulgarian state institutes and universities engaged in breeding tasks, their use continues to be very limited, which is a significant reason for reducing their use in broad agricultural practice. The same applies to the institutions responsible for the registration and control of seed and planting material (IASAS) and animal reproductive material (IASRJ). The root of the problem lies in the lack of standardization of the systems and protocols used between the two groups of institutions, due also to the differing requirements for the functioning of each of them.

That is why I definitely believe that the present work is a serious step towards overcoming this critically important shortcoming, which is a significant obstacle to the progress of Bulgarian breeding.

3. Purpose, tasks, hypotheses and research methods.

The aim of the dissertation submitted to me for review is to establish a molecular marker system and to propose it for reliable and reproducible identification of genetic diversity, both in plant and animal forms and microorganisms. The author's **main hypothesis** is that from the many known marker systems, one can be selected that allows effective use both for breeding purposes and for control purposes in variety testing and seed production in cultivated plants, as well as in breeding and maintaining the genetic status of local breeds.

To achieve the set goal, the following tasks have been formulated:

1. To conduct a comparative test of a set of marker systems for identifying genetic diversity in plant species (cotton, tomatoes, tobacco, barley, etc.), eukaryotic phytopathogens (phytophthora and fusarium fungi) and animals (goats).
2. To establish the possibilities for applying the studied marker systems for detecting loci for quantitative traits of specific importance for local populations.
3. To characterize the possibilities for applying the selected markers in variety testing, variety maintenance and conservation of the existing biodiversity of local plant forms and animal breeds.

Material and methods are accurately, clearly and logically reflected in the dissertation work. The wide spectrum of different types of eukaryotic organisms (plants - cotton, tobacco, tomatoes, pepper, barley, wheat, corn; plant pathogens - phytophthora fungi and Fusarium and goats as representatives of animal organisms) and a variety of modern molecular markers such as RFLP, AFLP, SSR and ISSR shows that the author is looking for the common and principled beginning between the molecular markers that could prove to be the most successful and effective in the studied organisms.

The static analysis methods and software used are selected and adequate to the methods used.

4. Visualization and presentation of the results obtained.

The dissertation submitted to me for review is illustrated with 17 photos, 63 figures and 33 tables, which well present both the methodology and the results of the application of the various molecular techniques used in the dissertation, as well as the variations in the phenotypic manifestations of the traits in different types of organisms. Particularly useful for the discussion are the presented genetic maps and groupings of individuals from different types of populations in different organisms, which give a clear idea of the applicability of the various marker systems in the context of Bulgarian genotypes.

5. Discussion of the results and literature used.

Due to its considerable volume, including the use of several marker systems in 11 species of organisms, the presentation of the results, which occupies 95 pages, is appropriately separated from the discussion on them (37 pages). This allows, within the framework of the discussion, to draw generalizations that are valid for the entire group of organisms studied. Since the organisms themselves are representatives of many different phylogenetic groups (plants, animals, eukaryotic microorganisms), the presented results and the discussion have a broader validity and significance, which makes the defense of the presented hypothesis well-argued. The arguments presented by the author are discussed in the context of a significant volume of national and international studies, included in the list of 280 titles of literature.

As it became clear, the present study presents the differences in the application of several types of molecular markers. In particular, it demonstrates the ability of ISSR as a tool for identifying varieties in a number of crops and two autochthonous goat breeds. Since the ISSR system has been proposed for a long time, but has not been sufficiently studied so far in the Bulgarian context, here the capacity of a set of primers to produce polymorphism in the main local crops and breeds for the country is assessed. Nevertheless, further studies will be needed to refine the parameters of the technique before it can be applied to its full capacity and routinely in RHS (distinctness, homogeneity and stability) testing in the approval of varieties by IACS, protection of breeders' rights, sorting of breeders' collections in proving the value and value of gene banks in plants, animals, microorganisms and other species.

6. Contributions of the dissertation work.

Scientific contributions

1. Based on a number of parameters (polymorphic information content, uniformity of distribution along the length of the genomes, reproducibility, reliability, etc.), a molecular marker system has been identified that has the capacity to meet the requirements of various areas of use.
2. Methods have been developed for the use of the ISSR marker system in a number of cultivated plants, two eukaryotic phytopathogens, and two autochthonous animal breeds.
3. Within the framework of this work, for the first time in our known literature, the ability of the ISSR marker system to identify intraspecific diversity in phytophthora fungi has been proven, which opens up new opportunities for the development of more effective systems for detection and identification of this group of phytopathogens.
4. The ability of a set of dominant (i.e. AFLP, ISSR, CAPS) and co-dominant (RFLP, SSR) molecular marker systems to identify genetic diversity in a range of plant and animal organisms of local origin has been characterized.
5. Through the use of comparative analysis, the advantages and disadvantages of each system have been identified depending on the planned direction of use - for selection, breeding or for the purposes of registering and maintaining the biodiversity of the relevant organisms.

Scientific and applied contributions

1. A molecular marker system (ISSR) has been identified, which has a wide range of applications in breeding programs of local forms of plants and animals, as well as for the detection and identification of phytopathogenic organisms.
2. The applicability of the ISSR marker system for analyzing genetic diversity in a large set of plant, microbial, and animal organisms has been demonstrated, thus proving its universality in terms of use in eukaryotes in such a context.
3. By performing comparative analyses, the effectiveness of the application of the ISSR marker system in the selection programs of a number of crops of national importance and in two autochthonous goat breeds has been proven.
4. The potential of the ISSR marker system for effective use for both breeding purposes and for the purposes of state variety testing, approbation and seed

control (in plants) and for the independent certification and breeding of local animal breeds has been demonstrated.

5. The compliance of the ISSR marker system with the system of criteria used by UPOV to determine the applicability of various marker systems for the purposes of PCR testing has been characterized in detail, thus proving its applicability in the IACS and IASR systems.
6. New marker/trait associations have been obtained for a number of economically valuable traits in local cotton and tomato varieties.
7. A significant number of molecular markers have been mapped in cotton, significantly enriching the existing genetic map with loci that have significant effects in local agro-ecological conditions.
8. A significant number of loci for quantitative traits have been identified in local samples, and those with pleiotropic effects on a number of qualitative and quantitative traits have also been identified.
9. The genetic diversity in two autochthonous breeds (Bulgarian Vitoroga Longhair and Kalofer Longhair) of goats at the level of hereditary material has been characterized, and recommendations have been given for the future breeding of both breeds, so as to reduce the risks of inbreeding, potentially leading to inbreeding depression.

With the contributions made, the author of the present dissertation convincingly shows how modern genetic and selection research and university education should be developed not only at the Agricultural University of Plovdiv. This makes the doctoral student a prominent and authoritative scientist and teacher and a good example for the younger generation of scientists in this field in our country to follow.

6. Critical notes and questions.

Essentially, I have no critical comments or questions for the author of this dissertation.

8. Published articles and citations.

In connection with the presented dissertation work, the author has presented 19 publications, in 13 of which he is the lead or sole author. The presented list of citations includes 152 citations of 1 article. In an additional list (of the full-text citing publications) another 12 citations of 8 other articles are presented. Thus, the total number of citations presented in connection with the dissertation is 164 (of 9 publications).

The presented abstract objectively reflects the structure and content of the dissertation.

CONCLUSION:

Based on the various research methods studied and applied by the doctoral student, the correctly conducted experiments, the generalizations made, and relevant conclusions, I believe that the presented dissertation fully meets the requirements of the Law on the State of the Republic of Bulgaria and the Regulations of the Agricultural University for its application, which gives me the necessary reason to evaluate it **POSITIVELY.**

I would like to suggest that the esteemed Scientific Jury also vote positively and award Bojin Maksimov Bojinov the scientific degree **"Doctor of Science"** in the scientific specialty Genetics .