REVIEW

of the doctoral thesis presented for the acquisition of the scientific degree "Doctor of Sciences" in the field of higher education 4. "Natural Sciences, Mathematics and Informatics ", Professional field 4.3. "Biological Sciences", Scientific specialty: "Genetics"

Author of the thesis: Professor, Dr. Bojin Maksimov Bojinov,
Department of Plant Physiology, Biochemistry and Genetics at the Agricultural University, Plovdiv.
Topic: "Use of molecular markers in research on genetic diversity and DNA profiling "
Reviewer: Asssoc. Prof. Samir Izetov Naimov, Doctor of Sciences, University of Plovdiv "Paisii Hilendarski", appointed as a member of the scientific jury by Order No. RD-16/753, 12 June 2025, by the Rector of the Agricultural University

1. Brief presentation of the candidate.

Prof. Dr. Bojin Maksimov Bojinov was born on November 30, 1965, in Plovdiv, where he spent most of his professional career. His education began with a degree in agricultural engineering in 1990 from the V. Kolarov University of Agriculture in Plovdiv. In 2000, he received a doctorate in plant breeding and biotechnology. In 2005, he was habilitated as an associate professor in genetics, and in 2024, he received the academic title of professor. His professional career includes various academic and administrative positions. From 1990 to 1995, he worked at the Institute of Cotton and Durum Wheat (ICDW) in Chirpan as a research associate, participating in the creation of new cotton varieties. From 1995 to 2008, he successively held the positions of assistant, senior assistant, chief assistant, and associate professor in the Department of Genetics and Selection at the Agricultural University of Plovdiv, where he was actively involved in teaching and research activities and supervised master's and doctoral students. From 2008 to 2016, he served two terms as dean of the Faculty of Agronomy. Then, from 2016 to 2020, he was head of the Department of Genetics and Selection, and since May 2024, he has been head of the Department of Plant Physiology, Biochemistry, and Genetics.

From 2016 to 2020, he was head of the Department of Genetics and Selection, and since May 2024, he has been head of the Department of Plant Physiology, Biochemistry, and Genetics. In these roles, he is responsible for the organization of educational, scientific and administrative activities, as well as for the accreditation of doctoral programmes and the development of academic staff. Prof. Bojinov is fluent in English, Russian and French.

2. Comparison of the minimum national requirements with the results of the candidate's scientific activity for the acquisition of the scientific degree "Doctor of Science".

Based on the regulated minimum national requirements that candidates for the scientific degree "Doctor of Science" must meet and the analysis of scientific output, as well as the scientific research activity carried out by Prof. Dr. Bojin Bojinov, it is established that the candidate fully meets the minimum requirements for the relevant indicators.

Based on the regulated minimum national requirements that candidates must meet in order to obtain the academic degree of "Doctor of Science" and the analysis of scientific output, as well as the scientific research activity carried out by Prof. Dr. Bojin Bojinov, it is established that the candidate fully meets the minimum requirements for the relevant indicators.

According to the requirements of the Regulations for the Development of Academic Staff at the Agricultural University, a total of 450 points are required for the mandatory indicators for obtaining the scientific degree of "Doctor of Science" (Annex to Article 1a, paragraph 1), a total of 450 points are required, and Prof. Bojinov presents information on a total of 608 points, which exceeds the minimum national requirements for obtaining the scientific degree of "Doctor of Science" by nearly 1.5 times. The candidate participates in the procedure with 9 scientific articles, four new cotton varieties, and numerous scientific works-dissertations, scientific articles, etc., as well as over 150 independent citations.

3. Relevance of the topic.

The topic of the dissertation is extremely relevant, as it examines the application of molecular markers that have found application and proven effectiveness in modern genetics and selection. Since the early 1990s, these technologies have been widely used in various biological systems, including plants, animals, and microorganisms, for the purpose of analyzing genetic diversity, accelerating breeding processes, and protecting intellectual property. Despite advances in technology and a significant reduction in the cost of using DNA-based methods, their application in Bulgarian practice remains limited. This is due both to the lack of standardization among the responsible institutions and to differences in their functional requirements. Against this background, this dissertation is timely and necessary. It combines the author's many years of practical experience with different types of molecular markers applied to a wide range of organisms and offers concrete solutions to overcome the identified limitations in a national context. In this regard the dissertation is timely and necessary. It combines the author's many years of practical experience with various types of molecular markers applied to a wide range of organisms and offers concrete solutions to overcome the limitations identified in the national

context. Particularly valuable is the contribution to the development and proposal of standardized approaches to DNA profiling that can be used both in scientific selection programs and in the procedures for registration, testing, and control of varieties and breeds.

4. Information about the dissertation

Prof. Bojinov's dissertation is 256 standard typed pages long and is structured in the generally used format. The work begins with an introduction and literature review covering 35 pages, followed by the sections "Aims and objectives" and "Materials and methods," which are developed over the next 54 pages of the thesis. The results of the study are described in 94 typewritten pages and discussed in the following 37 pages of the study. The dissertation concludes with formulated conclusions and contributions of the work, as well as a detailed list of the literature used.

5. Purpose, objectives, hypotheses, and research methods.

The purpose of the dissertation is to develop and propose a molecular marker system applicable for reliable and reproducible identification of genetic diversity in plant, animal, and microbial organisms. The system should be effective, scalable, and accessible so that it can be used in breeding practices as well as in state control procedures, variety registration, and genetic resource conservation. To achieve this goal, the author formulates and performs the following main tasks:

1. Conducting a comparative analysis between different marker systems (dominant and co-dominant) to assess genetic diversity in representatives of practically significant plant varieties and animal breeds, as well as phytopathogenic microorganisms.
2. Investigating the possibilities for detecting QTL (quantitative trait loci) that are important for the selection of local forms.
3. Assessing the applicability of the studied markers in variety maintenance, variety testing, and biodiversity conservation.
4. Analysis of the possibilities for marker transferability between different genetic maps.
5. Identification of markers associated with qualitative characteristics in populations with high intraspecific variability.
6. Establishment of the effectiveness of the proposed system in studying diversity at different taxonomic levels – from intraspecific to interspecific.

In accordance with the set goal and tasks, various methods were applied in the study, such as: Isolation of genomic DNA from plant and animal tissues and from phytopathogen cultures;

PCR-based analyses using RFLP, AFLP, SSR, CAPS, and ISSR markers; QTL mapping and genetic map construction using specialized software (MapMaker, MapDisto), etc.

The methodological approach is comprehensive, with well-selected and diverse biological material, including major agricultural crops, animal breeds unique to the country, and pathogenic fungi important for agricultural practice.

The selection of biological objects is well-founded, representative, and functionally related to the topic of the dissertation. It provides a solid basis for conducting an in-depth comparative analysis and ensures a significant contribution in both theoretical and applied aspects. The study covers varieties and lines of cotton (Gossypium hirsutum and G. barbadense), tobacco (Nicotiana tabacum), tomatoes (Solanum lycopersicum), peppers (Capsicum annuum, C. chinense, etc.), paulownia (Paulownia tomentosa and hybrids), barley (Hordeum vulgare), wheat (Triticum aestivum), and corn (Zea mays). Two Bulgarian goat breeds were also included in the study: Thracian long-haired and White Bulgarian dairy. Between 15 and 20 individuals were analyzed for each breed. The fungal pathogens are represented by two genera: Phytophthora and Fusarium. They are included as fungal pathogens of agronomic importance, and research on them is focused on molecular identification and assessment of genetic variability.

Illustration and presentation of the results

The dissertation is duly illustrated, combining various types of visual aids for data presentation and analysis. The dissertation includes 63 figures and 32 tables. The results of PCR analyses, electrophoresis, dendrograms, and statistical graphs are also presented, illustrating the results of molecular studies. Most of the figures are original and document the author's experimental results. The illustrations in the dissertation are of a high standard and successfully fulfill their functions—to illustrate complex genetic information, to facilitate the perception of the results, and to provide visual support for the conclusions drawn.

6. Discussion of results and literature used.

The section "Discussion of results" is a well-structured and analytically rich part of the thesis, in which the author thoroughly discusses the molecular marker systems used—SSR, CAPS, ISSR, etc. The discussion is organized both by marker type and by their application in different biological systems: cultivated plants, phytopathogens, and animals. This approach creates high informative value and allows for a real comparison between the theoretical characteristics of the methods and the practical results obtained in the studies. The use of ISSR markers is particularly strongly argued, presented as a universal, accessible, and highly

informative tool applicable in selection, variety testing, and the conservation of genetic diversity. The author highlights the advantages of these markers in various objects – from tomatoes and cotton to local goat breeds and phytopathogenic fungi – and clearly formulates the criteria for their practical applicability (e.g., polymorphism, reproducibility, cost). SSR markers are noted for their high efficiency in QTL analyses and genetic mapping, especially in crops such as cotton and tomatoes. However, they are characterized by a higher complexity of analysis and a need for better equipment and preliminary information about the target genomes. CAPS markers are considered suitable for specific allelic differences, but with limited application due to their labor intensity and lower level of polymorphism.

When discussing the results, the linking of the results to specific recommendations for breeding practices in Bulgaria makes a positive impression. The author duly argues that ISSR markers can be integrated into state control systems for varieties and breeds. This is a valuable contribution that goes beyond the academic framework and offers real applicability in breeding and biodiversity.

The literature used in the text demonstrates a high academic style. A wide range of sources is covered – 280 in total, 279 of which are in Latin. Both classic works (Nei, 1973; Botstein et al., 1980; Vos et al., 1995) and contemporary publications reflecting the current state of research. The cited sources cover methodological, applied, and regional studies, which emphasizes the interdisciplinary nature of the work.

8. Contributions of the dissertation.

The dissertation contains significant theoretical and applied scientific contributions related to the development, adaptation, and validation of molecular marker systems for analyzing genetic diversity in different groups of eukaryotic organisms—plants, animals, and phytopathogens. The author has formulated five theoretical and nine applied contributions. The contributions are accurately formulated and reflect the results described in the dissertation. In my opinion, contributions 3 and 5, as well as applied contributions 1 and 6, are particularly significant.

9. Critical comments and questions.

I have no critical comments on the dissertation. I believe that it would be useful to publish parts of the study as a monograph related to the dissertation, aimed at a wider audience.

10. Published articles and citations.

The candidate participates in the procedure with nine scientific articles: two with quartile

1, six with quartile 3, and one with quartile 4. The competition documents also include four new cotton varieties developed by Prof. Bojinov. Evidence of 152 independent citations is attached, and only one of the publications, which I assume the author has identified as the most significant for him.

11. Abstract.

The abstract is well-structured and consists of 48 pages. It is structured in accordance with generally accepted requirements and summarises the structure and content of the dissertation. It is illustrated with 25 tables and 21 figures. It is clearly written and describes well the results obtained, as reflected in the dissertation. The abstract provides an adequate overview of the scope of the research conducted and the significance of the results obtained.

CONCLUSION:

Based on the critical review of the dissertation and the materials attached to the procedure, I believe that the presented dissertation fully meets the requirements of the ZRASRB and the Regulations of the Agricultural University for its application, which gives me reason to evaluate it POSITIVELY.

I would like to suggest that the esteemed Scientific Jury also vote positively and award Prof. Dr. Bojin Maksimov Bojinov the scientific degree of 'Doctor of Science' in the field of higher education: 4. Natural Sciences, Mathematics and Informatics; professional field: 4.3. Biological Sciences; scientific specialty: Genetics