OPINION

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, Faculty of Agronomy, The Agricultural University, Plovdiv.

Topic: "Use of molecular markers in research on genetic diversity and DNA profiling"

Reviewer: prof. DSc Ivan Iliev Atanassov, Agrobioinstitute, Agricultural Academy, Field of higher education 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-16/753, 12 June 2025, by the Rector of the Agricultural University.

1. Relevance of the topic.

The development and application of various types of molecular markers has a fundamental contribution to the advancement of modern genetics and breeding. Today, molecular markers are widely applied in all biological systems, microorganisms, plants, and animals, in a wide range of scientific and applied fields and research including: analysis of genetic diversity of populations and collections of genetic resources, mapping of QTL loci for valuable economic traits, accelerated breeding in plants and animals, protection of intellectual property, assessment of varietal identity and homogeneity of seed and planting material, etc. In Bulgaria, the applications of molecular markers are still very limited and there is a justified need to expand their use in breeding programs for plants and animals, characterization and utilization of available genetic resources, as well as their inclusion in the procedures for registration, testing, and control of seed and planting material from plant varieties and animal breeds. All this makes the topic of the dissertation relevant and with opportunities for further development and applications in new scientific and applied research.

2. Aim, objectives, hypotheses, and research methods.

The dissertation has a clearly defined and formulated objective to establish and test the effectiveness and reproducibility of the application of a molecular marker system for characterizing genetic diversity in various species of plants, animals, and microorganisms, which can also be successfully applied for the purposes of control in variety testing and seed

production in plants, breeding of local animal breeds, as well as in selection programs. To achieve the stated objective, three main tasks and four sub-tasks have been planned and executed, including: application of ISSR, AFLP, CAPS, and SSR markers for characterizing genetic diversity in populations and collections of genetic resources among a wide range of plant species and crops (cotton, tobacco, tomatoes, peppers, barley, wheat, corn, and Paulownia), local breeds of goats and isolates of some of the most widespread and economically important plant pathogens Phytophthora and Fusarium; application of tested marker systems for detecting loci for quantitative traits; and assessment of the applicability and effectiveness of ISSR markers in variety testing, variety maintenance, as well as conservation of biodiversity of local plant forms and animal breeds. The selected breeding materials, sources of the biological samples studied, and experimental methods are detailed, supported, and significantly contribute to the successful execution of the objectives and goals of the dissertation work.

3. Illustration and presentation of the results.

The dissertation is excellently and properly illustrated with 33 tables, 63 figures, and 17 photos, which correspond to and represent the essence of the research, the obtained results, and conclusions from their discussion. The inclusion of a sufficiently large set of photos from electrophoresis and fragment analysis of ISSR fragments from various studies well illustrates and presents the methods used and the experimental results of the main group of studies related to the application of ISSR markers and achieving the goal set by the dissertation.

4. Discussion of results and references.

The dissertation includes a large volume of experimental results from individual studies on various biological subjects and the use of different marker systems. Accordingly, the discussion of the results is excellently structured in relation to the biological subjects and the marker systems used. The main part of the section "Discussion of Results" is focused on a detailed discussion of the results from the various applications of ISSR markers in the presented studies, which significantly contributes to achieving the objective of the dissertation. The bibliographic reference includes a total of 280 titles, of which 279 are in Latin script and 1 in Cyrillic.

5. Contributions.

The dissertation includes a total of 13 conclusions, 5 scientific and 9 scientific-applicative, which fully reflect in the presented results and discussion.

Scientific contributions

The dissertation work has significant scientific and scientific-applied contributions. The main part of the formulated scientific contributions is related to the systematic application of ISSR markers for characterizing genetic diversity in a large number of different biological subjects (plants, animals, and microorganisms), combined with a comparative analysis and assessment of the obtained results in relation to the results of applying other marker systems on these

subjects. In my opinion, they constitute a significant overall contribution of the dissertation work, enabling its use by a wide range of specialists when making decisions on the application of ISSR markers in specific research and the subsequent use of the results obtained. In this field, there is also a contribution related to the demonstrated effectiveness of ISSR markers for characterizing the intraspecific diversity of fungal pathogens with potential for further widespread application in various types of fungi.

Applied scientific contributions

The scientific-applied contributions of the dissertation work include two main groups of contributions:

- Contributions related to results and demonstrated possibilities for effective practical applications of ISSR markers for characterizing and assessing the genetic diversity of various economically significant biological objects (crops, animal breeds, phytopathogens), as well as applications in variety testing, approval, and seed control in crops and certification and breeding of local animal breeds. Based on the presented results, conclusions, and formulated contributions, the author proposes the use of ISSR markers in the activities of the control bodies IACAC and IACPЖ.
- Contributions related to saturating the genetic map of cotton through mapping new molecular markers and identifying loci for valuable economic traits in local cotton varieties, as well as identifying markers associated with economic traits in tomatoes.

6. Critical comments and questions.

I have no critical comments on the dissertation.

7. Publications and citations..

Prof. Bozhinov participated in the procedure with a total of nine scientific articles: two with Q1, six with Q3, and one with Q4, as well as with four newly registered cotton varieties. The documentation for the procedure includes evidence of 152 independent citations of one of Prof. Bozhinov's publications, while a review of publicly available sources on the internet indicates a large number of citations for other publications by the author: 328 citations in Scopus and 658 citations in Google Scholar. The presented abstract objectively reflects the structure and content of the dissertation.

CONCLUSION:

Based on the presented research, the obtained results, the discussion and the formulated conclusions and contributions, 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 to 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**

Date: 29.08.2025
Plovdiv

REVIEWER: .....
(prof. DSc Ivan Atanassov)