OPINION

on the dissertation for the degree of Doctor of Science in: field of higher education 4. Natural Sciences, Mathematics and Informatics, professional field 4.3. Biological Sciences, scientific specialty "Genetics".

Author of the dissertation: Prof. Dr. Bojin Maximov Bojinov, 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: Prof. DSc Anelia Veneva Iantcheva, Agrobioinstitute, AA, 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-16-753/12.06. 2025 by the Rector of the Agricultural University.

1. Relevance of the problem.

The dissertation presented by Prof. Dr. Bojin Bojinov for the degree Doctor of Science covers a topical issue for modern biological and agricultural science, namely the application of molecular markers for analyzing genetic diversity and DNA profiling in plants, animals and plant pathogens. In modern agricultural science, the use of molecular markers supports the work of scientists and breeders in developing modern breeding programs in plants, through the use of "marker assisted selection" (MAS) and in animals breeding programs with the selection of suitable parents for crossbreeding. In addition to improving and accelerating the selection process, molecular markers determine the genetic identity of the studied materials, providing intellectual protection to breeders. The relevance of the dissertation work and the obtained results are characterized by fundamentality and applicability in modern agricultural science.

2. Aim, tasks, hypotheses and research methods.

The purpose of the dissertation work is determined very clearly to identify a reliable molecular marker system that can define the genetic diversity in the studied objects; plant, animal and fungal pathogens; to be easily applicable, so as to allow its use in testing and control of varieties and breeds. To achieve the goal, clearly formulated tasks and sub-tasks have been set. For the purposes of the study, a wide range of molecular marker systems was used, including both dominant (AFLP, ISSR, CAPS) and codominant marker systems (SSR, RFLP). Along with the numerous marker systems, the number of used research objects is also impressive: plants - cotton, tobacco, tomatoes, pepper, wheat,

barley, corn, the tree species Paulownia; fungal pathogens of the genus *Phytophthora* and the genus *Fusarium*, as well as animals - autochthonous goat breeds.

3. Visualization and presentation of the obtained results.

The dissertation work, as well as the abstract for the thesis, are illustrated with figures and tables of a very good quality. The exception are the photos of some of the gels reflecting the obtained results, which are not sufficiently contrasting. The obtained results are presented clearly, tracing plant, animal and fungal objects of the study. The results processed with appropriate statistical programs, which makes possible to reliably analyze the obtained results.

4. Discussion of the results and used literature.

The discussion section of the results is clearly described, giving an accurate idea of the possibilities of the studied marker systems, revealing their advantages and disadvantages depending on the direction of research in different objects - plants, fungal pathogens and animals. A marker system has been proposed, which is characterized by wide possibilities for reliable determination of genetic diversity. This marker system is capable of differentiating varieties and breeding lines, which gives it possibility to be used in testing for Distinction, homogeneity and stability, to identify metabolic profiles in different varieties and the differentiation of isolates in the studied fungal pathogens. The studied ISSR marker system can identify significant genetic diversity among autochthonous goat breeds. All this is also supported by the relatively easy methodology and reproducibility of the results obtained. In the discussion, the obtained results are compared with similar ones from literature sources.

5. Contributions of the dissertation.

Scientific contributions

I accept the five theoretical contributions mentioned, they characterize the significance of the presented dissertation and the originality of the research conducted.

Applied contributions

The nine applied contributions presented, reveal the potential of the obtained results for the define ISSR marker system in the development of breeding programs, analysis of genetic diversity in various organisms, for the purposes of state variety testing, approbation and seed control, for the independent certification and breeding of local animal breeds. A large number of molecular markers in cotton have been mapped, and the genetic diversity in two Bulgarian autochthonous goat breeds has been characterized.

6. Critical notes and questions

I have no critical notes and questions. I have a recommendation for Prof. Bojin Bojinov to write and prepare the methodical part of the dissertation work, as a laboratory aid for carrying out analyzes using different molecular marker systems for different objects of plant, animal and fungal pathogens.

7. Published articles and citations.

The results presented in the dissertation have been published in journals indexed in the global SCOPUS database, and a list of 152 citations to one of these publications is presented. The presented abstract of the dissertation objectively reflects the structure and content of the dissertation work.

CONCLUSION:

The targeted research conducted by Prof. Dr. Bojin Bojinov based on the use of various molecular marker systems in plant and animal organisms and fungal pathogens leads to scientific and applied results, which determine the originality, significance and innovative nature of the dissertation work. Based on the conclusions and the contributions made, I believe that the presented dissertation work meets the requirements of the Law on the Development of Academic staff in Republic of Bulgaria and the Regulation of the Agricultural University for its application, which gives me reason to evaluate it **POSITIVELY**.

I would like to propose that the esteemed Scientific Jury also vote positively and award Prof. Dr. Bojin Maximov Bojinov the scientific degree **"Doctor of Sciences"** in the scientific specialty "Genetics"

Date: 27.07.2025

Sofia

Author of the opinion:

/prof. Anelia Iantcheva DSc/