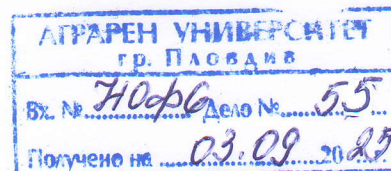


# REVIEW



on a dissertation for the degree of **"DOCTOR OF SCIENCES"** in: *Field of higher education: 4. Natural sciences, mathematics and informatics; Professional field 4.3. Biological sciences; Scientific specialty "Genetics"*

**Author of the Doctoral thesis:** *Prof. Dr. Bozhin Maksimov Bozhinov from the Department of Plant Physiology, Biochemistry and Genetics at the Agricultural University - Plovdiv*

**Topic of the Doctoral thesis:** *"Use of Molecular Markers in Genetic Diversity Studies and for DNA Profiling"*

**Reviewer:** *Prof. Dr.Sci. Diyana Lilova Svetleva, PhD, retired from the Agricultural University, Plovdiv; (Department of Genetics and Plant Breeding); appointed as a member of the Scientific jury by Order № RD-16-753/12.06.2025 signed by the Rector of the Agricultural University – Plovdiv*

## 1. General part.

Set of documents and materials presented by Prof. Dr. Bozhin Maksimov Bozhinov is complete and correctly presented in accordance with Articles 58 and 59 of the Regulations for Implementation of Law on Development of Academic Staff at the Agricultural University - Plovdiv.

## 2. General data on career and thematic development of the candidate.

Prof. Dr. Bojin Bojinov was born on 30.11.1965 in the town of Chirpan, Haskovo region.

He completed his higher education with a master's degree in 1990 at the Higher Agricultural Institute "Vasil Kolarov" (now Agricultural University) in Plovdiv with a specialty "Agronomist engineer" (Diploma Series A 87, No. 004625, issued 07.1990).

From 1990 to 1995, after successfully held competitions, he was appointed as a researcher associated in III, II and I degrees (equivalent to Assistant, Senior and Chief Assistant) in Cotton Breeding section at the Institute of Cotton and Durum Wheat, Chirpan.

After that (since 1995) his scientific career continued at Department of Genetics and Plant Breeding at the Faculty of Agronomy in Agricultural University, Plovdiv.

In 2000 he successfully defended his Doctoral thesis and obtained the Educational and Scientific Degree "Doctor of Philosophy (PhD)" in the scientific specialty "Breeding (Plant biotechnologies)", (Diploma No. 26902, issued on 23.08.2000). He was habilitated as an "Associate Professor" in 2005 (Certificate of the Higher Attestation Commission No. 23335, issued on 30.12.2005) and as a "Professor" - in 2024 (Diploma No. P 052, issued on 29.11.2024)



His path of development in Department of Genetics and Plant Breeding at the Faculty of Agronomy passes through several administrative positions. From 2008 to 2016 he was Dean of the Faculty for two terms, and from 2016 to 2020 - he was Head of the Department.

After the closure of the Department of Genetics and Plant Breeding at the Agricultural University, some of the lecturers, including Prof. Bozhinov, were transferred to the newly formed Department of Plant Physiology, Biochemistry and Genetics, of which he was elected as a Head in May 2024.

His organizational and leadership skills are complemented by the fact that he was a member of Temporary and Permanent Scientific and Expert Committees of the Scientific Research Fund for 3 terms and 2 terms. During the current term, he is a member of the Executive Council of the Fund with direction of Agricultural Sciences.

During the period 2013-2024, Prof. Bozhinov participated in 5 national (on 3 of them he was a moderator) and 5 international scientific forums dedicated to issues of improving breeding, agricultural development in various countries and application of modern molecular techniques and technologies in this field.

He is a member of a large number of prestigious scientific organizations - FAO Inter-regional cooperative research network on cotton; International Cotton Genome Initiative (ICGI); European Federation of Biotechnology (EFB) and Public Research and Regulation Initiative (PRRI).

Total duration of the scientific work experience of Prof. Bozhinov to date is 34 years, 29 of which are at the Agricultural University, Plovdiv.

He has very good computer literacy and he is proficient in many software programs and products.

Prof. Bozhinov uses in a high level English and Russian, and in a good level French.

### **3. Comparison of the minimum national requirements with the results of the candidate's scientific activity for acquisition of scientific degree "Doctor of Sciences".**

Based on the regulated minimum national requirements that candidates must meet to acquire the scientific degree "Doctor of Science" and from analysis of the scientific production, as well as scientific research activity carried out by Prof. Bozhinov, it is established that he meets the minimum requirements for relevant indicators.

According to requirements of the Regulations for Development of Academic Staff at the Agricultural University, with mandatory indicators for acquiring the scientific degree "Doctor of Sciences" (annex to Article 1a, paragraph 1) a total of 450 points, Prof. Bozhinov presents information about total obtained - **608**. This shows that it exceeds the minimum national requirements for acquiring scientific degree "Doctor of Sciences" by 1,35 times.

Distribution of points by indicators is as follows:

**By group of indicators A – Doctoral thesis for awarding the educational and scientific degree "Doctor" (minimum number for the criterion 50 points).**



In 2000, Prof. Bozhinov defended a Doctoral thesis titled: "Research on the possibilities of using *in vitro* methods in cotton breeding" for awarding of educational and scientific degree "Doctor" - **50 points**.

**By group of indicators B - Doctoral thesis for awarding of the scientific degree "Doctor of Science" (minimum number for the criterion 100 points).**

Prof. Bozhinov has prepared and presented for defense a dissertation project entitled: "Use of molecular markers in genetic diversity studies and for DNA profiling" for awarding the scientific degree "Doctor of Science" - **100 points**.

**By group of indicators D – (minimum number for the criterion 200 points, total 352 points are received).**

**7. Scientific publication in issues that has been referenced and indexed in world-renowned databases of scientific information with an impact factor.**

Nine articles in English are presented, which were published in the period 2003 - 2024 and are included in the Scopus database. They were published in prestigious international journals with impact factor/rank and quartiles in WoS/Scopus Q1, Q3 and Q4. Two of them are in quartile Q1 and are published in co-authorship with foreign colleagues. Six are in quartile Q3 and one is in quartile Q4, which is independent – **252 points**.

**9. An invention, patent or utility model for which a protected document has been issued in due course (Created lines and varieties, breeds/races of animals).**

Prof. Bozhinov presented certificates for 4 cotton varieties created during the period 2004 - 2020 (Cultivar Beli Iskar 800, Certificate with patent number 10556/31.03.2004; Cultivar Beli Lom 393, Certificate with patent number 10555/31.03.2004; Cultivar ITP-Veno, Certificate with patent number 10793/30.09.2008 and Cultivar Belitsa, Certificate with patent number 11210/30.04.2020) - **100 points**.

**By group of indicators E - 11. Citations in scientific publications, monographs, collective volumes and patents, referenced and indexed in world-renowned databases with scientific information (Web of Science and Scopus), (minimum number for the criterion 100 points, total 106 points are received).**

Fifty three citations of the article: Lacape, J. M., Nguyen, T. B., Thibivilliers, S., Bojinov, B., Courtois, B., Cantrell, R. G., Burr B., & Hau, B. (2003). A combined RFLP SSR AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. *Genome*, 46(4), 612-626" are indicated, found in publications in reputable scientific journals - **106 points**.

#### **4. Relevance of the problem.**

Relevance of the doctoral thesis is mainly determined by the following facts:

❖ Molecular markers provide specific advantages in determining genetic diversity and for improving plants and farm animals with respect to individual, economically important traits, as well as with respect to their DNA profiling.

❖ In the molecular studies of many plant, animal and phytopathogenic organisms, various markers and marker systems have been applied - both dominant



(AFLP, ISSR, CAPS) and codominant (RFLP, SSR), but very rarely have been given recommendations which of them can find rapid, accurate and clear application, as well as accurate reproducibility in different laboratory conditions.

❖ After using ISSR markers and their more in-depth study in different organisms, it was found that ISSR techniques are a better tool for analyzing the origin of gene blocks. ISSR markers do not require prior knowledge of the target sequences, they are well reproducible and provide high polymorphism.

❖ SSRs or microsatellites are described as short tandemly repeated DNA sequences (2 to 5 bp in length) widely distributed in the genome of eukaryotic organisms. Due to their abundance, high polymorphism between individuals in a population or in closely related genotypes, as well as Due to their multiallelic and codominant nature, microsatellite markers are used to analyze diversity between individuals.

❖ Gene mapping allows for MAS (marker assisted selection) – the use of markers in breeding work.

From the above, it is clear that Prof. Bozhinov very correctly selected the molecular systems with which he worked in various organisms in order to obtain a sufficiently accurate and informative assessment.

## **5. Purpose, tasks, hypotheses and research methods.**

Prof. Bozhinov very consistently and categorically justifies the necessity and importance of a comprehensive study of the problems considered in the "Literature Review" section, developed on 31 pages. The literature review is comprehensive and complete. It is entirely focused on the topic of the doctoral thesis. It covers the most important aspects of the problem and logically provides grounds for conducting these studies. On this basis, Prof. Bozhinov formulates the goal of his doctoral thesis: "To establish a molecular marker system that can be proposed for reliable and reproducible identification of genetic diversity in microorganisms, plant and animal organisms. This system should be able to be used for breeding purposes and for control in cultivars testing and seed production in plants, as well as in the breeding of various animal breeds".

In connection with achievement of this goal, 3 specific tasks and 4 subtasks have been set. In the process of developing the set goal and tasks, hypotheses were raised, and their specific crystallization is summarized in the presented conclusions doctoral thesis.

Methodology of the study is described in great detail on 48 pages. In my opinion, the volume is very big and a large part of this section could have been transferred to the "Results" section.

The studies were carried out on a wide variety of organisms. Plant: in agricultural crops - cotton, tobacco, tomatoes, barley, pepper, wheat, corn and the tree species - paulownia; Eukaryotic plant pathogens - phytophthora fungi and *Fusarium* sp.; Animal organisms - goats.

A very large number of plant crosses, hybrids and cultivars, 22 isolates of *Phytophthora* sp., 8 isolates of *Fusarium* sp. and 2 autochthonous breeds of goats – Kaloferska vitoroga dalgokosmesta and Bulgarska vitoroga dalgokosmesta.



Different molecular marker systems were used in the studies – **RFLP** (Restriction Fragment Length Polymorphism); **AFLP** (Amplified Fragment Length Polymorphism); **ISSR** (Inter Simple Sequence Repeat); **SSR** (Simple Sequence Repeats) and **CAPS** (Cleaved Amplified Polymorphic Sequence). An analysis of gene linkage was performed.

Number of DNA samples from different organisms is sufficiently large. Results have been statistically processed using appropriate methods, which allows for obtaining of reliable results.

## **6. Visuality and presentation of the obtained results.**

Presented doctoral thesis is a product of conducted extensive scientific research investigations. Its structure follows the generally accepted plan for this type of scientific work and includes following sections: Introduction, Literature Review, Aim and Objectives, Material and Methods, Results, Discussion, Conclusions, Contributions and References.

A total volume of doctoral thesis is 251 pages. Results obtained from the conducted studies are included in a "Results" section and are described on 94 pages. They are interpreted correctly and very thoroughly. Results are commented on in following areas: Phenotypic variation, DNA extraction, levels of polymorphism of molecular markers and profiles of samples, levels of genetic diversity. They are richly illustrated with 17 photos, 63 figures and 33 tables. The photos and figures are of relatively good quality. In my opinion, the only exceptions are figures with numbers from 44 to 48, which are not very clear and informative.

## **7. Discussion of results and used literature.**

Section "Discussion" is developed on 37 pages. It discusses what genetic relationships are found within and between cultivars and breeds; what are the consequences of distorted allelic segregation in cotton; what contribution does genetic mapping make to breeding and especially to implementation of so-called marker-assisted selection (MAS) and what is the applicability of ISSR markers in DHS (Distinction, Homogeneity and Stability) testing.

List of used literature includes 280 sources, presented on 54 pages. Of these, only one is in Bulgarian, and the rest are in Latin. I consider it appropriate to note that the largest number of literary sources are from the period 2000 - 2017 (165 issues – 58,93 %), which means that the most intensive work in the field of research area, included in the doctoral thesis, was done in this period. From the last 8 years (2018 - 2025) 43 sources have been included, which constitute 15,26 %. Number of sources published by Bulgarian authors is 11 (3,93 %). The statistics of literary sources give reason to conclude that the doctoral thesis of Prof. Bozhinov addresses a problem that is relatively new for Bulgaria. It is important and interesting from the point of view that the research was conducted exclusively with Bulgarian geneplasm from agricultural plant objects and from two autochthonous goat breeds.



## **8. Contributions of the doctoral thesis.**

In my opinion, it would be good, at the end before conclusions, to have a summary in which a parallel could be drawn between results obtained in the different studied organisms – to represent their similarity or their different.

Conclusions and Contributions sections are developed on a total of 7 pages.

Author of doctoral thesis has made 13 conclusions, which rather represent important summaries of the obtained results aimed at supporting future investigations in the area studied by him and with different types of organisms. Prof. Bozhinov has outlined contributions of the doctoral thesis, which he has divided into two groups: Theoretical (5 issues) and Applied (9 issues).

I consider contribution 2, from the group of theoretical contributions, to be methodological and contribution 3, from the same group, to be original. Contributions 6, 7, and 9 from the group of applied contributions are also original in my opinion.

I really liked the section "Used own investigations", which includes 23 articles developed by him in collaboration with his colleagues.

## **9. Published articles and citations.**

Nine articles in English are presented, which were published in the period 2003 - 2024 and are included in Scopus database. They were published in prestigious international and Bulgarian journals with quartiles in WoS/Scopus Q1, Q3 and Q4. Two of them are with quartile Q1 and are published in co-authorship with foreign colleagues. Six are with quartile Q3 and one with quartile Q4, which is independent. Total SJR (Scimago Journal and Country Rank) of Prof. Bozhinov is 4,084.

In two articles, Prof. Bozhinov is the first author, and in the remaining six articles he is in fourth and higher position.

Investigations described in articles have been carried out at the required level. Appropriate and modern methodology has been used. The results obtained are analyzed correctly and their discussion is supported by the opinions of other researchers who have published articles with studies close to the topic.

Presented declaration of originality proves that analyses included in doctoral thesis, the results obtained and summaries made are with personal participation of Prof. Bozhinov.

Prof. Bozhinov has indicated only 53 citations (which as a scientometric result are expressed as 106 points) of the article - Lacape, J. M., Nguyen, T. B., Thibivilliers, S., Bojinov, B., Courtois, B., Cantrell, R. G., Burr B., & Hau, B. (2003). A combined RFLP, SSR, AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. *Genome*, 46(4), 612-626. They were found in publications by foreign authors in reputable scientific journals. Total number of citations noted for this article is 152, which is a high attestation to its quality.

## **10. Abstract.**

Abstract is presented in Bulgarian. It is well-formatted in a volume of 48 pages. It is well structured and reflects in a summarized version the structure and content of doctoral thesis. It is illustrated with 25 tables and 21 figures. Abstract is written clearly and well illustrates obtained results, reflected in doctoral thesis. It is presented according to all the requirements of the law for such a publication.



## **11. Critical notes, questions and recommendations.**

### Notes:

- ❖ Titles of tables are written in very small font, which makes it difficult to read;
- ❖ Some of the tables (for example 7, 9, 19, 20 and 30) are divided into two pages. The same applies to figures 26, 27 and 41;
- ❖ Figure 39 is very unclear and lacks informative.

### Questions:

- ❖ Why are studied some qualitative and quantitative characteristics only in cotton, tomatoes and goats, and not in all organisms on which molecular analysis has been performed in doctoral thesis?
- ❖ Why were used different ISSR primers for the studied organisms?
- ❖ In Figures 29 and 31, for start 9 is written, that this is control. What is this control and why there are no distinct bands in it?

### Recommendations:

- ❖ I suggest Prof. Bozhinov try to look for a statistical relationship between induced bands, with a certain molecular weight from the corresponding identical ISSR primers, applied to some of the studied organisms. In my opinion, interesting results would be obtained.

## **CONCLUSION:**

Presented doctoral thesis contains scientific, scientifically applied and applied results that represent an original contribution to science and meet all the requirements of Law on Development of Academic Staff in Republic of Bulgaria (LDSRB), Regulations for Implementation of the LDSRB and relevant Regulations of Agricultural University Plovdiv.

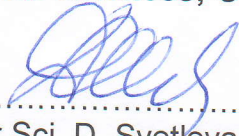
Doctoral thesis of Prof. Bozhin Bozhinov is dedicated to a current and significant problem for plant breeding and breeding of farm animals. It reflects in-depth knowledge, professional skills and opportunities for analytical presentation of own results and adequate argumentation of the relevant conclusions. He has presented 9 publications in connection with the developed doctoral thesis, which are included in Scopus database. Two of them are with quartile Q1 and are published in co-authorship with foreign colleagues. Six are with quartile Q3 and one with quartile Q4, which is independent. This is a very good certificate for the level of conducted investigations, adequacy of applied research methods and level of the obtained results, which are part of the doctoral thesis.

Prof. Bozhinov has presented certificates for 4 cotton cultivars created during the period 2004 - 2020 (Beli Iskar 800; Beli Lom 393; IPTP-Veno and Belitsa).

Doctoral thesis reviewed by me contains important information that can be used by the Executive Agency for Cultivar Testing, Approbation and Seed Control (EACAS), Sofia, when testing new lines and cultivars of cotton, tobacco, tomatoes, barley, pepper, wheat and corn.

Due to the above mentioned, I confidently give my **POSITIVE EVALUATION** for conducted investigations, presented by the above-reviewed doctoral thesis, abstract, achieved results and contributions. I **propose to the members of esteemed Scientific jury to award the scientific degree "DOCTOR OF SCIENCES"** to Prof. Bozhin Maksimov Bozhinov, PhD in the field of higher education: **4. Natural Sciences, Mathematics and Informatics**; Professional field: **4.3. Biological Sciences**; Scientific specialty: **"Genetics"**.

**Date:** 15<sup>th</sup> of August 2025  
City of Plovdiv

**REVIEWER:**   
(Prof. Dr.Sci. D. Svetleva, PhD)