REVIEW

by Prof. Dr. Tsanko Savov Gechev
Plovdiv University "Paisii Hilendarski

on the PhD thesis of Sibel Aziz

in Area of Higher Education: 4. Natural sciences, Mathematics, and Informatics

Professional field: 4.3. Biological sciences

PhD Programme: Genetics

Author: Sibel Dzevded Aziz
Title: Investigation of genetic variability in vegetable crops using molecular markers
Scientific PhD supervisors:
Prof. Dr. Teodora Atanasova Staykova, Plovdiv University
Prof. Dr. Nasia Borisova Tomlekova, Maritsa Vegetable Crops Research Institute

1. General review of the presented materials

With the Order No. РД-21-457 from 02.03.2023 г. by the Rector of Plovdiv University "Paisii Hilendarski, I was appointed as a member of the scientific evaluation committee for the defense of the PhD thesis entitled "Investigation of genetic variability in vegetable crops using molecular markers" in the Area of Higher Education: 4. Natural sciences, Mathematics, and Informatics, Professional field: 4.3. Biological sciences, PhD Programme: Genetics.

The author of the PhD thesis is Sibel Dzevded Aziz – regular full-time PhD student at the Department of Development, Plovdiv University "Paisii Hilendarski", and PhD supervisors Prof. Dr. Teodora Staykova from Plovdiv University "Paisii Hilendarski"and Prof. Nasia Tomlekova from the Maritsa Vegetable Crops Research Institute.

The set of paper materials presented by Sibel Aziz is in accordance with Article 36 (1) of the Regulations for the Development of the Academic Staff of the PU, includes the following documents:

- application form to the Rector of Plovdiv University “Paisii Hilendarski” for opening a procedure;
- Curriculum Vitae in European format;
- protocol of the Department, stating the readiness to open the procedure and the preliminary discussion on the PhD thesis;
- abstract;
- declaration of originality and authenticity of the attached documents;
- certificate of compliance with the minimum national requirements;
- list of publications;
- dissertation work;
- copies of the publications on the topic of the dissertation work;

Remarks to the documents:

I have not seen a list with the citations to her publications. The article Aziz S, Kantoğlu, KY, Tomlekova N, Staykova T, Ganeva D, Sarsu F (2021) Characterization of tomato genotypes by simple sequence repeats (SSR) molecular markers. Biharean Biologist 15 (2): 142-148 has two citations to date.

2. Brief biographical data about the PhD student

Sibel Aziz is born on 12.04.1993 г. She graduated BSc degrees in “Bioinformatics” and “Teacher in Biology” at the University of Plovdiv “Paisii Hilendarski” in 2016 and MSc “Biotechnology Microbiology” at the University of Plovdiv “Paisii Hilendarski” in 2017.

From 12.01.2017 to 01.05.2019, S. Aziz works as Expert in Biology at the Maritsa Vegetable Crops Research Institute in Plovdiv. After that, she works as Assistant Professor at the University of Plovdiv “Paisii Hilendarski”, where she works until now.

Sibel Aziz is enrolled as PhD student at the University of Plovdiv “Paisii Hilendarski” on 01.03.2019.

Since 2017 г.,. Sibel Aziz has two short-term specializations in Turkey (in the Center for Atomic Energy and the University of Hadjettepe), funded by projects of the International Atomic Energy Agency, coordinator Prof. Tomlekova. During the PhD study and the specialization abroad, Sibel Aziz learnt a number of molecular biology and bioinformatics techniques, including RAPD (Random Amplified Polymorphic DNA), ISAP (Inter-SINE amplified polymorphism), SSR (Simple

Sequence Repeats), ISSR (Inter Simple Sequence Repeats), and SCAR (Sequence-characterized amplified region).

3. Relevance of the topic and appropriateness of the set goals and tasks

The topic of the PhD study is relevant scientifically and in terms of applied research. The vegetable crops investigated (tomato, potato, common bean) are among the most important crops in Bulgaria and in the world. Bulgaria has a large diversity of cultivars, which are not fully studied in terms of their molecular biology and genetics, and in terms of their responses to the environment. Investigating their genetic diversity is of great importance to their future utilization in Bulgarian agriculture.

The specific tasks of the PhD study are directly connected with the characterization of this genetic diversity in a large number of cultivars, accessions, and mutant lines of tomato, potato, and common bean.

4. Knowledge of the problem

By reading the PhD thesis, especially the literature review and the discussion part in it, is that the PhD student is very familiar with the problem. The literature review is comprehensive, covers the modern state of art of vegetable crops selection, the applications of induced muthagenesis, the use of molecular markers and their application in the three vegetable crop species.

The reference list contains 323 articles, most of them published in the last few years. The discussion is blended with the results and is integrated with the current state of art very well, upgrading our knowledge in this field.

5. Methodology of the study

The molecular biology methods chosen in the PhD study (application of the marker systems: SSR and ISSR, based on microsatelites; ISAP based on polymorphism of the short mobile elements SINE and COS II), together with computational/bioinformatics and statistical analyses (databases NCBI, Sol Genomics Network, Pulse Crop Database; progremmes NTSYSpc-2.2j and SPSS), allow achieving the goals set and obtaining an adequate solution to the PhD objectives.

6. Characteristic features and evaluation of the thesis

The literature review, as noted above, is comprehensive and complete. It is structured in six parts: Importance of vegetable crops, Application of induced mythagenesis in plant biotechnology, Molecular-genetic markers and their application (parts 1-3) and investigating the genetic variability in tomato, potato, and common bean using molecular techniques (parts 4-6). There are six figures in the literature review. What I am missing is the application of the SNP markers in the GWAS (Genome Wide Associated Study) analysis – approach that is widely applicable for discovering genes and loci associated with important traits in vegetable crops.

The Methods section is comprehensive and includes description of plant material (cultivars, accessions, mutant lines – including tables and figures with their phenotype), detailed protocols of the PCR reactions, tables with the used SSR, SINE-ISAP, and COS II markers, as well as the bioinformatics databases and statistical programmes.

The first section of the results is dedicated to testing the applicability of the SSR, ISSR, ISAP, and COSII markers in tomato, potato, and common bean. The results show that these markers are suitable for the objectives of the PhD thesis. The second section of the results is dedicathe ted to investigation of genetic variability in tomato, potato, and common bean by using molecular markers. Results show specific DNA profiles of the particular cultivars and mutant lines, as well as phylogenetic trees indicating genetic distance between the selected cultivars, accessions, and mutant lines. This genetic variability is illustrated very well with 17 tables and 40 figures. In tomato, the most effective technique for identification of polymorphism is SSR, followed by ISSR, COS II, and ISAP. In potato, the most effective are ISAP and ISSR. In common bean, the most effective is the ISSR technique.

In my opinion, the work done by the PhD student is huge. The investigations are authentic.

7. Contributions and significance of the study for science and practice

The contributions of the PhD thesis can be divided into four categories: original scientific contributions, contributions toward applied research, confirmatory contributions, and contributions to the methodology.

Among the original scientific contributions, it is worth noting the use of ISAP method to investigate the genetic diversity of tomato and potato for the first time, which demonstrated the potential to identify polymorphisms. Another original contribution is the use of ISSR to investigate genetic polymorphism in Bulgarian potatoes, as well as Bulgarian mutant lines of common bean derived from the cultivar “Mastilen 11b” with valuable agricultural properties. Another original

contribution is also the use of the COS II marker system for investigating the genetic polymorphism of Bulgarian tomato cultivars and lines.

The application of SSR for early identification of tomato specimen is a contribution toward applied research. The polymorphism determined in this way can be used in future breeding programmes.

Another applied research contribution is the use oand ISAP и ISSR for genotyping mutant potato lines, which can be used for speeding up the mutational selection among them. Another contribution ate the specific SSR profiles in common bean with potential application for early identification of Bulgarian specimen, speeding the breeding programmes, protection of authorship, and transfer of genetic material.

The genetic diversity of M6 lines of common bean, determined by ISSR markers, can speed registration of new mutant cultivars.

Among the confirmatory contributions, it is worth noting the effectiveness of ISAP for identification of Bulgarian potato mutant lines, confirming the effectiveness of SSR for establishment of high polymorphic tomato profiles, and the effectiveness of ISSR for studying the genetic diversity of common bean. Introduction of four molecular marker techniques, based on microsatellites (SSR and ISSR), retrotransposons (ISAP), and SNPs (COS II) in selected Bulgarian cultivars and F1 hybrids from the collection of the Maritsa Vegetable Crops Research Institute is another methodological contribution. Another such contribution is the establishment of SSR, ISSR, and ISAP in potato mutant lines, created and maintained in the Maritsa Vegetable Crops Research Institute, as well as SSR and ISSR in selected Bulgarian common beans specimen. SSR markers, characteristic for the tomato genome, are introduced for the study of potato.

8. Evaluation of the publications based on the dissertation .....

Three publications originate from the PhD thesis. Two of the publications are scientific articles in journals with Q3 and Q4, respectively, with S. Aziz as first author, and the third publication is book chapter where the PhD student is co-author. Two of the publications have no citations yet (one of them is accepted for publication). The third one, published in 2021, has two citations, which indicates international visibility. The other two articles are very recent (2022 and 2023), so their true visibility will become clear in the future.

All three articles are authentic, with no overlapping results.

9. Personal contribution of the PhD student

My impression is that the PhD student Sibel Aziz has significant contribution for all scientific articles as well as for the whole PhD thesis. The amount of work on the PhD thesis is substantial, which indicates that S. Aziz is hard working and efficient.

10. Abstract

The Abstract is in accordance with the rules of Plovdiv University "Paisii Hilendarski" and correctly reflects the main results reported in the PhD thesis. The content of the thesis and the overall quality are at a very good level.

11. Critical remarks and recommendations

I have no critical remarks.

12. Personal impressions

My overall impression of the PhD thesis is very good. Sibel Aziz managed to get familiar with a very interesting topic and achieve results that have not only fundamental scientific value but also have potential for practical applications. Her two specializations abroad enriched her and contributed to her professional development.

I know the two Phd supervisors (Prof. Staykova and prof. Tomlekova) personally. I can frankly say that they are among the best scientists in their fields and they are also excellent tutors, which enabled them to transfer their knowledge and skills to the PhD student.

13. Recommendations for future utilization of the PhD results

My recommendation to the PhD student as well as to the two supervisors is to use this PhD thesis as a start of a more elaborate cooperation between the University of Plovdiv "Paisii Hilendarski" and the Maritsa Vegetable Crops Research Institute in the area of molecular analysis of vegetable crops and vegetable breeding.

CONCLUSION

In conclusion, having in mind the analysis above, I can say the following:

1. The PhD thesis has original scientific, applied research, and methodological contributions, which are clear advancement in the field.
2. The PhD thesis complies with the Law for Development of Academic Personnel in Republic of Bulgaria, the Regulations for its implementation, and the Regulations of the University of Plovdiv "Paisii Hilendarski".
3. The PhD thesis demonstrates that the PhD student Sibel Aziz had acquired deep theoretical knowledge and professional skills in Genetics, as well as ability to conduct independent research.

Taking into account the above evaluation, I give my **positive evaluation** for the PhD thesis (and its results and contributions) and **I propose to the esteemed Scientific Evaluation Committee to award the PhD degree** to Sibel Dzevded Aziz in the Area of Higher Education: 4. Natural sciences, Mathematics, and Informatics, Professional field: 4.3. Biological sciences, PhD Programme: Genetics.

07.04.2023 г.

Reviewer: .....

(подпис)

Professor Dr. Tsanko Gechev

(*ак. дл., н. ст., име, фамилия*)