REVIEW

From: **Prof. Dr.Sci. Diyana Lilova Svetleva, Ph.D.** - retired from the Agricultural University, Plovdiv on a Doctoral thesis for awarding the educational and scientific degree "doctor" in the *Area of Higher Education 4. "Natural Sciences, Mathematics and Informatics*; *Professional field 4.3."Biological Sciences"*; *Scientific specialty "Genetics"*

Author of the Doctoral thesis: **Sibel Dzhevdet Aziz**
Title of the thesis: **"Study of genetic variability in vegetable crops representatives through molecular markers"**

Scientific supervisor: **Prof. Teodora Atanasova Staykova, Ph.D. - Plovdiv University "Paisiy Hilendarski", Faculty of Biology, Department of "Developmental Biology"**

Prof. Nasya Borisova Tomlekova, Ph.D. - Institute of Vegetable Crops "Maritsa", Plovdiv

1. General description of the presented materials.

Description:

By order № RD-21-457 dated 02.03.2023 of Rector of the Plovdiv University "Paisii Hilendarski" (PU), I have been appointed as a member of a Scientific jury to ensure a procedure for the defense of a doctoral thesis titeled: *"Study of genetic variability in vegetable crops representatives through molecular markers"* for acquiring the educational and scientific degree "doctor" in the area of higher education: 4. Natural sciences, mathematics and informatics; professional field: 4.3. Biological Sciences; scientific specialty: Genetics.

Author of the thesis is Sibel Dzhevdet Aziz - full-time Ph.D. student at the Department of „Developmental Biology“, supervised by Prof. Teodora Atanasova Staykova, Ph.D. from the Plovdiv University "Paisii Hilendarski", Faculty of Biology, Department of "Developmental Biology" and Prof. Nasya Borisova Tomlekova, Ph.D. - Institute of Vegetable Crops "Maritsa", Plovdiv.

Set of materials, presented by Ph.D. student Sibel Aziz on paper and electronic variants, is in full compliance with Art. 36 (paragraph 1) of the Regulations for Development of Academic Staff of PU. All references are signed and contain reliable information.

Doctoral student Aziz has met the national scientometric requirements for the defense of her dissertation. With a required 30 points, she has collected a total of 42 points.

2. Brief biographical data for the doctoral student.

Sibel Dzhevdet Aziz was born on 12.04.1993.

She completed hers secondary education in 2012 at “Hristo Botev” Secondary School in Nikopol.

Hers higher education she completed at the Faculty of Biology of Plovdiv University "Paisiy Hilendarski", and in the period 2016-2017 she graduated with three majors - "Biology Teacher", "Bioinformatics" and "Biotechnological Microbiology".

During the period 2017-2019, she worked as an expert biologist at the Institute of Vegetable Crops "Maritsa", Plovdiv where from 2019 she was elected as an assistant in the scientific specialty "Genetics". She works in the scientific laboratory of "Molecular Biology" at the institute, where she conducts molecular research on various vegetable crops.

From 01.03.2019, after a successful competition, she was enrolled as a full-time doctoral student in the scientific specialty "Genetics" at the Faculty of Biology of the Paisii Hilendarski University of Plovdiv.

Ph.D. student Sibel Aziz is very active in improving her knowledge and practical skills. Through the IAEA-Vienna, she carried out two specializations at the Atomic Energy Center and Hacettepe University in Ankara, Turkey. There she carried out studies of genetic variability in representatives of the family *Solanacea* through molecular markers and proteomic studies for the detection of mutations in vegetable crops .

To improve her qualifications and to support her research, Ph.D. student participated in 3 international courses under the RER/5/024 program. Participated in 8 training courses at the University Center for Work with Young Scientists, Doctoral and Postdoctoral Students at the University of Plovdiv. Participated in 5 seminars organized by the Union of Scientists in Bulgaria - Plovdiv branch, "Mathematics and Informatics" Section, as well as in two *online* seminars - organized by the Swedish Agricultural Academy (Växtnoden) and COST Action PlantEd, and the other by the Olink platform.

Sibel Aziz participated very actively as a member in the development of 3 international, one European, one national project for the "Scientific Research Fund", Ministry of Education and Culture and 4 tasks from projects for the Agricultural Academy, Sofia. This gives her the opportunity to improve her skills in conducting scientific research and analyzing the results obtained from them. Fruit of this activity is the award for "Best Report" - first place in the "Biology and Agrobiology" section at the International Conference for Young Scientists, awarded by the Union of Scientists in Bulgaria - Plovdiv branch, received in 2020.

Sibel Aziz has participated in 2 international and one national conference.

She has very good organizational skills. During the period 2014 - 2017, she actively participated as an organizer and co-organizer of a number of events at the Student Council of PU "Paisiy Hilendarski". She is a member of the Union of Scientists in Bulgaria - Plovdiv branch and the Management Board of the Center for Doctoral Students and Postdoctoral Students "Academia luventutis" to Plovdiv University.

She is part of the team of the Association of Young Scientists in Bulgaria.

She speaks English and Turkish at a very good level. Uses various statistical programs freely.

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

A classic structure was used to shape the Doctoral thesis.

Following sections are the largest in terms of volume:

✓ Literature Review section is written in 44 pages. It includes 6 sections, in which the studies done and described in the literature so far in the field of Doctoral thesis development are very competently presented and discussed;

✓ Obtained results and their interpretation are elaborated on 95 pages;

✓ Literary sources included in the Doctoral thesis development are described on 22 pages in the "Bibliographic reference" section.

I was very impressed that in the section "Literature review" after each section, for the studied vegetable crops, a short summary is made that shows what are the future directions for research.

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

❖ Despite the observed phenotypic diversity in tomato, potato and bean, low genetic variability has been found at the molecular level. This is due to the continuous "selection pressure" applied and the use in the breeding work of a limited number of varieties and lines with a high cost price, according to the requirements of the farmers;

❖ Joint application of molecular investigations, as a support base, with the implementation of traditional breeding activity significantly accelerates the obtaining of new varieties with high economic and biological qualities;

❖ Application of various molecular techniques to assess the genetic potential of a given crop species increases and accelerates breeding efficiency. For this reason, in recent years, molecular studies have been applied to many agricultural crops and an increasing number of informative molecular markers have been used;

❖ Correct choice of appropriate molecular-marker systems affecting the highly variable regions in the genome is of great importance to establish the genetic heterogeneity;

❖ On the other hand, the establishment of suitable DNA markers for genotyping, makes it possible to sift out the phenotypic changes that are result of the environmental factors action, so breeding process is based mainly on genetic variability.

Ph.D. student Aziz very consistently and categorically substantiates the need and importance of a complex study of the problems discussed in "Literature review" section and on this basis shapes the goal of hers Doctoral thesis development. Five specific tasks set for its achievement convincingly demonstrate the routine and creative approach of Ph.D. student to solving the scientific problem.

3. Knowing of the problem.

List of cited literature includes 323 sources, of which 320 are in Latin and 3 in Cyrillic. It is particularly important to note that a number of the cited sources (92 items) were published in the last 8 years, which emphasizes the relevance of studies by Ph.D. student Aziz problem.

Discovery, investigations and inclusion in the Doctoral thesis development of such a rich volume of literary sources, which are very precisely included in analyzes of obtained results and writing literature review, shows that Ph.D. student Aziz has managed to penetrate into the depth of the investigated problems.

It is noteworthy that only 19 scientific publications were written by Bulgarian authors, which shows that for Bulgaria this problem is relatively new and in the future the Ph.D. student will be able to continue hers investigations in this area.

My general opinion is that the Ph.D. student shows a very good knowledge of studied topic and shows competence in analyzing problems under consideration.

4. Research methodology.

Methodical setting of conducted investigations is chosen and set correctly, which is very important for obtaining accurate and reliable results.

The experiments were carried out with three important vegetable crops - tomatoes, potatoes and beans choosent as plant matherial.

Following of them are selected for this purpose:

- Four hybrids (F_1); 3 Bulgarian varieties and 4 breeding lines of **tomatoes** from the species *Solanum lycopersicum* L. and two varieties obtained by interspecies hybridization (*Solanum lycopersicum* L. × *Solanum pimpinellifolium* L).; (*Solanum lycopersicum* L. × *Solanum chilense* L.).
- Sixteen induced EMS - mutant lines of **potato** (*Solanum tuberosum* L.), their parents and controls.
- Sixteen induced with ethyl methane sulfonate (EMS) mutant lines of **garden bean** (*Phaseolus vulgaris* L.) from the population of variety "Mastilen 11 b" from the collection of Institute of Vegetable Crops "Maritsa", Plovdiv.
- Ttwenty local samples and selection lines common bean (*Phaseolus vulgaris* L.).

Selected collections were investigated using marker systems: SSR and ISSR (microsatellite-based), ISAP (short mobile element polymorphism-based), SINE and COS II (restriction polymorphism).

Applied mathematical-statistical methods for the analysis of the obtained results are based on different software products:

- NTSYS-2.2j software program was used to analyze the composite arrays of all amplified fragments, allowing identification and grouping of profiles that included corresponding number of samples.
- To estimate genetic distance, dendrograms were constructed using UPGMA group method of unweighted pairs with arithmetic means. Software program SPSS Statistics 26 was used.
- PIC value was calculated in an *online* program (www.gene-calc.pl).

Another statistical method was used for multivariate scaling (ALSCAL) using software program SPSS Statistics 26. This is a graphical method to visualize proximity and distance between studied genotypes.

Above mentioned in the review convinces that Ph.D. student has quite rightly selected a very rich arsenal of appropriate methodological and statistical approaches for the analysis of obtained results, their interpretation and graphic presentation, which very well complements correct solution of the tasks to be performed.

4. Characterization and evaluation of the Doctoral thesis.

Presented Doctoral thesis is a product of extensive research work.

Structure of thesis development follows the generally accepted arrangement of sections in this type of scientific work: Introduction, Literature review, Aim and tasks, Material and methods, Results and discussion, Conclusions and Contributions, Bibliographic reference and a List of publications in connection with the Doctoral thesis.

Doctoral thesis includes 26 tables and 51 figures, of which 3 are photographs of very good quality.

The "Results and Discussion" chapter represents most essential and largest part of the thesis. It covers 4 main sections and 13 subsections, which include all research conducted and the molecular techniques used in the studied crops - tomatoes, potatoes and beans.

I believe that fourth section, which includes the results of a study of genetic variability in studied vegetable crops, is particularly significant. On the basis of conducted analyzes and constructed dendrograms, relationships and distances between genotypes included in the study are clearly and categorically outlined, which will show to breeders which pairs of them can be used in future breeding programs to obtain best combinations of the desired traits.

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

In the "Conclusions" chapter, 10 conclusions, which I accept unconditionally, are presented in a convincing manner and with categorical evidence. They are an illustration of the original, scientific and scientific-applied contributions in Doctoral thesis.

Of original scientific nature are contributions related to application of molecular methods, for the first time in Bulgaria, for study of varieties and accessions from collection of the Institute of Vegetable Crops "Maritsa", Plovdiv of vegetable crops studied by the Ph.D. student, as followed:

- ISAP and molecular-marker system COS II for study of genetic diversity in tomatoes;

- ISSR - for study of genetic variability in potatoes (mutant lines and their initial forms) and beans with valuable economic traits.

Following contributions of a scientific and applied nature have been outlined by the Ph.D. student:

Possibility of using SSR markers for early identification of tomato and bean accessions has been demonstrated, while ISAP and ISSR markers can be used for genotyping potato and bean mutant lines. Through their application, the genetic diversity presented or induced can also be ascertained. This shows that work in breeding studies with SSR, ISAP and ISSR markers can accelerate breeding process in order to obtain new varieties of tomatoes, potatoes and beans.

I believe that the results obtained and conclusions drawn by Ph.D. student Aziz in hers Doctoral thesis can be used by other authors in developing breeding strategies to obtain new varieties with improved qualities.

8. Evaluation of scientific publications published in connection with the Doctoral thesis.

In connection with the topic of Doctoral thesis, Ph.D. student has submitted a list of 3 scientific works published in the last three years.

Included in the list are two articles published in SIR-ranked journals (Q4 and Q3), as well as a co-authored book chapter accepted for publication in Advanced Crop Improvement, Volume 2 – Case Studies of Economically Important Crops.

All publications are in English. In two articles, Ph.D. student Aziz is the first author (66.7%), and as a co-author in the book she is in the second position (33.3%). This proves her leading role and her personal involvement in developing ideas for writing these scientific publications.

Investigations described in the articles are performed at a high level. An appropriate and modern methodology was used. Obtained results are properly analyzed and their discussion is supported by the opinions of other researchers who have published articles with studies close to their topic.

9. Personal participation of the Ph.D. student in conducted investigations.

Submitted declaration of originality proves that analyzes included in the Doctoral thesis, obtained results and made summaries are with personal participation of the Ph.D. student Sibel Aziz.

10. Abstract.

Abstract is well-formed in a volume of 32 pages. It is structured well and reflects in a summarized version the structure and content of the Doctoral thesis. It is illustrated by 5 tables, 7 figures and a diagram that clearly shows the research methods applied.

Abstract is clearly written and well reflects obtained results of the Doctoral thesis. It is presented in accordance with all the requirements of the law for such a publication.

11. Critical notes and recommendations.

Questions:

- Why did you decide to do investigations with three different vegetable crops that have completely different morphological and genetic characteristics?
- Why were not applied to the three vegetable crops all studied markers from different molecular techniques and to all studied genotypes?
- Can you recommend some of the applied molecular markers to be used as universal to reveal genetic polymorphisms in almost all or other vegetable crops and why?

Notes:

- Contributions of a methodological nature are not formulated correctly, because they do not prove what exactly has been changed by the Ph.D. student and what is new in the methodology of investigations;
- Most of the presented photos of gels are not of very good quality. Obtained bands are not clearly and contrastively visible (especially figure 24). I assume that polymorphism readings were done directly on the gels, in order to obtain more accurate results;
- There are some technical omissions and errors in the text;
- In the bibliographic reference for the cited literary sources under numbers 57 and 238, the years of publications are not indicated.

Questions asked and notes made by me are made so that in the future Ph.D. student can think about more opportunities to improve her investigations. I am in awe of the comprehensive research that has been carried out, which has been achieved as a result of very hard and focused work on the part of the Ph.D. student. She has achieved a lot considering that a doctorate is the earning of an Educational and Scientific degree "Doctor". So, Ph.D. student Sibel Aziz quite successfully covered both levels - she gained new, interesting and important knowledge and she coped quite successfully and accurately with the experiments conducted.

12. Personal impressions.

I do not know Ph.D. student personally and I have no personal impressions of her work, but I am very impressed by the presented Doctoral thesis.

13. Recommendations for future use of dissertation contributions and results.

Doctoral thesis presented by Ph.D. student Sibel Aziz is comprehensive and a sufficient number of varieties and genotypes of vegetable crops - tomatoes, potatoes and beans - have been studied. Statistically processing of the obtained results was carried out, which were correctly interpreted and logical conclusions and conclusions were drawn. Some of the mentioned contributions can serve as a basis for continuing research in this area by the Ph.D. student and some of her colleagues.

Considering original nature of some of the contributions in Doctoral thesis, I would recommend that Ph.D. student continue her investigations with only one culture, but to include a larger number of genotypes from other Bulgarian and foreign collections, as well as with some wild representatives of this species. To look for some specific relationships between morphological characters important for breeding and applied molecular markers. This will contribute to a real combination of molecular techniques with traditional breeding techniques and will help to shorten the breeding process for studied agricultural crop.

CONCLUSION

Doctoral thesis contains scientific, scientific-applied and applied results, which represent an original contribution to science and meet all requirements of Law on Development of Academic Staff in Republic of Bulgaria, Regulations for Implementation of the Low and relevant Regulations of University of Plovdiv "Paisii Hilendarski".

Doctoral thesis shows that Ph.D. student Sibel Aziz possesses in-depth theoretical knowledge and professional skills in the scientific specialty "Genetics", demonstrating qualities and skills for independent conduct of scientific investigations. She presented three articles in connection with developed doctoral thesis, two of which were reported at scientific conferences.

Due to the above, I confidently give my **POSITIVE ASSESSMENT** for conducted investigations, presented by the above-reviewed doctoral thesis, abstract, achieved results and contributions, and ***I propose to the honorable Scientific jury to award educational and scientific degree "doctor" to*** **Sibel Dzhevdet Aziz** in Area of higher education: ***4. Natural sciences, mathematics and informatics***; Professional field: ***4.3. Biological Sciences***; Scientific specialty: ***Genetics***.

30-th March, 2023 Reviwer: ……
City of Plovdiv (Prof. Dr.Sci. D. Svetleva, Ph.D.)