

EVALUATION STATEMENT

of dissertation for acquiring the educational and scientific degree "Doctor" in: field of higher education 6. Agrarian Science and Veterinary Medicine, professional field 6.1 Plant science, Major: *Genetics*.

Author of the dissertation: *Assistant Todorka Angelova Srebcheva* – full-time PhD student in the Plant Physiology, Biochemistry and Genetics Department of the Agrarian University, Plovdiv

Topic of the dissertation: *Analysis of Genes, Responsible for the Synthesis of Capsaicin in Peppers (Genus: *Capsicum*)*.

Evaluation statement, prepared by: Prof. Dr. Daniela Ganeva Ganeva, PhD, Maritsa Vegetable Crops Research Institute, Plovdiv, field of higher education: 6. Agrarian Science and Veterinary Medicine, professional field 6.1 Plant science, science major Selection and Seed Production of the Crop Plants, nominated as a member of a Scientific Jury by order №RD-16-948/09.27.2022 from the AU rector.

1. Relevance of the problem.

Pepper is one of the main vegetable crops in our country. The large germplasm collection and diversity in peppers is a good opportunity for conducting scientific research. The high productivity, excellent taste and nutritional qualities, high adaptability consider the pepper attractive for various production directions. Most of the researchers in Bulgaria work with sweet peppers. The research on hot peppers is relatively low. The synthesis of the *capsaicinoid capsaicin*, which determines the chilly taste of the fruits is unique to the species of genus *Capsicum*. The analysis and control of the genes responsible to produce capsaicin could lead to the increase of the genetic diversity and the creation of pepper forms possessing different alkaloid content levels as well as forms without the alkaloid. Phenotype assessment, genetic linkage, the use of DNA based technologies such as molecular marker systems, sequencing and bioinformatic instruments is of major importance for speeding up the pepper breeding process. The dissertation considers current problem with scientific and applied importance.

2. Objective, tasks, hypotheses, and research methods.

The topic of the dissertation is currently important, the aims and objectives are precise and clearly formulated. Five tasks are set, which fully meet the objective to determine the allelic status in loci determining capsaicin levels of Bulgarian pepper forms

and to identify presence of known and/or novel loci modifying capsaicin synthesis in them. The state-of-the-art methods used are properly selected based on the literature review and are consistent with the nature of this research and contribute to the successful achievement of the objective. Successful hybridization between selected parental lines was performed and the inheritance of capsaicin in F1 and F2 was studied. The hybrid nature of F1 generation was proven after organoleptic and laboratory analyses. The heterozygous state of the *Pun1* gene was confirmed, the hybrids were genotyped *Pun1/pun1-1*. In F2 of the crosses between *C. annuum* L. cv Familia x *C. chinense* Jacq. – Habanero pepper and *C. annuum* L. cv Delicacy x *C. frutescens* L. has been shown to involve a single gene (*Pun1*) controlling fruit pungency. In F2 of the cross *C. annuum* L. - cv Plovdivska kapia x *C. chinense* Jacq. (Habanero pepper), the presence of a second gene determining the absence of hotness has been demonstrated. The relationship between genetics and capsaicin biosynthesis in parental lines, F1 and F2, is investigated. Appropriate statistical methods were used to prove the results obtained.

3. Illustrations and presentation of the results obtained.

The dissertation is written in 130 pages, in a very good scientific style and the terminology used is correct. It is structured correctly and contains all the necessary sections according to the classical scheme. The research results are well presented and illustrated in 11 tables and 47 figures, some of which are photographs. The dissertation contains formulated conclusions, implications, and recommendations.

4. Discussion and literature cited.

The presented literature review of 267 titles is evidence of the doctoral student's awareness of the topic under study.

The PhD student has carried out a large amount of experimental work, increased theoretical and practical training, mastered, and applied new modern methods of analysis. Genetic and biochemical methods have been used to characterize biodiversity in pepper.

The results obtained have been analysed and interpreted competently, in a very good scientific style and using adequate terminology. The results obtained have been proven using appropriate statistical methods.

The results of the experiments and analyses carried out are summarized in eight conclusions, interesting both for their fundamental nature and for their scientific and practical orientation - they complement and enrich the knowledge and enable practical application to facilitate and accelerate the breeding process.

The dissertation developed is of high scientific and applied value, which makes it relevant and practically applicable to other crops.

5. Contributions of the doctoral dissertation.

The results received allowed the PhD student to formulate five scientific and four scientifically applicable contributions which I fully accept. For the first time the allelic state of the *Pun1* gene in the Bulgarian pepper varieties – Plovdiv Kapia, Familia and IZK Delikates, of the species *C. annuum* L. has been determined. Tracing down the inheritance of the genes and their mutant alleles responsible for the lack of pungency in the F₁ and F₂ generation in the cross-breeds *C. annuum* L. – cv Plovdiv Kapia x *C. chinense* Jacq. (Habanero pepper), *C. annuum* L. – cv Familia x *C. chinense* Jacq. (Habanero pepper) and *C. annuum* L. – cv IZK Delikates x *C. frutescens* L. The analyzed species and varieties of pepper could be used as a potential donor in future breeding programs.

The scientific and applied contributions are related to the application of a methodology to determine the allelic status of the *Pun1* gene, which is a good strategy for testing seed lots in purity and quality programs. For the first time, a successful hybridization was performed between the Bulgarian sweet pepper cultivars Plovdivska kapia, Familia and IZK Delicates from species *C. annuum* L. as mother component and the pungent species *C. chinense* Jacq. (Habanero pepper) and *C. frutescens* L. as male component.

The data received from the sequencing and processed through bioinformatic tools provided the foundation needed for conducting further analyses of the genes responsible for the synthesis of capsaicin in peppers (genus *Capsicum*).

6. Critical notes and questions.

I do not have critical notes or questions. The PhD student has fully considered the recommendations I made during the internal presentation.

7. Published articles and citations.

The PhD student has presented four scientific articles which are directly linked to the results from the dissertation. She is a leading author which proves her personal input.

The presented synopsis of the dissertation follows correctly the structure and the contents of the PhD thesis.

CONCLUSION:

The dissertation of the PhD student assistant **Todorka Angelova Srebcheva** proves her ability for performing independent research studies. She can execute correctly experiments in lab and field conditions and use various modern methods. She can also interpret well the results received and draws conclusions.

This dissertation meets the requirements of The Law for the Development of the Academic Staff in Republic of Bulgaria and the Regulations of the Agrarian University for its implementation, which allows me to give my **APPROVAL**.

I also suggest to the honorary scientific jury to also vote positively and award assistant **Todorka Angelova Srebcheva** with the academic and scientific degree "Doctor" in Genetics.

Date: 07.11.2022..... **STATEMENT PREPARED BY:**

Plovdiv



(Prof. Dr. Daniela Ganeva)