



REVIEW

by Assoc. prof. DSc Samir Izetov Naimov, University of Plovdiv "Paisii Hilendarski " on PhD thesis, entitled "Analysis of genes involved in capsaceine synthesis in pepper (genus *Capsicum*)" by Teodorka Angelova Srebcheva, PhD student in the department of Plant physiology, Biochemistry and genetics

1. Brief presentation of the PhD student.

Todorka Srebcheva was born in Kuklen, Bulgaria. At 2001st she graduates high school in Plovdiv, and during the period from 2011 to 2016 studies at the Agrarian University and obtains bachelor's degree in plant protection. During this period, she does her diploma thesis at the department of "Genetics and plant selection" under the supervision of assoc. prof. Bojinov. Later on, at 2018 Ms Srebcheva obtains a master's degree in molecular biology and biotechnology at department of Plant physiology and molecular biology at University of Plovdiv. Since 2018 she is a PhD student in genetics. Until 2021st Ms Srebcehva has successfully passed all obligatory exams and did fulfil all formal requirements in accordance with the Bulgarian law of higher education and science.

2. Relevance of the problem

The pepper is an economically important crop plant, grown around the world. One of it's specific qualities is the synthesis of capsaicine, is a unique alkaloid found primarily in the fruit of the *Capsicum* genus. It is what provides its spicy flavor the fruits and therefor is an important agronomic trat, subjected to intensive selection. A quick literature search using the word "capsaicine" reveals approximately 5000 hits, half of them are in the field of capsaicine synthesis or its genetic regulation. However only three articled do describe experiments on development QTL markers suitable for marker assisted selection. Since the research described in the PhD thesis, reviewed is focused on capsaicine synthesis, its inheritance, and possible implications for marker assisted selection I found the thesis of high importance and relevance to the demands of modern pepper selection.

3. Aims, hypothesis and research methods

The literature provided in the thesis is prepared in a good scientific manner, which indicated the applicant's knowledge and ability for critical reading. The main research goals are very well defined and reflects the current state of the art. The main aim of the study is defined as an investigation of the allelic structure of capsaicin synthesis related loci and screening for eventually new loci associated with the trait. In order to achieve the goal, the applicant has defined five concrete tasks which includes a number of crossings, backcrossing and molecular analysis of known trait associated loci. The research program is straight forward and professionally designed.

4. Scientific results and data representation

The research data obtained during the study are described in great details in the chapter "Results and discussion". The presence of the genes *pun1-1*, *pun1-2*, and *pun1-3* in local, Bulgarian cultivars pepper is studied and their allelic state is investigated in deep details. Six different types of pepper hybrids between those cultivars and *C. chinense* (Jacq) and *C. frutescens* (L) has been produced as follows: 1) *C. annuum*, cv. Plovdivska kapia x *C. chinense*, Habanero; 2) *C. annuum*, cv. Plovdivska kapia x *C. frutescens*; 3) *C. annuum*, cv. Familia x *C. chinense*, Habanero; 4) *C. annuum*, cv. Familia x *C. frutescens*; 5) *C. annuum*, cv. IZK Delikates x *C. chinense*, Habanero и 6) *C. annuum*, cv. IZK Delikates x *C. frutescens*. The resulted F1 and F2 hybrids are further analyzed for a their morphological, organoleptic, and genetic characteristics. The data obtained is subjected to a statistical analysis and the outcome is critically analyzed and discussed. Based on the ISSR profiles high-quality phylogenetic trees are constructed and the structure of the trees is further analyzed. The identity of the PCR products derived from molecular analysis is confirmed by Sanger sequencing, followed by BLAST analysis. Thirteen tables and 47 high quality figures are used for illustration of the results. All figures, used in the PhD thesis, are made by the applicant.

5. Discussion and literature review

The literature review of the thesis consists of 36 standard pages and provides a comprehensive overview on the inheritance and genetic control of the pepper spice determination. The physiological, climatic and genetic factors determining the capsaicine synthesis and accumulation are described in details. The theoretical background of the molecular biology methods,

used in the thesis, including more routine and high-throughput methods are described. The chapter provides sufficient amount of information related to RNAseq, QTLs, WGS, and general transcription factors, related to the subject of the thesis. The chapter is written in scientific but still easy and reader friendly manner. The literature review is based on 268 research articles, all written in English.

The research data obtained by the applicant and described in the thesis is critically discussed and compared to the findings of the other groups. In discussion the PhD student demonstrates deep understanding of the theoretical bases of capsaicin synthesis, and its inheritance and regulation. From the other hand Ms Srebcheva makes evident of deep knowledge on theoretical and practical aspects of the methods used during her PhD study. The main results from the study are summarized in eight carefully formulated and evidence supported conclusions.

6. Main contributions of the thesis

Based on the scientific findings and research conclusions made by the applicant five fundamental contributions and four contributions with potential application in the practice are formulated.

7. Questions and remarks

Since I had an opportunity to make an comments in the preliminary thesis review, and all my questions and remarks were correctly addressed, I have no additional questions. However, there is still need of improvement in documents preparation in general.

8. Publications

The applicant has presented four publications related to the thesis as follows:

1. Srebcheva, T., & Bojinov, B. (2018). Genetic control of α in pepper (*Capsicum sp.*) *Știința Agricolă*, (2), 57-63.
2. Todorka, S., & Bojin, B. (2019). Identification of allelic State of the Pun-1 gene associated with the capsaicin synthesis in selected *Capsicum* (pepper) species. *Научни трудове на Съюза на учените-Пловдив. Серия В: Техника и технологии*, 17, 257-260.

3. Srebcheva, T., & Kostova, M. (2022). Influence of the *pun1* gene on capsaicin synthesis in hybrid lines of the genus *Capsicum*. *Trakia Journal of Sciences*, 20(1), 37. DOI:10.15547/tjs.2022.01.005 Corpus ID: 248190910, ISSN 1313-3551 (online).
4. Srebcheva, T., Kostova, M. (2022). Study of the inheritance of pungency in a hybrid pepper lines (genus *Capsicum*). Изследване унаследяването на лютивината в хибридна линия пипер (род *Capsicum*). *Journal of Mountain Agriculture on the Balkans (JMAB) Journal of Mountain Agriculture on the Balkans*, 2022, 25 (1), 407-422 ISSN1311-0489 (Print), ISSN 2367-836 (Online).

One of the publications in fact is a minireview on capsaicin synthesis inheritance and on associated organoleptic qualities. Three of the articles are directly linked to the scientific findings of the applicant during her PhD study. In all cases Ms Srebcheva is a first author. The quantity and quality of the publications are sufficient to fulfil specific requirements necessary to obtain a PhD degree. The main findings and research conclusions are summarized in a separate booklet which is sufficient to provide good understanding of the main results of the applicant. No sign of plagiarism or unethical use of knowledge and knowhow was found.

Conclusion:

The reviewed PhD thesis is a original research work of Todorka Srebcheva. It is a complete high quality scientific study. The applicant has managed to obtain a high-quality research data with fundamental and practical importance. Most of the findings can be further exploited and eventually used in practice as markers for assisted plant selection. The PhD thesis fulfils all legal and specific requirements for obtaining doctoral degree. In this respect I would like to confirm my **POSITIVE** assessment and I would highly recommend awarding Ms Todorka Srebcheva a **doctoral** degree on field of high education: 6. Agrarian sciences and veterinary medicine; Professional area 6.1. Plant breeding, Scientific domain - genetics.

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Plovdiv

Reviewer:
(Assoc. prof. Samir Naimov DSc)