Kraków, March 28, 2023

REWIEW

of the doctoral thesis of Ms. Maja Szymańska-Lejman, MSc

titled "*Impact of DNA interhomolog polymorphism on meiotic crossover formation at the genome-wide and recombination hotspot scale in Arabidopsis thaliana*"

Introduction

Meiotic crossover recombination is a process of utmost biological importance. It has been a subject of investigation conducted throughout many years by numerous research groups, using a variety of model and non-model organisms. As it is fundamental to all eucaryota, general mechanisms have been recognized in yeast and animals, however more specific characteristics of factors governing meiotic crossover recombination in plants are still not fully elucidated. The PhD dissertation of Maja Szymańska-Lejman provided experimental clues to describe some of those factors, including the effects of global and local polymorphisms between parents and the importance of the *Msh2* gene on the crossover frequency depending on the local level of polymorphism. The research was performed on *Arabidopsis thaliana*, a model plant species widely used in plant molecular genetics and genomics, having a small genome with less-than-usual share of the repetitive DNA fraction. A significant methodological advance introduced in the dissertation, i.e. development and application of the *seed-typing* technology should also be noted.

Structure of the dissertation

The dissertation comprises 210 printed pages. Three papers published in reputable journals (*Nature Communications, EMBO Journal, Frontiers in Plant Science*), including supplementary materials, and one book chapter are the main constituents of the thesis. A section titled "Description of the results of the doctoral thesis" comprises the description of the scientific

profile of the candidate (one page), introduction (11 pages; divided into subchapters), aims of the thesis (one page), the main achievements of the four articles (14 pages), conclusions (one page), and the list of references (18 pages). Author's statement and co-authors' contribution statements are also included, along with one-page-long abstracts in Polish and in English, the list of scientific articles included in the dissertation, the list of abbreviations and information about funding. The documentation is complete, it complies with legal requirements and allows substantive evaluation of scientific achievements of the PhD candidate.

Evaluation of the scientific merit of the dissertation

The research aimed at the investigation of the impact of interhomolog polymorphisms in *A. thaliana* on meiotic recombination at genome-wide and local scales. It started with a comprehensive description of the state-or-the-art knowledge on meiotic crossover recombination mechanisms in plants. It was compiled in the highly informative review paper originally published in *Frontiers in Plant Science* (2018) and included in the dissertation. It provided a very thorough description of the general background and summarized recent achievements on the subject of interest. Ms. Maja Szymańska-Lejman specifically contributed to sections describing the general information about crossover mechanisms in plants and impact of double strand break (DSB) formation on crossover distribution, she also participated in developing the general concept of the article. I found the paper intellectually stimulating, I am expecting to discuss some issues in the course of the public defense of the PhD thesis.

Ms. Maja Szymanska-Lejman also contributed to the development of advanced methods allowing investigation of the process of meiotic crossover recombination by optimizing the genotyping-by-sequencing (GBS) strategy to study genome-wide crossover distribution in *A. thaliana*, developing a CRISPR/Cas9 genome edition protocol for efficient generation of deletion mutants in *A. thaliana*, and adapting the *seed-typing* method to study local (hotspot) scale crossover distribution. Application of those methods was her key input in the original research paper published in the *EMBO Journal* (2020) and the book chapter (published in 2022) describing the protocol of the CRISPR/Cas9 edition of the *A. thaliana* genome.

She used those methods in full swing in the original research paper published in *Nature Communications* (2023). I view the article as tour de force of the dissertation, as the PhD

candidate was the first author of the paper and largely contributed to the success of the study at all stages, including its design, laboratory work, visual presentation and writing. In the paper, she generated *A. thaliana* lines allowing studies on DNA polymorphism versus recombination, and subsequently used them to reveal the effect of structural variation (SNPs and indels) on crossover frequency at a local scale and to investigate the interplay between the action of *Msh2* and the local SNP density. The investigation was very well designed and benefited from the development of extremely short interval lines (ESILs) carrying recombination hotspots on *A. thaliana* chromosome 3 flanked with fluorescent reporters separated by less than 50 Kb. In particular, observations of three closely positioned crossover hotspots located in the pericentromeric region provided novel information on hotspot competition (or the lack of it).

The results reported in the articles included in the dissertation provide major novel contribution to the knowledge on meiotic recombination in plants. They include evidence of the lack of short-distance hotspot competition, involvement of Msh2 in local and genome-wide distribution of crossovers, confirmation of the heterozygosity effect on the local scale. The developed *seed-typing* method, combining visual identification of recombinant seeds followed by long-range PCR and NGS genotyping of the crossover hotspots in recombinant individuals, provides the research community with a valuable tool for future meiotic crossover recombination studies in *A. thaliana*.

Conclusion

The doctoral dissertation of Ms. Maja Szymańska-Lejman is of outstanding quality. The theoretical section of the thesis clearly shows her deep knowledge on the subject of investigation. Methodology-wise, she not only competently used a range of existing laboratory protocols, but also developed novel approaches enabling more effective studies on meiotic crossovers in *A. thaliana*. She is perfectly capable of working in a team comprising multinational/multi-institutional members. She is able to plan her research, prioritize goals and provide sound conclusions from complete and well-documented results. In short, she is a mature scientist with expert knowledge on the phenomenon of meiotic crossover recombination.

The dissertation fulfills all requirements indicated in the currently binding regulations (Ustawa Prawo o szkolnictwie wyższym i nauce; Dz. U. z 2018 r. poz. 1668, z późn. zm.). In

particular, it provides an original solution of a scientific problem (art. 187 ust. 2), it confirms the general theoretical knowledge of Ms. Maja Szymańska-Lejman in the discipline of biological sciences and her ability to conduct research (art. 187 ust. 1).

Thus, I put forward a motion to the Council of the Discipline of Biological Sciences of the Adam Mickiewicz University in Poznań to admit the PhD candidate, Ms. Maja Szymańska-Lejman, to further stages of the procedure. Also, taking into account the very high scientific level I put forward a motion to award the dissertation.

prof. dr hab. inż. Dariusz Grzebelus