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Reviewers report concerning PhD thesis entitled „Impact of DNA interhomolog polymorphism on meiotic crossover formation at the genome-wide and recombination hotspot scale in *Arabidopsis thaliana*” by Msc Maja Szymańska-Lejman

General assessment

The subject of the presented research is closely related to the longstanding scientific interest area of the Ziółkowski's team - the promoter of the work. So far the scientific consequence of the aforementioned research team allowed the development of unique and effective tools enabling the work on various aspects underlying the meiosis process in plants. Here I would like to point out that, apart from the candidate's individual contribution, the issues mentioned above have a significant impact on the undoubtedly high quality of the doctoral dissertation authored by Szymańska-Lejman, MSc.

The dissertation was prepared in the form of a collection of thematically related articles, in which Mrs. Szymańska-Lejman, MSc, is the co-author or lead author. The dissertation consists of two original research experimental works, the method paper describing the development of tools and the protocol, and the review paper. The results of the presented works were achieved thanks to the financial support obtained by the thesis supervisor (two projects of the National Science Center and the project of the Foundation for Polish Science) and by the author of the dissertation (three university-funded internal projects and the PRELUDIUM project funded by the National Science Center, in which the author was the primary investigator).

The structure of submitted document is typical for this type of dissertation. The description of the results begins with a chapter presenting the scientific profile of the candidate. This very accurate editorial device facilitates the formal review of the thesis and clearly presents the candidate's scientific career path. While analyzing the activity from the bachelor's thesis, through the master's thesis, or participation in student activities,

we can notice a very high interest of the candidate in the subject areas like genetic engineering, genomics or even some aspects of synthetic biology. The results of these works were published or were presented at conferences. Moreover, the project in the field of synthetic biology was awarded in a competition organized by MIT. All this indicates that the choice of a scientific career was not accidental.

This chapter is followed by the introduction part, in which the candidate outlines in a synthetic manner the subject of study and demonstrates her contribution to the progress of research in this area. Here it is worth emphasizing the clever editorial maneuver, like distinguishing the authors contribution by the blue font style. This well-edited and balanced chapter also shows that the works selected for the dissertation are not random. Not only the resulting papers, but also the developed tools, as well as the observations presented in the review work, are directly related to the dissertation.

The third chapter presents the objectives of the work and ways used to address them. Here I have to point out that the author has not presented the assumptions of the work or working hypotheses that could be subjected to further experimental verification, confirmation or rejection.

The subsequent chapters concern the description of the results obtained in individual publications that are integral part of the dissertation included in the form of reprints together with appropriate supplementary data (chapter 10). The obtained results and their interpretation are summarized in chapter eight, entitled Conclusions. This chapter is well developed, the conclusions are real conclusions - not just the summary of results.

The dissertation also contains the co-authors statements revealing their individual contribution to published works. That fulfils typical requirements for dissertations being a collection of subject-linked publications. Considering the above issues, I admit that the thesis has been prepared carefully in a form that is sufficient for a substantive assessment of the candidate's general knowledge, ability to independently conduct research and solve scientific problems.

Substantive assessment of the PhD dissertation

General understanding of biology and knowledge of the candidate in the field of exact sciences

The candidate demonstrates a very good understanding of the basic terms and rules of plant biology. That also includes aspects more directly related to the subject of studies

presented in her dissertation. This is reflected by a very well written introduction part that helps to understand result of the work where the PhD candidate describes both the process of meiotic recombination and more detailed aspects like the formation of so-called “recombination hotspots” or the influence of a single nucleotide polymorphism on genomic recombination. The abovementioned part of the dissertation presents the current state of knowledge, moreover, it is accurately confronted with the results obtained jointly by the PhD candidate and other research team members involved in this work.

Another point that proves the good orientation of the candidate in the field of studies is the fact that she is the first author of the review paper „ Where to cross over? Defining crossover sites in plants” published in 2018 in the Frontiers in Plant Sciences. In this work, in a very informative way, she addresses such issues as relation between the double strand breaks repair and chromatin structure regulatory mechanisms and the frequency of the recombination hotspots occurrence. Also she emphasise the role of heterozygosity and genetic polymorphism on the recombination frequency. Good understanding of the subject is further reflected in research publications included in this dissertation. Obtained results that are presented in these works prove extraordinary understanding and knowledge on the field of general plant sciences that in Poland are classified as the part of the exact sciences discipline.

The ability to conduct independent research

In the case of exact sciences evaluation of this aspect is rather difficult, mainly due to the fact that usually the experimental work is carried out by research teams, in fact frequently as a cooperative effort of many research teams. Here I must admit however, that the works included in the dissertation, interpretation and description of results provided by the PhD candidate manifest the great independence and individual approach of the researcher. In the work describing the relation between the genetic variability of the MSH2 gene region and the distribution of crossing-over sites the Genotyping-by-Sequencing (GBS) method was used (Blackwell et al., EMBO J, 2020). The author of this dissertation became familiar with this approach during one month research stay in the laboratory of Prof. Ian Henderson (University of Cambridge). Thanks to this the candidate has participated in the preparation of libraries and after their sequencing, she was able to carry out data mining analyses leading to description of the relationship

between the crossing-over frequency and the density of polymorphic region occurrence. That also helped to shed more light on the role of the MSH2 factor in promoting of the crossing over in heterologous regions juxtapositioned to more homozygous regions.

The PhD candidate participation in the next work included in the dissertation (Methods in Mol. Biol., 2022) is not massive – she was involved in the description of technical details of the procedure and the creation of some constructs allowing generation of knock-out mutants via the CRISPR/Cas9 gene editing. Here, however I would like to stress the fact that this participation was not accidental, since she was further involved in both refinement as well as the practical use of the system. This resulted in the generation of materials described in the main experimental work being a part of the dissertation (Szymańska-Lejman et al., Nature Communications, 2023).

The major, and in my opinion the most substantial achievement of the work by Ms Maja Szymańska-Lejman is a description of the influence of natural genetic diversity present amongst the *Arabidopsis thaliana* species on the activity of crossover hotspots. The work was published in the prestigious journal Nature Communications and Ms Szymańska-Lejman plays a leading role in it. She developed fluorescent system that was subsequently used for precise studies of the crossover frequency. The name of the system components given by the author “extremely short interval lines” is very accurate, since she has managed to engineer lines having fluorescent protein markers placed at a distance not exceeding 50 kb. It was this approach that allowed for mapping the crossover breakpoints in the F2 progeny of 242 Col-0 x Ler recombinants and identification of three recombination hotspots. As an inquisitive person I did some “research” to learn why these hotspots were called Aro, Coco and Nala. I had a lot of fun solving the mystery, since names were given in honour of dogs owned by team members. That additionally proves a huge commitment (also emotional) to scientific work. Going back to the essence of reported findings; the study shows that there is a high degree of conservation of hotspots between different *Arabidopsis thaliana* natural accessions, moreover these hotspots occur predominantly in regions of a high genetic variability (SNP-rich sites). Interestingly crossover brake-points tend to occur in non-polymorphic central regions of hot-spots. In further experiments with the *msh2* mutant the author shows that the detection of the local SNP region located in the vicinity of the hot-spot requires the action of MSH2 factor. This recognition triggers crossover process activation at the recombination hotspots. In this work individually or together with

other co-authors MSc Szymańska-Lejman conducted all experiments, except generation of the *msh2* mutant in *Ler* accession. The critical factor that gave a foundation to the whole work was generation of the seed-typing system. I will refer to this aspect in the next paragraph of the reviewers report.

After getting acquainted with the statements of all co-authors of the publications included in this dissertation I am able to conclude that the contribution of Ms Maja Szymanska-Lejman is significant and the experiments that she performed are crucial – in particular in the case of the most important work in this collection (Szymanska-Lejman et al., Nature Communications, 2023). Of course, what is understandable in the case of supervising and being the research group leader, the burden of setting the direction of research or general planning of experimental setup rested on dr hab. Piotr Ziółkowski (what is also consistent with his statement). All of this, however does not undermine the individual intellectual and practical contribution of the author of this dissertation.

The novelty of the scientific finding and problem solving

I allowed myself to start the evaluation of this aspect from the review paper co-authored by the candidate. Undoubtedly this is a good, well elaborated study going far beyond the summary of the current state of knowledge. The author shares with us individual opinions and points out issues worth further exploring in the future.

The CRISPR/Cas9 protocol co-authored by Ms Maja Szymańska-Lejman is also very original, since it uses very neat seeds-specific fluorescent selection system, that allows to obtain Cas9-free knock-out mutants.

The findings reported in other two research papers were achieved with help of the fluorescent seed systems facilitating meiosis frequency assessment that were obtained by the author of the thesis. In particular, the “seed typing” system (due to the small distance between markers) allowed to estimate the relationship between genetic variability (presence of SNPs) in *Arabidopsis thaliana* accessions and frequency of recombination hot spots occurrence. The combination of this approach with a well-chosen sequencing method and the use of gene editing tools (for example for the *msh2* mutant) paved the way to further localisation of crossover breakpoint sites and demonstration of the MSH2 factor participation in the recognition of local SNP-rich regions in the vicinity of hotspots.

The results of this basic research help to understand how plants, by regulating their genetic variability, adapt to the surrounding environment. As we can see, certain genomic regions showing greater variability act as sites of genetic rearrangements, thanks to which plants adjust to unfavourable environmental conditions, improve their competitive potential in the natural habitats or gain resistance to pathogens.

Summary of the assessment

The thesis meets all criteria pursuant to art. 187 of the Act of 20 July 2018 The Law on Higher Education and Science (Journal of laws 2022, item 574, as amended). Considering this and all other aspects revealed in the reviewers report I would like to recommend the thesis to be accepted and the candidate should be awarded the PhD degree in the field of exact and natural sciences, and the discipline of biological sciences.

Overall, the author demonstrates the outstanding scientific achievement and shows an appropriate understanding of the subject, therefore I would like to recommend the distinction for the thesis and the PhD candidate to the Scientific Board Biological Sciences Discipline of the Adam Mickiewicz University.

Kind regards

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