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Review report on PhD thesis submitted by Nadia Kbiri, MSc.

entitled

“Genetic factors in *Arabidopsis thaliana* meiotic recombination: Mapping new crossover modifiers and characterizing MutL complexes”

Assessment of the scientific problem addressed in the thesis

The doctoral dissertation of Nadia Kbiri, M.Sc. has been prepared at the Laboratory of Genome Biology, Faculty of Biology of the Adam Mickiewicz University, under the supervision of Professor Piotr Ziółkowski. The studies of the dissertation focus on genetic mapping of crossover modifiers using natural accessions and characterization of MutL complexes in *Arabidopsis thaliana*.

Meiotic crossover recombination ensures balanced segregation of chromosomes and contributes to genetic diversity in progeny. Meiotic recombination initiates with the formation of numerous DNA double-strand breaks (DBSs), but only a small number of these DSBs are repaired as chromosomal exchanges, termed crossovers, indicating that the number of crossovers is limited and regulated tightly. Crossover frequency and distribution are regulated by epigenetic factors that lead crossovers to locate preferentially in gene-rich euchromatin but suppress in heterochromatic pericentromeres and centromeres. The narrow range of crossover numbers (1-3 crossovers per homolog pair) and skewed distribution of crossovers are major obstacles to be addressed in modern plant breeding. Therefore, manipulation of crossovers is of vast interest in plant breeding to maximize genetic gain. Pro- and anti-crossover factors, epigenetic factors that control the number and distribution of crossovers in two conserved crossover pathways (class I and class II), have been identified in model organisms such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and *Arabidopsis thaliana*. However, the crossover modifiers and mechanisms that control the crossover number and position remain incompletely understood. Therefore, the questions raised by Nadia Kbiri in her doctoral dissertation are important and need to be addressed to elucidate further the molecular mechanism of controlling crossover formation and to accelerate breeding in diverse plants.

Assessment of the approach towards solving a scientific problem

Cytological analysis has previously revealed moderate variation in the number of crossovers in natural accessions of *Arabidopsis thaliana*, suggesting the existence of natural variation in crossover control. The groups of Ian Henderson (University of Cambridge) and Piotr Ziółkowski have used high-throughput measurement of crossover frequency to map recombination quantitative trait loci (rQTLs) in *Arabidopsis* accessions using a fluorescent seed reporter line (*420*). Mapping of rQTLs has led to the identification of *HEI10, TAF4b*, and *SNI1* using QTL map populations of Col-0, Ler and Bur accessions. In order to identify new natural variations of *trans*-acting crossover modifiers, Nadia selected 5 accessions (Col-0, Co-1, CDM-0, Neo-6, Per-1, and Oy-0) based on the climate temperature where the plants are growing, and crossed 420 Col-0 with the other four accessions to generate four rQTL mapping populations. Fine mapping of rQTLs depends on the size of the QTL mapping populations, crossover measurement and genotyping-by-sequencing (GBS) of individuals in the mapping populations. For rQTL mapping, Nadia measured crossover frequencies of 208 individuals per mapping population using the SeedScoring pipeline and performed GBS. In particular, Nadia used the Tn5 transposase to generate multiplexed GBS libraries for next-generation sequencing. *HEI10* was mapped as an rQTL in Col-0 × Co-1 and Col-0 × CDM-0 F2 mapping populations, while no significant QTL was detected in the Col-0 × Neo-6, Col-0 × Per-1, and Col-0 × Oy-0 F2 mapping populations. Importantly, Nadia has identified new rQTLs in the Col-0 × CDM-0 F2 mapping population. These two rQTLs need to be further mapped using F3 individuals to identify crossover modifiers.

In the other topic of her doctoral dissertation, Nadia has investigated the genetic effects of the MLH1/MLH3 complex (MutLγ) on crossover formation in *Arabidopsis*. Nadia used fluorescent seed crossover reporter lines (subtelomeric interval 420 and centromeric interval CTL3.9) to examine whether heterozygous and homozygous mutations of *MLH1/MLH3* genes or their overexpression can affect crossover frequencies in a dosage-dependent manner, similar to HEI10 E3 ligase, the pro-crossover factor. Nadia used several T-DNA insertion mutants of *mlh1* and *mlh3*, and also generated new knockout mutants for these genes using CRISPR/Cas9 technology. Upon measuring crossover frequencies in their heterozygous mutants, Nadia concluded that there were no dosage effects of the MLH1/3 genes on crossover formation, while homozygous mutations of *mlh1* and *mlh3* disrupted the formation of the majority of crossovers, leading to decreased pollen viability and seed fertility. Nadia generated transgenic plants that overexpress MLH1 or MLH3 under their own or *DMC1* promoter, and then examined crossover frequencies. Additional copies of MLH1/3 under the native promoter moderately increased 420 crossover frequency in some transgenic plants, but expression of MLH1/3 under the *DMC1* promoter decreased 420 crossover frequency, pollen viability and seed number per silique. Lastly, Nadia investigated the role for EXO1b in crossover formation using an *exo1b* mutant and transgenic plants, and observed that *EXO1b* overexpression led to an increase in *420* crossover frequency. It is interesting to observe that the transgenic plants overexpressing *EXO1b* show increased 420 crossover frequency, which needs to be confirmed by other approaches such as MLH1 counting and GBS-driven genomic crossover mapping.

Assessment of the doctoral dissertation

The doctoral dissertation consists of three chapters: Chapter 1 (general introduction to meiosis and the formation of crossovers), Chapter 2 (rQTL mapping), and Chapter 3 (genetic effects of the MutL genes on the formation of crossovers). Each chapter includes a list of references and a list of abbreviations. Chapter 1 provides general introduction to meiosis, pro- and anti-crossover factors. Meiotic progression and crossover factors that control the number of crossovers were well described in Chapter 1. Chapters 2 and 3 include introduction, material and methods, results, conclusion, and supplementary data.

In the introduction to Chapter 2, the aims and biological significance of rQTL mapping were well explained. Nadia provided detailed methods, such as automatic seed scoring, GBS library construction using Tn5 transposase, crossover mapping and analysis for QTL mapping in the material and methods section. Results were mainly presented as figures showing recombination and QTL plots and tables. In the discussion and conclusion of Chapter 2, Nadia has understood that fine mapping of rQTLs, including CDM-rQTLa and rQTLb needs to be further analyzed using F3 and backcrossed QTL mapping population.

In Chapter 3, Nadia focused on characterizing the MLH1/3 complex and EXO1 in terms of crossover frequency and fertility using knockout mutants and overexpression transgenic plants. Therefore, Materials and methods include detailed information of mutants, constructions for CRISPR/Cas9-driven mutants, and transgenic plants. Results include the plots showing crossover frequencies in genotypes, number of seeds per silique, and pollen viability, as well as the images for DAPI-stained metaphase I of pollen mother cells and

Alexander-stained pollens. The effects of MLH1/3 overexpression on crossover formation did not appear to be significant. However, the results of EXO1b overexpression were interesting, with a significant increase in crossover frequency. In the discussion section, Nadia explained why the MLH1/3 complex did not promote meiotic crossovers in a dosage-dependent manner and is different from HEI10, a major dosage-dependent pro-crossover factor, and how EXO1b can increase crossovers.

In this thesis, the automated measurement of the crossover frequency of individual plants using fluorescent seed report lines facilitated the mapping of rQTLs and the characterization of the MLH1/3 complex in *Arabidopsis*. The research presented in this thesis has unveiled promising new rQTLs and provided intriguing insights into EXO1b.

At this point, I would like to ask Nadia some questions while she defends:

1. Regarding the known and newly mapped rQTLs in Arabidopsis thaliana, could you explain or hypothesize the reasons for the emergence of natural variations in rQTLs and their roles in the population during the local adaptation and evolution of plant species?
2. Could you propose a hypothesis about the potential relationship between rQTLs and dynamic changes in plant ploidy?
3. Could you propose a hypothesis that outlines the advantages and disadvantages of hyper-crossovers in natural populations and crop breeding? Additionally, why do most species tend to have only 1-2 crossovers per bivalent?
4. Could you hypothesize the mechanisms by which the MLH1/3 complex is recruited to the designated crossover sites at late pachytene stage?
5. Could you hypothesize the timing and mechanisms by which the MLH1/3 complex activates the MLH1-3 endonuclease to resolve double Holliday Junctions (dHJs)?

Conclusion

In summary, Nadia's work in this thesis has not yet been published due to time-intensive nature of rQTL mapping and the investigation of the molecular mechanism of EXO1b using various approaches. Nevertheless, Nadia has done a tremendous amount of experimentation and analysis in this thesis during her time in Professor Piotr Ziółkowski's group. Her work needs to be continued to finalize the rQTL mapping and to explore the roles of the MLH1/3 endonuclease complex in crossover resolution. However, Nadia's findings provide invaluable insights into how new rQTLs may contribute to crossover control and how EXO1 activity may regulate the number of crossovers during meiotic recombination.

The work presented in this thesis meets all the requirements for a PhD, although it needs further investigation for publication. Therefore, I recommend that the Scientific Council of the Discipline of Biological Sciences of the Faculty of Biology at the Adam Mickewicz University in Poznan award Nadia Kbiri the title of PhD in Biological Sciences.

Sincerely,

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