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This is a review of M.Sc. Nadia Kbiri PhD thesis: Genetic factors in *Arabidopsis thaliana* meiotic recombination: Mapping new crossover modifiers and characterizing MutL complexes. PhD dissertation is written in English and contains 279 pages. It is divided into three chapters where Chapter 1 contains a general introduction and Chapters 2 and 3 describe results.

In my opinion, the Thesis is written in a clear way and in general a sufficient level of detail is provided. The thesis has an unusual structure with each chapter containing its own introduction, results section, discussion and conclusions. Given the very different nature of the results presented in Chapters 2 and 3, in my opinion, this is justified.

Chapter 1 general introduction as well as the specific introductions contained in Chapters 2 and 3 are well-written based on current literature allowing the reader to follow the succeeding result sections.

The methods sections are well structured with sufficient details provided to replicate the studies.

Chapter 2 describes Nadia Kbiri's analysis of QTLs responsible for natural crossover variability for 5 accessions crossed to Col-0. In my opinion, the study was designed and performed correctly and the results while not final are presented in a clear way with paths towards the next steps clearly indicated. I have several questions about this part that I would like to discuss with the PhD candidate during the defence:

On page 108 a limitation of QTL mapping approach is mentioned: which is the fact that fluorescent markers are not available in all the studied accessions. As a result, studies were done in Col-0 crossed with selected accessions limiting the possibility to detect crossover modifiers from more diverse Arabidopsis accessions. The author concluded that: "This limitation can be overcome by using a GWAS approach for example." I guess that is a simplification as I do not see how still using the fluorescent markers in Col-0/Ler only one can bypass this limitation using GWAS. I would like to ask the candidate to explain this point.

I would also like to ask if given the close proximity of some of the putative QTLs to a known modifier HEI10 a cross between *hei10* mutant with FTL cannot be used to eliminate its influence?

In summary, this part while not yet conclusive as no new modifiers have been found clearly shows that the PhD candidate is skilled in plant molecular biology and has done a good job conducting a challenging QTL mapping experiment.

Chapter 3 describes the analysis of MUTL genes' effect on meiotic crossover recombination. I am not an expert in recombination so I will refrain from commenting on the conclusions about the role of different subcomplexes in crossover control and comparisons to known mutants. In this respect, I would only like to

note that in my opinion to make conclusions about the strength of observed phenotypes they have to be observed side by side like in the case of for example figure 18. This is not always the case and, in some experiments, conclusions are made in regards to the strengths of observed phenotypes between experiments.

Chapter 3 starts with a description of published results on HEI10 dosage effect on recombination frequency. Here I would just like to mention that Figure 10 and Figure 10 legend do not mention explicitly that +/Oe and Oe/Oe are HEI10 overexpresses. While this can be inferred it would make reviewers' life easier if it was mentioned directly. The next set of figures describes the effect of MLH1/3 and PMS1 haploinsufficiency on the recombination rate. A decreased recombination rate was observed in the 420 interval in MLH1-2 +/- plants. This phenotype was lost in subsequent generations and not observed in additional MLH1 alleles tested. The applicant concluded that MLH1 does not show haploinsufficiency with respect to 420 interval recombination rate control.

Interestingly MLH3-1 +/- heterozygote showed a consistent reduction in 420 recombination a slight increase in 3.9 recombination rate and no change in any other tested interval. Importantly this phenotype persisted despite 3 backcrosses. Surprisingly none of the two others tested MLH3 alleles showed similar effects. Here the author concludes that MLH3-1 allele changes in 420 and 3.9 intervals recombination's effects "are due to local rearrangements in the *mlh3-1* mutant within the region corresponding to the 420 interval". While it is possible it is relatively easy to rule out by a backcross to WT. Next, the author acknowledges that part of the following results was done on the *mlh1-3* allele before the problems with rearrangements were discovered and that the following results will be presented taking into account the possible impact of additional mutations on the observed phenotype. This is to be appreciated but I did not find a reference to this point in the following parts of the thesis. Can you please clarify which experiments are affected?

Next, the author addresses the role of MLH in recombination in the homozygosis state. A clear reduction in recombination using 420 as well as other intervals is observed in two alleles of MLH1. For MLH3 some alleles show a reduction of recombination rate at multiple intervals while *mlh3-3* allele shows a peculiar bimodal distribution of recombination frequency. The author concludes that: "the phenotype can be due to the location of the T-DNA in the intron... T-DNA could be spliced out.. or maintained". While I agree that a T-DNA in the intron can be spliced out or maintained it is difficult to see how that could be consistently happening one or the other way in different plants. Is the applicant aware of any such example in the literature? And even if so then why no such phenotype is observed in 3.9 interval where other alleles of MLH3 do show a clear reduction in recombination? Could an alternative explanation be that *mlh3-3* is still segregating?

In the next section, the applicant analysed chiasmata and bivalent counts. While I am not an expert on this method I was surprised to find no Wt control data shown side by side in Figure 18 describing the results.

After analysing pollen viability and silique length that are consistent with recombination defects in *mlh1* and *mlh3* the author moves on to analysis of *mlh1 mlh3 fancm* triple mutant. Both in the case of pollen viability and seed per silique number there is a reversal in the triple mutant compared to *mlh1 mlh2* double mutant. I would like the author to discuss the biological implication of this phenotype as I did not find relevant discussion in the thesis.

My next set of questions is focused on the overexpression experiments. Here the applicant overexpressed MLH1 and 3 from native and DMC promoters and analysed the effect on recombination. Interestingly similarly to what was observed in mutants mlh1/3 plants a reduction in 420 recombination is observed. In the discussion, the author proses a dosage model where to low or to high MLH1/3 levels would lead to

defects in recombination concluding that MLH1/3 protein levels need to be tightly controlled. Interestingly no reduction is observed in 3.9 region upon MLH1/3 overexpression. Can the author reconcile the results why would MLH1/3 protein level be more important for the 420 than for the 3.9 interval? Can this be explained by the lost effect of MLH1/3 expression on 420 recombination in a backcross? I guess the 3.9 was also introduced by a cross? To follow up on this the applicant noticed that when over-expression lines are backcrossed to Col-0 the MLH1/3 ox effect on 420 is lost. The applicant proposes that the backcross reduced the MLH1/3 expression to a level that can no longer affect recombination. This is a plausible explanation but it should be supported by an analysis of MLH1/3 expression in the backcross. Somehow surprisingly despite this, the applicant has generated MLH1/3 ox lines in *hei10* mutant and HEI10ox background and analysed the recombination frequency. Here I like to ask how sure are you that the disappearing effect of MLH1/3 ox in Col-0 cross is not relevant in *hei10* and HEI10ox cross? Also looking at corresponding supplementary figures (SFig5) I noticed that panel A lack biological replicates. Based on SFig5B one can see that MLH3 ox resulted in HEI10 overexpression. Given the dosage effect of HEI10 on recombination can you please comment on this? Also, can this potentially explain the effect seen in MLH3 HEI10 double ox?

The last set of experiments focuses on EXO1 a regulatory add-on subunit of the MLH1/3 complex. Here in an elegant approach to separate EXO1 nucleolytic function from the ability to stimulate MLH1/3 the applicant has constructed catalytically inactive EXO1b. I do however fill that referring to the muted version s EXO1b was very confusing compare for example figures 34 and 35.

To sum up this part, the author has done a very good job exploring MLH1/3 defects in recombination frequency. The experiments are comprehensive and involve multiple alleles as well as comprehensive phenotyping not only for recombination frequency but also seed number per silique, pollen viability, chiasmata and bivalent counts. The MLH1/3 expression experiments suggest a tight link between MLH1/3 expression level and recombination control.

In summary, both experimental parts of the thesis described in Chapters 2 and 3 are novel, well-designed and executed in a prudent way, resulting in valid conclusions. In my opinion the presented thesis shows that M.Sc. Nadia Kbiri is well-skilled in molecular biology and genetics, has a deep understanding of the subject and is well prepared for becoming an independent scientist. I conclude that the doctoral dissertation meets the conditions specified in Article 187 of the Act of 20 July 2018. Law on higher education and science, consolidated text Journal of Laws 2022, item 574, as amended.

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