Marseille, August 28th 2023

To whom it may concern,

Nadia Kbiri doctoral dissertation comprises a general introduction about meiotic recombination and two main result sections about her experimental work. The quality of the manuscript is excellent. Concepts and data are clearly presented in a way pleasant to read and easy to understand. The general introduction covers the key concepts that are addressed in the manuscript, and it is nicely complemented by additional introductions of the different results sections. Given the broad variation of meiosis and meiotic recombination, a bit more discussion about non-*Arabidopsis thaliana* (*A. thaliana*) could have been welcome. The result section is impressive with massive experimental results obtained during her doctoral work. This led to a method paper (Kbiri et al, Methods Mol Biol. 2022) and will for sure lead to at least an additional important manuscript.

The first part of the result section contains in fact two important sub-sections. The first sub-section consists of determining the natural crossover variability in *A. thaliana* by generating genome wide recombination maps in five different hybrids, notably to determine the effect of sequence polymorphism on recombination. Massive genome-wide genotyping has been performed during this study that revealed global conservation but also some local variations in the recombination maps that can probably be investigated further. The general trend of increased recombination near chromosome ends and just next to the centromeric recombination depleted zone seems conserved. While this trend appears to be established in *A. thaliana* when all 10 chromosome arms are combined, it may be important to note that several chromosome ends do not show such a recombination enrichment, and at least two chromosome ends are close to a centromere. Interestingly, Nadia's study also shows a positive correlation between the level of sequence divergence and the recombination frequency in *A. thaliana*, but only up to a certain threshold that seems similar in the five hybrids studied. Eventually, this first sub-section provides a wealth of information that already provided general conclusions, and which further analysis may provide deeper conclusions about the relationships between sequence polymorphism and recombination.

The second sub-section of the first part of the results also involved a lot of experimental work to find Quantitative Trait Loci (QTLs) modulating recombination frequency assessed using a reporter assay inserted on the left arm of *A. thaliana* chromosome 3. In addition to the well-known Hei10 recombination modulator, this work identified a few additional QTLs at rather broad scale. Although further work is needed to refine their characterization and identify the causative gene(s) and mutations, the key point is the existence of addition QTLs modulating recombination frequency independently of Hei10 providing a proof of concept of the approach used.

The second part of the result section focuses on the analysis of the so-called MutL gamma complex and the effect of overexpression of some of its members on *A. thaliana* meiotic recombination. The MutL gamma complex has a nuclease activity suspected to act on recombination intermediates to specifically generate crossover recombinants. Nadia's study is included in a broader interest of Nadia's mentor lab about the relationships between sequence polymorphisms, the mismatch repair machinery and meiotic recombination. In that

respect, to be completely thorough about the introduction, it would have been appreciated that the notion of heteroduplex DNA rejection after recognition by the mismatch repair machinery would have been discussed more, even if it turns out that its existence in *A. thaliana* is not clear. Despite this detail, this part also represents massive experimental work where different over-expressing constructs have been tested. Eventually, an important result of this part is that increased load of MutL gamma complex could promote recombination, suggesting that MutL gamma has a broader substrate specificity than that originally anticipated. Some results also point at a toxic effect of MutL gamma complex over-expression, reminiscent of the mutator effect of over-expressing only parts of the complex in budding yeast, for instance. For a non-plant specialist, it is not always clear to appreciate the generality of the results since not all the recombination intervals tested show the same behaviors. As suggested by Nadia, completing some of the results obtained with genome-wide analysis should clarify some of these issues. Finally, this last part contains a very exciting result, which is the potential strong pro-recombination phenotype of the over-expression of a nuclease dead version of EXO1b, a structural component of the MutL gamma complex.

Overall, I am impressed by the amount of experimental work done by Nadia and I am convinced that at least a major publication will come out of it. The way the results are presented and discussed clearly shows that Nadia is a mature student who masters most aspects of meiosis and meiotic recombination, in addition of being a plant biology specialist. For all these reasons, I fully support that Nadia proceeds to the final stages toward the award of the doctoral degree.

Bertrand Llorente, PhD