INSTITUTE OF PLANT GENETICS
POLISH ACADEMY OF SCIENCES

Strzeszyńska 34, 60-479 Poznań

Tel.: +48 616550200 or 616550255 E-mail: office@igr.poznan.pl www.igr.poznan.pl
VAT: PL 7811621455 REGON: 000326204 BDO: 000017736

dr hab Robert Malinowski
Associate Professor IPG PAS

Head of the Department of Integrative Plant Biology
Institute of Plant Genetics of the Polish Academy of Sciences
ul. Strzeszynska 34
60-479 Poznan POLAND

rmal@igr.poznan.pl

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Review on the dissertation

"Identification and genetic characterization of SNI1, a gene encoding the subunit of SMC5/6 complex, as a natural modifier of meiotic recombination in *Arabidopsis thaliana*"

by

Longfei Zhu

Without any doubts we can say that crossing over (CO) is amongst the most important mechanisms behind generation of the genetic diversity in living organisms on our planet. The CO phenomenon is a driving force of evolution that helps organisms to adjust to changing environments. It is obviously important for diversity in plants as well as the successful completion of meiosis – the essential process for a generative life of a plant that leads to seed production. Knowing the fact that currently we are facing numerous challenges having negative impact on plant cultivation and a crop yield, recognition and a deep characterization of factors involved in the CO process in plants could be a starting point for more practical attempts to increase crop durability and shape future agriculture to secure food supply.

In contrast to mitotic recombination whose major role is to repair lesions in DNA and generate error-free DNA molecules the primary goal of the meiotic recombination is the CO

process that allows local exchange of genetic material between homologues. This functional difference is further reflected in the fact that mitotic recombination occurs rarely and its location is random whereas meiotic one is frequent and has a place in so called hot spots where SPO11 and MTOPVIB topoisomerases mediate the formation of double-strand breaks (DSBs). Resulting DSBs can exchange DNA strands with the homologous chromosome to form a single-end invasion (SEI) and then a double-Holliday Junction (dHJ) structures. Then dHJs are resolved by a nick and ligation mechanism to give cross overs. Due to the importance of this process molecular toolbox used to accomplish it is relatively well conserved, however species specific differences in a number of particular components are observed. Despite the fact that large number of DSBs can form during meiotic recombination only a fraction of them is subjected to actual CO exchange and factors responsible for anti-crossover activity have been characterized both in animal and plant kingdoms. Meiotic recombination can be modified by genetic and environmental cues or factors influencing chromatin and chromosome structure maintenance. Living organisms can use a differences in meiotic recombination as a trait that allows to adaptive plasticity, therefore we can expect to see cross population diversity within species. This in turn gives us an opportunity to identify factors involved in the biological basis of meiotic recombination with help of molecular mapping and comparative genomic approaches. The experimental design used in PhD work of M.Sc. Longfei Zhu is based on a combination of molecular mapping and the use of the 420 fluorescent system for a high-throughput measurement of crossover frequency. The subject itself is a continuation of studies aiming at understanding of molecular and genetic mechanisms governing meiotic recombination in plants that were conducted by Ziołkowiski's group at the Faculty of Biology Adam Mickiewicz University in Poznan. Previous studies of this team aiming at deciphering genetic basis of a difference in a cross over rate between Col-0 and Ler accessions led to a discovery of two QTLs. One of them has been further identified as the *HEI10* E3 ligase (Ziolkowski et al., 2017) whereas the recognition of the second is the subject of Mr Longfei Zhu's study performed under the supervision and in the lab of the Associate Professor Piotr Ziólkowski.

The dissertation has a classical form, is divided into five chapters, namely: introduction, aims of the project, materials and methods, results and discussion. Together with the abstract, abbreviation part as well as the summary and references it contains just 68 pages.

In general proportions between particular chapters are correct, however the discussion part is far too short (I will go back to this issue later in my report).

The Abstract contains a synthetic description of results. One small remark that I want to make is that it would be beneficial to say precisely what was the character of tested *SNI1* gene mutations (knock outs, substitutions, deletions; in which way those mutations could affect protein function or interaction with other components).

After that we move to the Introduction part where the author describes current state of knowledge on the meiotic recombination mechanism dividing it into chapters describing initiation and further progression of the process (chapter 1.1), factors influencing it (chapter 1.2) as well as the role of other elements involved in a structural maintenance of the DNA molecule (chapter 1.3). In general all chapters reads well, however after more careful inspection I found some aspects that could be done more carefully. At first there is a lot of ambiguity introduced by the repetitive use of the same words. Scientific language must be concise but the use of synonyms is always appreciated. An example of a problem:

*"A global analysis of crop **yields** shows that despite the continuous increase in **yields**, **yields** either never improve, stagnate or collapse across 24–39% of maize-, rice-, wheat- and soybean-growing areas (Ray, et al. 2012)". [page 1]*

Words like "development" or "reshuffle" are also used too frequently and could be replaced with other, more context-specific substitutes.

Another editorial issue is that the author forms too definite statements that do not exactly reflect the true situation. One example is from page 2 where he writes:

"The underlying molecular basis for the variation in recombination rate remains unknown, especially in plant species."

In my opinion this needs rewriting since some experimental data describing particular factors and steps is already available and in fact the author describes it below in the same chapter.

Similar impreciseness (page 3):

"...mutation in either SPO11-1 or SPO11-2 results in complete sterility due to fail in meiotic recombination initiation, indicating they act together as a heterodimer (Hartung, et al. 2007)."

In fact Hartung et al. (2007) have shown that both SPO11-1 and SPO11-2 are indispensable components of the DSB-inducing protein complex, however no experimental

data on the character of interaction between these proteins was provided. The authors (as well as other followers in later works) suggest this scenario but as far as I am aware interaction has not been described.

The literature review provided by the author could also be more up to date. In the part describing plant SPO11 other species than Arabidopsis and Rice could be mentioned; for example: Wheat (Da Ines et al. Plant J 2020).

The part devoted to SMC5/6 complex is also not always up to date and does not go beyond previously published review by Diaz and Pecinka (Genes, 2018). Since that time some interesting works were published and the introduction part would benefit from including them. For example the involvement of the NSE4a in embryo development (Diaz et al., Plat Cell 2019) or works describing the role of the SMC5/6 complex in the maintenance of the gametophytic ploidy in Arabidopsis (Yang et al. Plat Cell 2021, Yang et al. Frontiers in Plant Science 2021) should be included.

Finally, partially due to the fact that I work in the institution whose activity aims at applied aspects of plant science, I would like to say that it is a pity that no chapter addressing the meiotic recombination control potential implementation in plant breeding has been included either in the introduction or a discussion part of the dissertation. This is an interesting avenue that has been reviewed recently by Taagen et al. (Trends in Plat Science, 2020) and in my opinion the author of the thesis could explore this field in the introduction or at least address it while discussing his results.

Second chapter that describes aims of the project is well written and the text is accompanied with the diagram describing the generation of the F2 population and two QTL's that were identified. The starting point as well as the aims of the work are clearly defined.

Materials and Methods part is well written and allows for the repetition of particular techniques in a different lab. I like particularly the chapter 3.1.4 describing the seed based system and how measurements were performed. Fig. 6 is very neat, informative and professional.

I have just few minor issues:

On page 21 (chapter 3.1.1) the Catania-1 (Ct-1) accession should be introduced somehow.

On page 22 reference to Tab. 2 for Alexander staining should be included.

On page 23 "inflorescence stem" rather than "stem" should be used.

For chapter 3.2.6 could you please explain why different reference genes were used for *SNI1* and for *PR1*?

Results part starts from the description of the generation of the F3 population that was an entry point of the work performed by the author. The F2 (Col-0 x Ler) population for the *QTL4* mapping contained only Ler chromosome 4 substituted (location of the QTL4). The mapping was initially performed using 11 simple sequence length polymorphism (SSLP) PCR-based markers located within previously defined genomic region where QTL4 has been discovered. This was correlated with the 420 CO measurements. This way two markers located close enough to be inherited together were found. Subsequently the F3 population (n=2280) heterozygous for the QTL4 region was generated and subjected to further mapping and this resulted in the identification of 325 recombinants showing CO events location within the region defined by used markers. For these recombinants 420 CO frequency was measured and more dense mapping performed. This allowed to narrow down the QTL4 region to 53kb stretch containing 26 genes. One individual from the F3 was used by the author of the dissertation to construct F4 population of 152 individuals and further mapping followed by the creation of the F5 where the H-27 individual possessing only 19.5 kb Col/Ler heterozygous region containing 6 genes was selected. Recombination frequency measurements showed that QTL4 must be located in one out of six genes within this region. The author hypothesized (correctly) that since there was a difference in recombination frequency between Ler and Col-0 accessions disruption in one out of six selected genes should also lead to a visible change in frequency. In order to pinpoint the gene they have performed an allelism test for crosses containing T-DNA mutation in each of six genes and only mutation in SNI1 gene showed such change. Further comparison of SNI1 cds region in two accessions Ler and Col-0 showed two non-synonymous substitutions. Complementation of the *sni-1* homozygous mutant carrying 420 segregating reporters with Ler or Col-0 variant of *SNI1* resulted in reversion of CO rate in both cases. Here I want the author to address the fact why no statistically significant difference in this response was observed between $SNI1^{Col-0}$ and $SNI1^{Ler}$?

In subsequent chapters the author has shown that the differential CO frequency related to *SNI1* is not caused by expressional changes of this gene and that the only one out of two observed substitutions within *SNI1* (I235V at the amino-acid level) is responsible for the

phenomenon. The authors assessed CO pattern changes that are caused by the mutation in the *SNI1* gene. They obtained similar results with two different methods (FTL-reporter based and genotyping by sequencing GBS). In both cases they found that mutation leads to elevated CO within distal region of chromosome arms whereas in pericentromeric regions lower CO frequency is observed. Here I would like to ask the author to address this issue and try to explain why we see such phenomenon.

Then the author studied CO interference in the *sni1-1* mutant. The interference was reduced comparing to the wild type control. Please comment it as well.

Since SNI1 was first found to be involved in SA responses in chapter 4.6 author made an attempt to dissect CO phenomenon caused by *sni1-1* from SAR-related reactions. SA treatment did not lead to any change in 420-measured CO frequency both in Col-0 and *sni1-1* mutant pointing out this way that CO response is not related to SAR. When innate immunity compromised *eds1* mutant was crossed with the *sni-1-1* only reversion in somatic aspects of phenotype were observed – that additionally proves independent role of the SNI1 in vegetative and generative processes. Here I would like to mention that it would be clearer if the EDS part immediately follows SA treatment experiments. Instead effect of DNA repair genes is described in the middle and that breaks a bit a logical flow. In the test of the DNA repair genes influence on the *sni1-1* meiotic phenotype I think it would be nice to have better quality pictures of plants including close-ups of siliques and flowers – at the first look *sni1-1/rad51* looks much different than *sni1-1.* Fertility defects could be documented better. Never the less authors conclude that mutations that are able to suppress vegetative phenotype of *sni1-1* do not affect meiotic phenotype. Here could you please explain why the *atr* mutation leads to further increase in CO frequency of *sni1-1* mutant?

In the chapter 4.8 with help of different mutants from the SMC5/6 complex author shows that SNI1 role is in fact related to a proper function of the complex. This as well as the next chapter devoted to relation of SNI1 and anticrossover factors is based on the genetic interaction study. So in my opinion both of them should be followed with proper interactomics studies and the role of particular factors as well as the mechanism of interaction should be further dissected in the future.

To summarize results part I can say that despite some minor comments I find the experimental design as well as the reported discovery to be a real scientific achievement. I

can clearly see lots of work that has been done at the genetic level ad my comments are mainly related to the fact that I am excited to see the follow up of this work.

When it comes to discussion part I must admit that it could be more elaborated. I particular the molecular mapping process including the choice of approaches used could be discussed and confronted with other existing works I the field. I also had some dilemma how to judge the fact that in large part of a discussion author decided to include new results (not described in the "results" chapter. Surely this work is very neat and complementary to results but perhaps it would be more appropriate to incorporate it with date from "results" rather than generating chapters almost exclusively devoted to new data.

In general I did not spot any inappropriate claims I the discussion part and my comments here are more related to the form and the lack of sufficient discussion of some results described by the author.

Summary of the review report

Overall, the thesis demonstrates an appropriate understanding of the state-of-the-art in the research area. The PhD thesis work written by M.Sc. Longfei Zhu is concise and robust. Perhaps bit short but results stand up for themselves. The work was published in the PNAS what independently proves the quality and the relevance of the scientific finding. I value this work very high, therefore I strongly recommend the thesis to be accepted and the candidate should be awarded the PhD degree.

dr hab. Robert Malinowski, prof IGR PAN