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Review of the Ph.D. thesis of Elodie Rey entitled 'Genomics of Alien Gene Transfer in Wheat'

Elodie Rey presents in her manuscript entitled 'Genomics of Alien Gene Transfer in Wheat' the results of her work done at the Institute of Experimental Botany under the supervision of Pr. Jaroslav Dolezel.

The manuscript is divided into four main parts: a brief introduction, a literature review, the objectives and results and conclusions. Appendices corresponding to the original papers and published abstracts (either oral presentations or posters) by Mrs. Rey follow the main text.

The literature review presents a state-of-the-art in three different topics. The first one is wheat, with its socio-economic importance, as well as the challenges related to wheat improvement, especially the lack of genetic diversity and the use of wide hybridization to tackle the genetic bottleneck. The second part is a description of the wheat and barley nuclear genomes based on the reference sequences produced recently by the IWGSC and IBSC. The third part is a comprehensive review of transcriptomic analysis, including the different techniques and bioinformatics tools to study gene expression.

This literature review is thorough and provides the reader with an up-to-date view of these three different aspects that is critical to understand and value the work of Mrs. Rey. In particular, I think that Elodie should consider publishing the review on transcriptome analysis in a peer-review journal or at least make it publicly available through a pre-print server.

The 'Objectives and Results' part introduces briefly the general aims of the work, namely: (1) establish a protocol for transcriptome analysis in wheat-barley alien introgression, and (2) study the impact of inter-genome interactions on gene expression in wheat-barley 7HL introgression line. Then, the results are presented in the form of abstracts of a book chapter, a peer-reviewed first author paper, three poster presentations, and two oral presentations. All of these communications, except the oral ones, are presented in the appendices.

The book chapter entitled 'Genomics of Wild Relatives and Alien Introgressions' has been published in the book 'Alien Introgressions in Wheat' (Molnar-Lang, ed.). It describes various tools to support alien introgressions in wheat as well as functional aspects of alien gene transfer.

The first author article entitled 'Transcriptome reprogramming due to the introduction of a barley telosome into bread wheat affects more barley genes than wheat' and published in Plant Biotechnology Journal in 2018, represents the core of the Ph.D. thesis. It describes the characterization of a wheat addition line carrying a barley 7HL chromosome. An in-depth transcriptome comparison has been conducted between the wheat parent, the barley parent and the addition line. It showed changes in the overall gene expression for both wheat and barley genes, the latter being more impacted. It also revealed an unsuspected 36-Mb deletion of the wheat parent.

Follows a brief conclusion summarizing the main findings of this work.

Overall, the manuscript is well written and pleasant to read. Though the analysis might look trivial for people who are not familiar with the Triticeae genomes, it represents a high-quality and thorough work. In particular, the care that has been taken to develop a reliable RNA-seq analysis method has to be highlighted as it illustrates a high scientific merit. In addition, the discovery of the 36-Mb deletion and the way Mrs. Rey managed to deal with it reflects her ability to cope with unexpected problems and challenges, which is, to me, of primary importance for a scientist. Beside the valuable literature review and very interesting results on the 7HL addition line, this work has far-reaching implications as it opens new perspectives to characterize the impact of wide hybridizations in wheat.

However, there are few minor concerns that have to be mentioned. The development of protocol for transcriptome analysis in wheat-barley alien introgression is clearly identified as one of the main objectives of the thesis. Although it is clear that this protocol has been designed successfully, it would have been good to see it in more details, for example as a figure in the 'Appendices' section. It would be very useful for future works on this topic. In addition, Mrs. Rey should consider making the analysis pipeline / code available on a web server. Finally, the manuscript is missing perspectives of this work. Even though characterization of alien introgressions is still in its infancy and it is easy to see what could be done, I would have liked to read Mrs. Rey's opinion on the most relevant directions to explore.

That being said, these minor concerns do not affect the quality of this work that fully deserves recognition and congratulations. Therefore, I give a very favorable opinion on the Ph.D. defense.

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List of questions or points to be discussed during the Ph.D. defense

Literature review

1- *"The low rate of genetic gain results from a limited access to novel alleles in wheat improvement due to narrow genetic diversity of the current elite wheat pool germplasm." (p12)*

It has been suggested that the genetic gain was still progressing constantly and that the yield stagnation was an effect of climate change. Could you comment on that?

2- *"was to introgress important genes and alleles from its wild relatives through wide hybridization" (p. 13).*

Don't you think that mining alleles in untapped wheat genetic resources (*e.g.* landraces) would be easier and more straightforward?

3- *"This is partly due to the crossability genes carried by cv Chinese Spring on the homeologous chromosomes group 5"* (p. 17)
Which genes? Crossability genes are often present in Asian landraces. Are there wheat accessions other than CS that have been used?

4- "*At the same time, wheat-barley chromosome (-arm) additions represent an excellent model system for identifying and understanding the molecular basis of functional interactions between the host genome and alien chromatin."* (p. 19)
Does the whole set of chromosome addition lines exist? Are there lines in which the wheat chromosome has been replaced by the orthologous barley chromosome?

5- *"Gene loss in homoeologous wheat genomes"* (p. 29)
What about gene loss in the barley genome?

Objectives & results

6- "*Understanding how the host and alien genomes interact at various functional levels (i.e. genomic, transcriptomic, proteomic, epigenetic, ...) is necessary to make the development of useful introgression lines more efficient and successful.*"

Could you elaborate a bit on that?

7- You mention the development of a protocol for transcriptome analysis as a major objective of your work. However little is said regarding this protocol. Could you please tell us a bit more? Which tools did you try? Which one did you select? Why?

8- Why did you choose the 7HL addition line and not another one?

Book chapter

9- "*However, low representation of wild wheat relatives in the SNP design process may limit the utility of the platforms in wheat alien introgression breeding (Wulff and Moscou 2014)*" (p. 358)

The Axiom 820K SNP array has been developed from a wide range of wheat relatives. It has been designed specifically to identify and track introgressions.

10- "*Despite the importance of Triticeae species for the humankind (Feuillet et al. 2008), attempts to sequence their genomes were delayed due to the size and complexity.*" (p. 359)

Do you know which wheat or wheat relative genomes are sequenced to date?

11- *'Genomic stress' due to polyploidization may activate TEs and promote their proliferation and mobility.*

Have you seen the article recently published in Genome Biology and Evolution by Göbel and collaborators? They show that TE expression is globally robust to hybridization.

12- "*A recent observation using 3D-FISH in wheat– rye chromosome arm introgression lines indicated that the rye alien chromosomes were positioned at the periphery of nuclei*"

Wheat chromosomes tend to adopt a Rabl configuration in interphase. How do alien introgressions affect this configuration?

Article Plant Biotech J

13- "*Of the 35 301 wheat genes, the vast majority (34 341, 97.3%) were not transcribed differentially between CS and CS + 7HL, still leaving 960 which were differentially transcribed.*"

Are the wheat DT genes orthologs of the 7HL genes?
Does it affect some alternative transcripts or the whole gene expression?

14- "*These greatly differing proportions of differential transcription (DT) implied that the effect of a non-native genetic background was much greater than the effect of the presence of an exotic telosome*"

Can it be that the wheat genome has already experienced polyploidization? Would you expect the same results if it was a wheat telosome in a barley background?

15- "*On average, a similar number of genes per chromosome were down-regulated (24) as were up-regulated (20), but the numbers were particularly high on chromosome 7A (117 down- and 29 up-regulated genes)*"

Looking at figure 2, it seems that there are more DT genes on chromosomes 2, 5 and 7. Are these chromosomes originating from the same ancestral chromosome?
It would have been good to have a percentage of DT genes per chromosome rather than an absolute number.

16- "*The terminal region of 7AL in CS + 7HL harbours a 36 Mbp deletion*"

Could it be related to the crossability gene identified on 7AL by Tixier et al. in 1998?

17- "*There was, therefore, no apparent effect of the deletion on the transcription of the 7B and 7D homeologs.*"

Can it be that there differential expression is changed in the 7HL line because of a compensation from the barley telosome?

18- "*The associated mean log FC value was -8.21, and the average transcript abundance in CS + 7HL was extremely low (mean FPKM: 0.25); 25 of the genes recorded an FPKM of zero in every replicate..*"

Only 25% have a FPKM of zero. Since these genes are supposed to be deleted, what is the impact of such results on the overall analysis?

19- "*Overall, therefore, there was no effect of the deletion on the level of transcription of the 7HL gene complement, although a large proportion of the 7HL segment harboured homeologs of the genes lost as a result of the deletion.*" BUT "*The distal deletion on 7AL is partially compensated for by orthologs on 7HL*"

Not sure to understand this correctly. Could you please explain a little bit?

20- "*Biological relevance of alterations in the pattern of wheat and barley gene transcription*"

What is the expression breadth of up- and down-regulated genes in wheat and barley?
Since the analysis is done on one tissue at a given time point, could the set of genes be completely different in another condition?

21- "*for the rest, their altered transcription intensity implies either the absence of a fully functional regulatory environment and/or an effect of gene dosage due to the presence of their wheat homeologs.*"

What would you suggest to discriminate between the two?

22- "*The genes which were differentially transcribed were distributed throughout the wheat genome and along the entire length of chromosome 7HL, implying a level of stochasticism.*"

Would you expect differences between 7HL addition, substitution and translocation lines?

Conclusions

23- Which perspective do you see to your work?

24- How can your results lead to a better use of alien introgressions in wheat breeding programmes?

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12 June 2018

Review of the Ph. D. thesis of Elodie Rey: "Genomics of Alien Gene Transfer in Wheat"

Bread wheat is an allopolyploid organism that unites the genomes of three progenitor species in a single nucleus. More generally, genome evolution in the Triticeae tribe (comprising the major cereal crops wheat, barley and rye) has proceeded by frequent inter-species hybridization and polyploidization events. Plant geneticists and breeders have taken advantage of the comparatively low crossability barriers between related species to generate artificial hybrids by targeted crosses, sometimes requiring elaborate technique (such as embryo rescue) to obtain viable progeny. The best-known outcome of these effort has been the establishment of Triticale, a hybrid between wheat and rye, as a novel crop. In addition to creating new crop species, crosses between wheat and its relatives followed by subsequent backcrosses with euploid wheat as the recurrent parent have been used to generate introgression lines harboring alien chromatin in the form of chromosomes, chromosome arms, or smaller genomic segments. There has been a large interest in utilizing these introgressions lines in crop breeding as – in the best of cases – these lines show an agronomic performance similar to that of the recurrent parent, but also exhibit beneficial characters such as a pathogen resistance or resilience to abiotic stress. However, often alien introgressions come with a cost: alien genes linked to traits of interest negatively affect plant performance. Moreover, wide hybridizations may have unexpected consequences such a dysregulation of gene expression or reversal of transposon silencing.

The thesis of Elodie Rey focusses on understanding the consequences of alien introgressions in bread wheat by using a transcriptomic approach. She studied an addition line carrying the entire chromosome 7H of barley cultivar Betzes in the background of the full chromosome complement of the wheat reference cultivar Chinese Spring. Her methodology is state-of-the art: she took advantage of recently developed genomic resources for wheat and barley, namely annotated (draft) reference sequence assemblies. High-throughput RNA sequencing is currently the most commonly used method for studying gene expression. The bioinformatics analyses, involving read alignment, quantification of transcript abundance and enrichment analysis of gene ontology terms. A key result of her analyses was that differential gene expression was largely delimited to the introgressed barley chromosome: 42 % of barley genes were differentially expressed, whereas transcript abundance of only 3 % of wheat genes was altered. This is an important and unanticipated result that certainly warrants further investigation.

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The thesis itself is clearly structured and concisely written. The introduction (which is mainly an expanded, more detailed version of the book chapter in Appendix I) places the work into the context of traditional wheat genetics and explain the manifold aspects of the new genomic technologies. The results are presented in Appendix II, a paper recently published in Plant Biotechnology Journal. The figures and tables are instructive and the conclusion fully supported by the data. The layout is visually appealing and the quality of the English language as good as in any scientific paper. Without doubt, the Ph. D. work of Elodie Rey is a well-done effort, and her thesis an important addition to the scientific literature on wheat genetics.

After this general assessment, I want to focus on one particular aspect of thesis not mentioned so far. Elodie Rey and her co-workers detected a large deletion in the distal region of chromosome 7A, which was supported by lack of gene expression in this region, flow-cytometric measurements of chromosome size, in situ hybridization and genetic marker analysis. This was unexpected as prior cytogenetic work had characterized the barley 7H addition line as containing an intact set of Chinese Spring chromosomes. The three specific questions that I would like to discuss with the defendant mainly focus on this aspect of the thesis:

1. In several places in the thesis, the concept of "genomic shock" is evoked. This is certainly a charismatic term to elude to what may happen after two genomes that have diverged for millions of years come to interact in the same nucleus instantaneously. But what is actually meant by genomic shock? Which processes (may) occur following interspecies hybridization and what is the evidence for drastic changes at the whole genome-level in wheat and other systems? How have some polyploids managed to survive the shock without major rearrangements at the DNA sequence level?
2. The "hidden" deletions on 7A should make us wary about the traditional cytogenetic stocks. In the published literature there is at least one other example of such a surprise: Wicker and colleagues (Plant Cell, 2011) had to exclude chromosome 1D from the comparative sequence analysis of wheat and barley group 1 chromosomes, because the purported 1D double-ditelosomic line was heavily rearranged. What would be recommendations for users of cytogenetic stocks? Now that we have reference genome sequence assemblies for wheat and its relatives, and a battery of affordable high-throughput methods, what would be strategies to update and refine the characterization of cytogenetic stocks?
3. The present thesis has taught us that wide hybridizations have unexpected – and one can add – unwanted consequences. The new genome editing toolbox and the rising field of genomics in crop wild relatives suggest an alternative strategy to alien introgression: clone your genes of interest in the relatives and edit them into the wheat genome. This would avoid the laborious and time-consuming process of wide crosses and overcoming linkage drag. Will cytogenetic stocks still play a role in wheat breeding and genetics in twenty years from now?

Gatersleben, June 12th 2018