Reviewer's report

Martin Kovačik

Analysis of transcriptive dynamics in developing barley seeds

Assessment

During his doctoral thesis, Martin Kovačik developed an transcriptome atlas for the different tissues present in the barley seeds, from 4 to 32 days after pollination. The seeds have three distinct tissues with distinct genetic identity: the maternal sporophytic tissue and two tissues issued from fertilization, the endosperm and the embryo. The endosperm is produced after fertilization of a diploid maternal cell by a haploid sperm cell, and is therefore triploid. The embryo is a product of fertilization with equal paternal and maternal genome quantity. Given these differences, their transcriptome profile may be distinct. Moreover, the maternal and paternal origin of each genome may affect their transcriptional activity, by epigenetic regulation (DNA methylation, imprinting, chromatin structure). This background knowledge is described in the Literature Review chapter. And the candidate significantly contributed to extend this knowledge with his doctoral work.

The thesis is composed of a Literature Review chapter, a Result chapter of three parts describing the results obtained during the doctoral studies, a Discussion chapter, and a Conclusion Chapter. The dissertation also includes the list of publications and abstracts to conferences contributed by Martin Kovačik. The full text version of the publications is provided as appendices. Overall the dissertation is well written, with minor remarks (see below) for the last part reporting on the results. From a formal point of view, I am missing a chapter summarising the material and methods used in the dissertation thesis.

In his Literature Review chapter, the candidate presents the current knowledge on seed structure focusing on monocots and provided details of the barley seeds, material used during his work. Seed development is divided into three stages from early development to maturation phases, detailed in Figure 2. However, here, it would have been appreciated to have the relation between these stages and days after pollination, a reference to seed age used later. During endosperm development, events of endoduplication happen. Some details answering the follow-up questions could have been appreciated: Would you know if it happens in all types of endosperm? And if it is epigenetically controlled? The following parts of the Literature Review focussed on the barley genome and its activity/regulation. I believe the part on epigenetics mostly described the molecular components of *Arabidopsis thaliana.* Are those known in barley?

The Result chapter consists of three parts. The first part described a protocol published in JoVE (no published video?). It is an essential prerequisite of the following parts and I believe a useful protocol for researchers working on barley or other related species. It is mentioned that embryos are too tiny to be extracted before 8 DAP. Was a mechanical isolation of the young embryos tested? Something like described for Arabidopsis by Raising et al. (also in JoVE) in 2013? The second part uses the isolated material to perform a transcriptomic analysis of the different tissues at different time points. This is an important analysis that could bring cues for follow-up studies on grain development. Such atlas were also published for dicots (*Brassica napus* by the Belmonte lab and *Arabidopsis thaliana,* for example), a comparative analysis may be informative about the specificity of barley grains, for example. In the last part, the analysis focuses on PRC2 and epigenetic profiles. The text of the dissertation is sometimes less clear than the text in the publication with some shortcuts. For example, page 43, “a comparative analysis of our ChIP-seq data”: what ChIP-seq?, what protein/antibody was used for IP? and what was the experimental setup? Why 16DAP endosperm was chosen as sample?

The dissertation further contains a Discussion chapter and the full text of the contributed publications. Martin Kovačik significantly contributed (first authorship) to the JoVE protocol and the work published in Plant Cell (great story!), with minor contributions to four other research articles.

Here are some questions I would ask the candidate:

1. You briefly mentioned in the Literature Review that PCD processes occur during seed development (page 9). In Maize seed, Thomas Widiez identified an endosperm domain named Endosperm Adjacent to Scutellum (EAS), marked by the expression of specific genes, and showed that the EAS cells in proximity of the embryo undergo cell death to allow for the embryo to grow (Doll et al., 2020, Plant Cell doi: 10.1105/tpc.19.00756). Did you find any evidence in the transcriptomic analysis of the endosperm indicating that a similar process may happen in barley seeds? Can you extrapolate (or provide evidence) on the role of epigenetic regulators or genome imprinting mechanisms in such development process (e.g., communication between the embryo and the endosperm to allow for embryo growth)?
2. Technical questions related to the tissue isolation protocol: What is the starting amount of material (number of seeds and mg of fresh material) yielding how much total RNA? What is the duration of the procedure? And it seems that you do not use the same grains for the isolation of the different tissues, correct?
3. LEC1 was chosen as a marker for embryonic samples. However, Song et al. 2021 (Nature Communications, doi: 10.1038/s41467-021-24234-1) showed that Arabidopsis *LEC1* is expressed in the endosperm and transported to the embryo via plasmodesmata. LEC1 was found in a QTL controlling barley grain size, affected by grain filling and thus endosperm development (Hong et al. 2023; IJMS doi: 10.3390/ijms24054932). Can you discuss these publications in context of your results? Did you observe any other discrepancy between published data and your transcriptome atlas?
4. The WGCNA analysis identified that more genes are expressed in early and late time points, therefore, those developmental phases “exhibit more dynamic transcriptional reprogramming” (page 38). Could you comment on the biological relevance of such transcriptional behavior? Could it be a bias of the experimental setup?
5. The barley seed transcriptome was analysed by comparison to what is known in other species (for ex. p 40). Did you identify any pattern of expression you did not expect? If you would follow up your analysis by functional genetics, which genes would you pick to study their function and why?

Conclusion

The scientific contribution of the candidate is significant and innovative. He developed a protocol for tissue isolation in barley seeds and delivered a transcriptome atlas of the development barley seeds. It opens the path to many functional and genetic studies related to the regulation of seed development, including seed size and the impact of stresses. The candidate demonstrated his abilities to conduct research and interpret his data in context of the state-of-the-art. I recommend the acceptance of the dissertation to the doctoral committee of the Faculty of Sciences of the Palacky University of Olomouc.

Helene Robert Boisivon