Posudek disertační práce/Doctoral thesis evaluation of Mgr. Martin Kovačik

Doktorského studijního programu P1527 biologie, studijního oboru Molekulární a buněčná biologie

Školitel/Supervisor: doc. Mgr. Aleš Pečinka, Ph.D.

Název práce/Thesis title: Analysis of transcriptome dynamics in developing barley seeds

Oponent/Opponent: prof. Ing. Petr Smýkal, Ph.D., Katedra botaniky, PřF UP Olomouc

The topic of this thesis focuses on the deep analysis of barley seed gene expression throughout its development. This is already appreciated as it uses important crop instead of model plants. However, this offers also advantages, such as relatively larger tissues. In angiosperms, the mature seed consists of several genetically distinct and yet interacting tissues: diploid embryo derived from the fusion of maternal and paternal gametes, triploid endosperm with two maternal genomes, and then mother plant-derived seed coat, or as in the case of cereals seeds consisting of the pericarp. Thanks to the advancement of genomic technologies, the author was able to develop the protocol of tissue dissection followed by RNA isolation for sequencing analysis of spatiotemporal gene expression in a series of 4 to 32 days grains after pollination.

There were three major aims of the thesis. The first to develop a protocol of tissue dissection to provide high-purity material for further analysis. The second is to perform transcriptomic analysis using high-throughput sequencing and subsequent bioinformatic analysis. The third one is to study genomic imprinting and more specifically study the role of PRC2 complex and H3K27me3 modification across the genome in endosperm tissue.

The thesis starts with 27 pages of **Literature review**, providing sufficient insight into cereal crops, and grain development in more detail including the description of known crucial genes involved in this process. Then there is a description of the barley genome structure including epigenomics relevant for the study. An important part includes also genomic imprinting, an epigenetic phenomenon leading to the differential expression of alleles based on their parental origin. This is a relatively new and important topic. This has both theoretical as well as practical aspects (e.g. heterosis effect in breeding). The beauty of plants, in contrast to animals, is that it can be studied by reciprocal crosses using the same parents. This phenomenon leads to Polycomb group proteins, forming PRC1 and PRC2 complexes, studied in the barley. Finally, the theoretical parts end with a brief description of gene expression studies. Perhaps this is the weakest part of the introduction, being rather brief. Instead of describing some already outdated methods, like Northern hybridization or microarrays, it could better include more up-to-date ones, such as single-cell analysis, not just typical Illumina-based NGS using short reads.

This is followed by **Results,** which are not just simple additions of published papers, but rather newly written text related to three research aims. It is describing the development of a protocol for high-purity tissue isolation from barley seeds throughout the development. This includes extensive tissue dissection, especially challenging for younger stages. The purify was checked by flow cytometry as well as qPCR assessment with known tissue-specific genes. I can add that RNA isolation from some tissues rich in starch or phenolics is not trivial.

Subsequently, isolated RNA was subjected to RNAseq analysis and resulting reads were processed. Although not explicitly mentioned, this has been done by Mgr. M. Kovačik and possibly some colleagues. Notably, the data were not only published in well impacted paper (The Plant Journal) but also made publically available on Barley ePlant browser. Analysis shows the high number of differentially expressed genes both between tissues (e.g. embryo and endosperm) and developmental stages. With a peak at early stages and gradually decreasing. Altogether this provides a great dataset for further studies. Not simply only gene expression itself was analyzed but also the motives for transcription factor binding were analyzed. Selected transcripts were followed by in situ hybridization. Having in mind the third aim, of genomic imprinting, the further focus was on endosperm expressed genes. Since endosperm in not just a single homogenous tissue, there are several domains recognized, which could be revealed by in situ hybridization. Thus the genes were grouped according to these.

The final part investigates the role of histones and the Polycomb repressive complex (PRC2). Transcriptome analysis showed 152 out of 175 barley histone genes being expressed during grain development. Focusing on PRC2 complex H3K27me3 immunostaining and ChlP-seq was performed and correlated with gene expression.

These results were published in peer-reviewed journals with Mgr. M. Kovačik as first author. First to establish a tissue isolation was provided in a methodological paper in the *Journal of Visual Experiments*, being thus a toolkit for further studies. Second, comprehensive transcriptomic analysis of barley seeds in the prestigious *The Plant Cell*.

In addition, there are further 4 published papers where Mgr. M. Kovačik is co-author. Namely, *JXB* published study of endoreduplication dynamics, *Genes (Basel*) published study of endopolyploidy variation across the environmental gradient, another *JXB* on chromosome organization in endoreduplicated nuclei of barley seeds, and promotor (promoterome) analysis of barley embryos (*Comput Struct Biotech J*).

The thesis is finalized by the **Discussion part**, which critically discusses the findings in light of other published studies. I also appreciate to include also problematic difficult steps, and comments of each studied topic, providing information for followers.

The author´s experience is documented by an extensive list of references, properly cited in the text. Altogether, the study provides a comprehensive toolkit and data source for not only seed biology but also barley breeding. Supplements are reprints of all publications, abstracts and posters, presenting the complete author´s research outcome.

Altogether this yielded 2 original research papers which M. Kovačik is the first author, published in peer-reviewed Q1 or D1 journals. In addition, there are 4 papers with M. Kovačik being a co-author, related to the topic. No doubt that all these results will be highly cited and used. This demonstrates the author´s capability to conduct research, evaluate the results, and finalize them for publishing. It also shows the authors´ ability for extensive bioinformatics analysis of the datasets.

Questions- comments-remarks:

- The study is based on single barley genotype cv. Morex. How much variation (in expression) can be expected throughout the current barley cultivars, namely six- vs two-rows, spring or winter types, and specific barley genotypes from Nepal, Tibet or Ethiopia? Any known data or comments on this?
- Related to this, being myself interested in domestication, it would be very interesting to have a similar dataset on wild barley progenitor.

- Logically the focus is on embryo and endosperm, being the results of parental hybridization and also breeders focus. However, what about the maternal pericarp. By having reciprocal crosses and parental alleles, could be detected some communication between endosperm-embryo and pericarp?
- Although being technically and financially challenging, there is a clear future of single-cell transcriptomic (for maize endosperm, Yuan et al. Nature Commun volume 15, 34 (2024). What is the author's opinion on this?
- Another aspect is biological variation, with essentially every seed being individual and distinct (Krzyszton et al. Plant Commun 2024 Feb 12;5(2):100732). Using single seed transcriptomic.
- Regarding the genomic imprinting, was there a developmentally related pattern, in other words, does the alleles expression change during development? It is mentioned in Results, that about 40% of candidate genes were verified to be imprinted. This used a reciprocal cross between cultivated Morex and wild barley (HOR12560) and 8 DAP stage endosperm. Resulting in 21 genes, of which 18 were tested. This is a relatively low proportion of expressed genes. And what about their expression in diploid embryo?

Summary of the evaluation:

The author has demonstrated the ability of independent experimental work, analysis, and interpretation of the results obtained and the submitted dissertation meets the requirements for the defense of the Ph.D. degree.

April 3, 2024

prof. Petr Smýkal, Ph.D.
Department of Botany, Faculty of Science, Palacky University, Olomouc, Czech Republic