Review of Tenaillon *et al.*: Transcriptomic response to divergent selection for flowering time in maize reveals convergence and key players of the underlying gene regulatory network

In the manuscript the authors study early and late flowering maize inbred lines that have been obtained from selection experiments. The authors aim at understanding the responses to phenotypic selection at the transcriptome level, at adding new information to the gene regulatory network of flowering in maize and at testing for convergence of selection at the level of individual genes between different lines. The authors conducted an RNAseq study in agronomical field conditions on shoot apical meristems in different developmental stages. Expression of some genes were also measured by qRT-PCR.

Timing of flowering is economically important trait in many crop species. The genetic basis of flowering time variation has been and still is widely studied. Traditionally these studies focused on the function of individual genes, but during the last years RNAseq studies have become an important method for identifying gene groups that are differentially expressed between selected lines.

The strength of the paper is that the experiment is done in the field conditions and in my opinion this is the aspect that the authors should emphasize more. In general, the paper reads well, is nicely structured and it is easy to follow the story. However, there are several issues that should be addressed to improve figures and the readability of the paper:

1 **Some general comments:**
   - In the manuscript "FT" is used for abbreviation of "floral transition". FT is commonly used for FLOWERING LOCUS T. In the context of flowering and its genetic basis, I would not use FT for anything else, to avoid misunderstandings and confusion. For instance, the sentence "ZCN8 encodes a florigen protein that migrates through the phloem from the leaf to SAM triggering via its accumulation, the reprogramming of the SAM to FT" in the introduction can be very confusing.
   - Include the full gene name when the gene is mentioned the first time; both in the abstract and in the main text.
   - The material used in the study is obtained from the selection experiments called Sacley's DSE. Authors' role in those experiments should be made more clear.
   - The focus of this paper is on flowering time variation. Were there any other phenotypes co-varying with flowering time? Moreover, to me the difference between early and late lines is not that big (based on Figure 1, only about 10 days in the MBS line, about 16 days in the F252 line). Please discuss this.
   - Although gene expression studies (especially RNAseq) in the field conditions are still quite rare, some of them have been published. They should be cited and discussed in the paper. Moreover, are there other RNAseq studies on maize? If they exist, also they should be discussed. Explain why this experiment was done in the field conditions.
2 **Abstact**:
   - Delete "we obtained" (line 4).
   - What are "realistic field conditions"? Please specify or delete "realistic".
   - The sentence "we validated the reliability of performing RNA-sequencing in uncontrolled conditions" is not clear.
   - Describe why the used genes were selected for qRT-PCR.
3 **Introduction**:
   - Intro is nicely structured and compact.
   - The same sentence starts both the Abstract and the Introduction. Please modify one of them.
   - Second paragraph: based on the text it is not clear if DSEs refer to all six selection experiments or only to the two on flowering time (8^{th} and 9^{th} line).
   - The sentence "sucrose levels in sources leaves and carbohydrates export to sink tissues" is not clear.
   - End of Introduction: it should be explained why those genes were selected for qRT-PCR.

4 Results:

- Second line: “DES” -> “DSE”
- First paragraph: please make it more clear that the used flowering time data is from another study, not from the one presented here.
- If the Material and Methods section will stay at the end of the manuscript, more details of the field experiment are needed in the results section. For instance, the years are not explained, location of the field experiment is not mentioned etc. I would personally put the Material and Methods between Introduction and Results to avoid redundancy.
- “We determined the timing of FT of each progenitor as the earliest stage at which we observed a majority of transitioning SAMs”. What does the majority mean in this case? Please be more clear. It would be better to use the proportion of meristems being vegetative/transitioning/being reproductive (also in Table 1).
- “FT occurred later in MBS than in F252 consistently with the flowering time difference between these two inbreds.” Is this difference statistically significant?
- Section “Genome-wide patterns of gene expression as determined by RNA-seq”
    - Were the same individuals used for both phenotyping and RNAseq?
    - Please name the samples used in the pairwise correlations so that they can easily be identified in Table 1.
    - Some data (and statistics) on clustering in Figure 1 should be included.
- Section “DE genes and targets of selection”
    - I find the two first paragraphs very list-like. The second paragraph repeats the numbers of Table 2. Is there a way to make it more readable?
- Section “Expression of 5 genes as determined by qRT-PCR and correlation with RNA-Seq measures”
    - Mention the names of the studied genes already at the beginning of the section.
    - Please explained why those five genes were selected for qRT-PCR. Connect to the previous section (“Functional relevance of DE genes”). Were these genes among the DE genes in the RNAseq study?
    - The organs used for qRT-PCR are not clear for those who do not work with maize. What are the immature and mature parts of leaves? Pictures would help.
    - The sentence “We also incorporated with the amplification of each gene control samples for F252 and MBS for which all organs were pooled” is not clear.
    - “While we were able to detect ZCN8 expression in the SAM via qRT-PCR (Figure 5B)”: Figure 5B -> Figure 4B

5 Discussion

- In general, I think the Discussion is a bit too long. Maybe it could be shortened and made more compact.
- There is a conflict between sentences “As expected, expression varied more between lines than among developmental stages” (page 15) and “We have shown that the meristem developmental status is the main source of differential expression” (page 22).

6 Material and Methods

- Some environmental data on the field site during the field experiment should be added. The coordinates of the field site would also be informative.
- When were the seeds sown and/or the plants planted? Were the plants let to germinate in the field conditions or somewhere else? If somewhere else, the conditions for the pre-growing should be described. How many plants were planted? How was the experimental set up – were the plants randomized, were there blocks?
- Check that all parenthesis are opened and closed in the “qRT-PCR assays and statistical procedure” section.
- Add a comma between the R primer of ZCN8 and the RAP2.7 gene.
- In expression studies the timing of sample collection is important. Although this study focuses on developmental genes which are often either “on” or “off”, some of them might have circadian expression patterns. It should be mentioned what time of the day the samples were collected and how this issue was considered.

7 Figures:

- Fig. 1: How were days to flowering measured? In which conditions? Days from where? Add the abbreviations used in the text to the figure (FE, FL etc.).
- Fig. 2: Specify which sample is which (has to be comparable to Table 1, at least it has to be clear which samples are from early/late lines). Are the symbols correct? Conflicts with Table 1: e.g. in Table 1 there are 7 F252 samples for RNAseq, but in Figure 2 only 6.
- Fig. 3: please add the axis names, meaning of colors and explain the diagrams.
- Fig. 4: explain the colors used in the figure.
- Fig. 5: this figure should be cited in the results section, not only in the discussion. Check that the used colors correspond to the ones mentioned in the figure legend. Add A-F in the figure. B and C figures are swapped, to my understanding.
- Fig. S1: adding FE, FL, FVL, ME and ML next to the red circles of their progenitors in the figure would be informative.
- Fig. S2: the y-axis is missing. The x-axis in the first figure is missing. Different leaves are shown for different lines and it makes it difficult to compare the figures. To me it is not completely sure how the figure should be read. Does it mean that for instance in FL line (year 1), in all plants with 6 leaves the SAM is vegetative, and in ~20% of plants with 9 leaves the SAM is transitioning and in remaining ~80 the SAM is reproductive? The number of pooled samples should be mentioned in the figure legend.
- Fig. S3: specify which sample is which (see Table 1).
- Fig. S5: “When known relationships” -> “When known, relationships”; Are the explanations of black dots and arrows correct? Explain GRN and GDB.
- In Figure 1 the flowering time is shown as days and in Figure S2 as developmental stage. How are these connected to each other? Can you compare those phenotypes?
- (Microscopic) pictures of plants in different developmental stages would be informative for people not familiar with phenotyping in maize.

8 Tables:

- Table 1: Explain V, T and R. The reproductive stage (year 1) is not reached for FVL, ME and ML. Why?
- I could not access the Supplementary Tables and those materials are not reviewed. Please make sure they are available.