

Title: Genome-wide identification and expression profiling of DREB Genes in *Saccharum spontaneum*

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Reviewer's report:

General Comment:

Li and colleagues performed the genome-wide analysis of the DREB gene family in *Saccharum spontaneum*. The research topic is of interest, but the methods are not novel for the community, and the work is not based on an original hypothesis. On the other hand, it serves its purpose, justifying this genome-wide study. For that reason, I suggest the following minor revisions:

Specific remarks:

-Clarify the purpose of this genome-wide study in the Abstract and not only in the main text.

-All the sections, including Abstract, Introduction, Materials and Methods, Results and Discussions, are presented in the correct order. However, the Materials and Methods and legends need to be revised to include all the details to be reproducible. Important information has not been given.

- The "Plant materials" section needs to be more informative. I understand that the description of sample material was done previously by Li and colleagues, 2020, but a brief description or an additional table describing the material is desirable. It is also necessary to describe in details the varieties and treatments in "They collected the primary meristem of heartleaf (2-3 cm) in three sugarcane varieties under different degrees of drought treatment for RNA-seq library construction" (line 330).

-The "Expression profiling analysis of DREBs in *S. spontaneum* based on RNA-seq" section needs to be re-written in details to include the version of the software that was used for each case, and also the parameters that were employed or state that standard ones were used". It is also not clear the source of the "reference gene models" for the alignment with the reads.

- In the "Experimental validation of DREB gene expression level by qRT-PCR" section, the authors don't justify using a single reference gene, failing to follow the MIQE guidelines (<https://doi.org/10.1373/clinchem.2008.112797>). And also, don't describe the quantification method used. Justification is needed.

-Apart from the "Phylogenetic analysis" section, the authors fail to address the softwares' versions (or day of access) and used parameters in all the sections. This needs to be revised.

-What is the justification for the tree scale in Figure 1? And for the choice of the labels DREB1s and DREB2s instead of DREB1 and DREB2?

Minor comments:

-There are grammatical inaccuracies throughout the text and figure legends, for example: "the black line represents" (line 748); and inappropriate language like "they" (line 330) and "collected" (line 345). As a result, the authors need to revise the entire text.

-There is a problem in Figure 3. It seems to be congested, and there is a need to put in numeric order the motifs on the right side.

-The heatmap needs to be detailed in the Figure 8 legend. And a unit needs to be included for the expression values in (a).

-The choice of colours in the Additional file 2 must be justified in the figure's legend.

-The different datasheets have to be described in the Additional file 5 legend.

-Fix the link in line 394.

Overall, the work has potential interest for the community but has to be more precise in the methodological information and in the authors' goal to perform the study.