

Reviewer Report

Title: Comparative Proteomics Analysis of Tibetan Hulless Barley under Osmotic Stress via Data-Independent Acquisition Mass Spectrometry

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Reviewer name: Kosovi Kljira

Reviewer Comments to Author:

Dear Authors,

Reviewer comments GIGA-D-19-00339

The manuscript entitled "Comparative proteomics analysis of Tibetan hulless barley under drought stress via data-independent acquisition mass spectrometry" represents an excellent proteomics and bioinformatics study aimed at an identification and functional characterization of proteins differentially responding to osmotic stress represented by 21% PEG-6000 treatment on young plants of two Tibetan hulless barley cultivars with different drought tolerance, a tolerant XL and a sensitive DQ cultivar. The manuscript surely deserves publication due to presentation of novel proteomic data followed by their bioinformatic analyses aimed at an investigation of the identified proteins possible biological functions (Figure 3) and biological networks (Figure 6).

However, I have a few important comments how to improve the quality of the present manuscript:

Terminology:

Osmotic stress vs drought stress: The authors used 21% PEG-6000 treatment for simulation of drought stress. However, I recommend to refer to PEG-6000 treatment as an osmotic stress, not drought stress since drought means a lack of water in soil resulting in decreased soil water content while PEG-6000 treatment can reveal differential effects on plants due to its different nature. Thus, to be precise, I recommend to use the term "osmotic stress" instead of "drought" for PEG-6000 treatment.

Differentially abundant proteins vs Differentially expressed proteins: I recommend the authors to use the term "differentially abundant proteins" instead of "differentially expressed proteins" since Proteomics methods determine protein relative abundance which always represents a result of two opposite processes, protein biosynthesis ("protein expression") and protein degradation.

Materials and methods:

In Materials and methods, the source of seeds of the two cultivars of Tibetan hulless barley, drought-sensitive DQ and drought-tolerant XL, has to be given. The authors should write from which institution the seeds were obtained.

RT-qPCR analysis: In Figure 5, the authors present their original data on gene expression levels of six core genes in plant defense response. However, no basic information on the methodology of RT-qPCR including the sequences of forward and reverse primers and the housekeeping gene is given in Materials and methods. The authors have to add basic information on RT-qPCR methodology corresponding to the results presented in Figure 5.

In Figure 5 legend, loess method is mentioned for fitting a set of data points with smooth curves; however, no reference on loess method is given in Materials and methods.

Results:

I would recommend the authors to add a graphical abstract or Figure 7 or a summarising table providing a summary of the differences in response to osmotic stress between the two Tibetan hullless barley cultivars, DQ and XL, at proteome level, based on the results of the present study.

Discussion:

I think that the authors should discuss their results in a broader context of other proteomic studies focused on drought or osmotic stress response in barley or wheat such as Ford et al. 2011, Wendelboe-Nelson and Morris 2012, Ashoub et al. 2013, Ghabooli et al. 2013, VÃ-tÃjmvÃjs et al. 2015 *Frontiers in Plant Science* 479, and others.

Formal comments on the text:

Use SI units for volume, i.e., use dm^3 instead of l , cm^3 instead of mL and mm^3 instead of ml .

Abstract, line 4: Do not use contracted forms in Scientific text, i.e., write "Thus, it is critical to explore..." (not "Thus, it's critical to explore...").

Background, page 2, line 4: Remove the word "be" in the sentence "The droughty agricultural areas are estimated to double by the end of the 21st century..."

Background, page 3, line 15: Correct the term "salicylic acid" (not "salicylic").

Materials and methods, page 4, line 22: Modify the word form "centrifuge" to "centrifuged" in the sentence "After centrifuging..., 100 mm^3 of ABC (0.05 M NH_4HCO_3 in water) was added into the filter unit and centrifuged at 14,000 g."

Materials and methods, page 4, line 24: Modify the word form in the words "after centrifuge" to "after centrifugation."

Materials and methods, page 4, line 36: Add a comma both preceding and following the word "finally" in the sentence "...and, finally, 100% buffer A for 15 min."

Materials and methods, page 6, line 18: Correct the typing error in the term "heatmap package" (not "pheatmap package").

Analyses, page 7, line 2: Add "a" preceding the words "previous study", i.e., "...and this is in accordance with a previous study..."

Analyses, page 7, line 7: Modify the heading "Pairwise differential abundance analysis" according to my note on DAPs vs DEPs in terminology.

Figure 5 legend, line 1. Correct the typing error in the word "plant" (not "pant") in the term "plant defense response."

Final recommendation: Minor revision.

Methods

Are the methods appropriate to the aims of the study, are they well described, and are necessary controls included? Choose an item.

Conclusions

Are the conclusions adequately supported by the data shown? Choose an item.

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