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How do roots respond to osmotic stress? a transcriptomic approach to address this question in a non-model crop.

INTRODUCTION

Drought is a complex phenomenon that is relevant for many crops. Performing high-throughput transcriptomics in non-model crops is challenging. The non-model crop where our workflow has been tested on is banana (*Musa* spp.), which ranks among the top ten staple foods (total production over 145 million tons in 2013 (FAOstat)[1]). Bananas need vast amounts of water and even mild-drought conditions are responsible for considerable yield losses[2]. To characterize drought in the roots of different banana genotypes, we designed a lab model based on osmotic stress (5% PEG treatment for 3 days) and performed mRNA-seq analysis[3]. Using Illumina technology, 18 cDNA libraries were sequenced producing around 568 million high quality reads, of which 70-84% were mapped to the diploid reference genome[4]. We show that the applied stress leads to a drop in energy levels inducing a metabolic shift towards (i) higher oxidative respiration, (ii) alternative respiration and (iii) fermentation (Figures 1, 2 and 3). We also analyzed the expression patterns of paralogous genes belonging to the same gene families and detected possible cases of sub-functionalization (Figure 4).

RESULTS

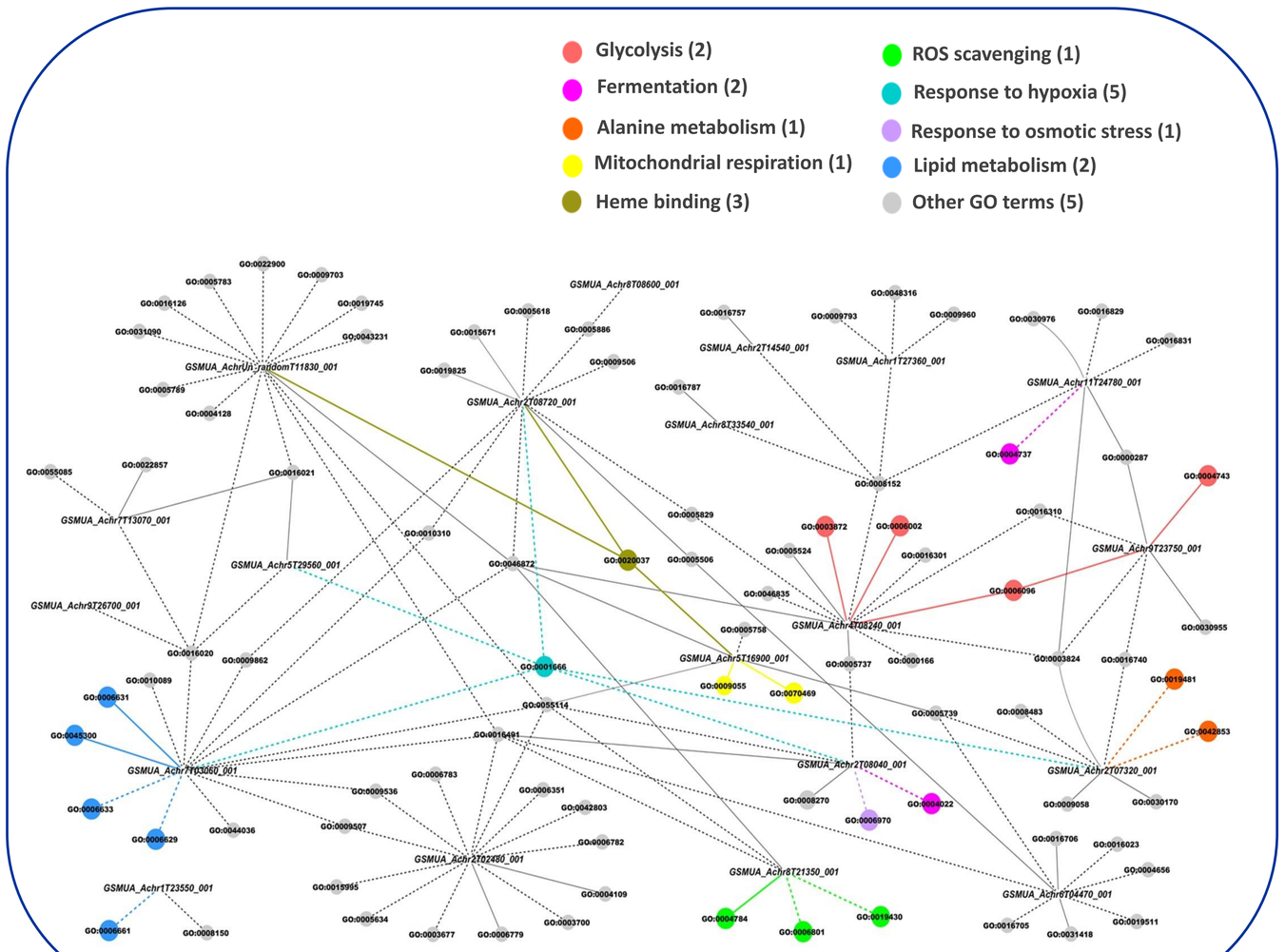

Figure 1. Interaction network of candidate genes and associated GO terms depicting key root processes affected under mild osmotic stress. 20 *Musa* genes (indicated in italics) commonly up-regulated in the 3 tested genotypes were validated by qRT-PCR in an independent PEG experiment and annotated functionally. Relevant GO terms are highlighted in different colors and the number of genes associated to them is indicated between brackets. Solid lines: GO terms assigned via Uniprot (http://www.uniprot.org/). Dashed lines: GO terms assigned via cross-species annotation.

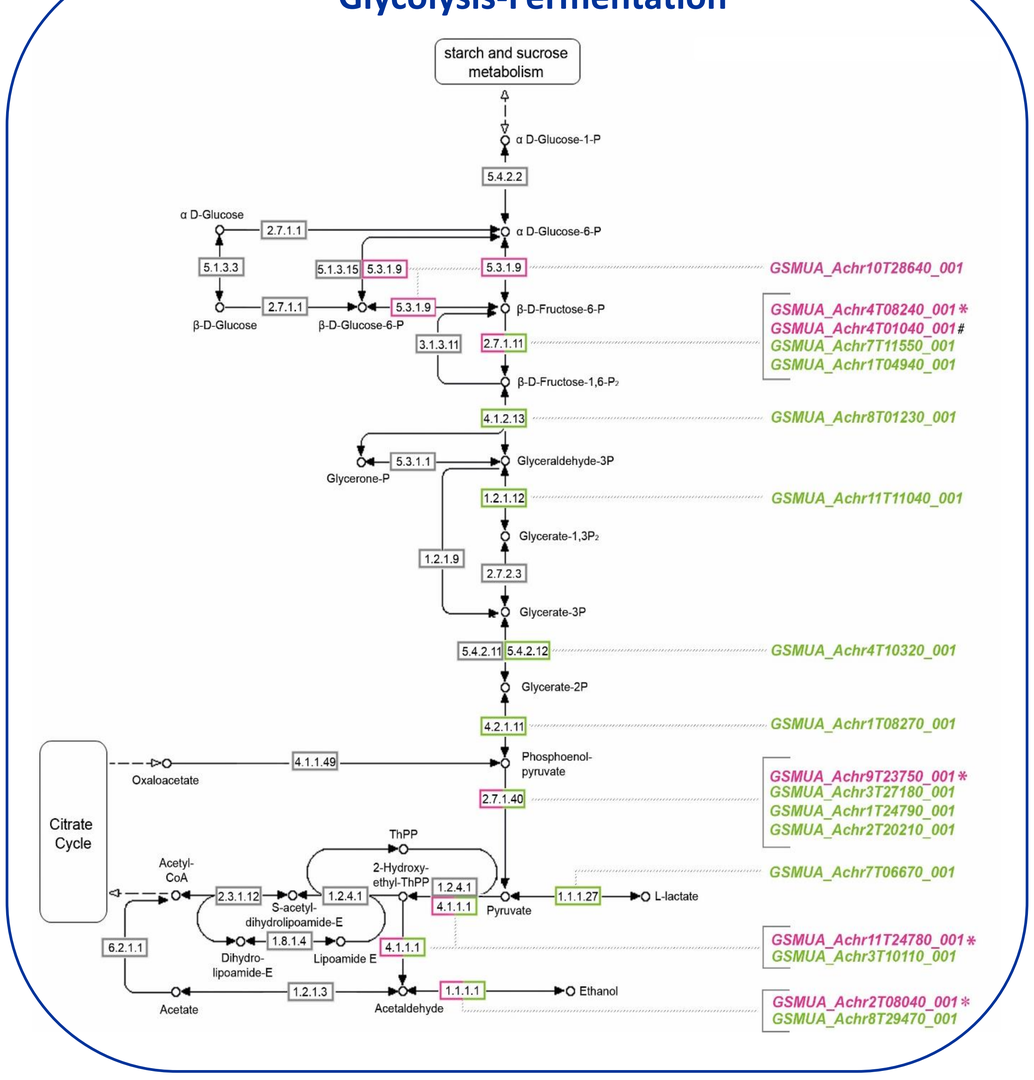

Figure 2. Glycolysis-fermentation associated enzymes and corresponding transcripts up-regulated in banana root under mild osmotic stress. Enzymes and transcripts coded pink were significantly induced in the 3 genotypes; those coded green were significantly induced in 1 or 2 genotypes. *genes validated by RT-qPCR. #not detected at false discovery rate≤0.05. 5.3.1.9: Glucose-6-phosphate isomerase; 2.7.1.11: 6-phosphofructokinase; 4.1.2.13: fructose-bisphosphate aldolase; 1.2.1.12: glyceraldehyde-3-phosphate dehydrogenase; 5.4.2.12: phosphoglycerate mutase; 4.2.1.11: phosphopyruvate hydratase; 2.7.1.40: pyruvate kinase; 4.1.1.1: pyruvate decarboxylase, 1.1.1.27: L-lactate dehydrogenase; 1.1.1.1: alcohol dehydrogenase.

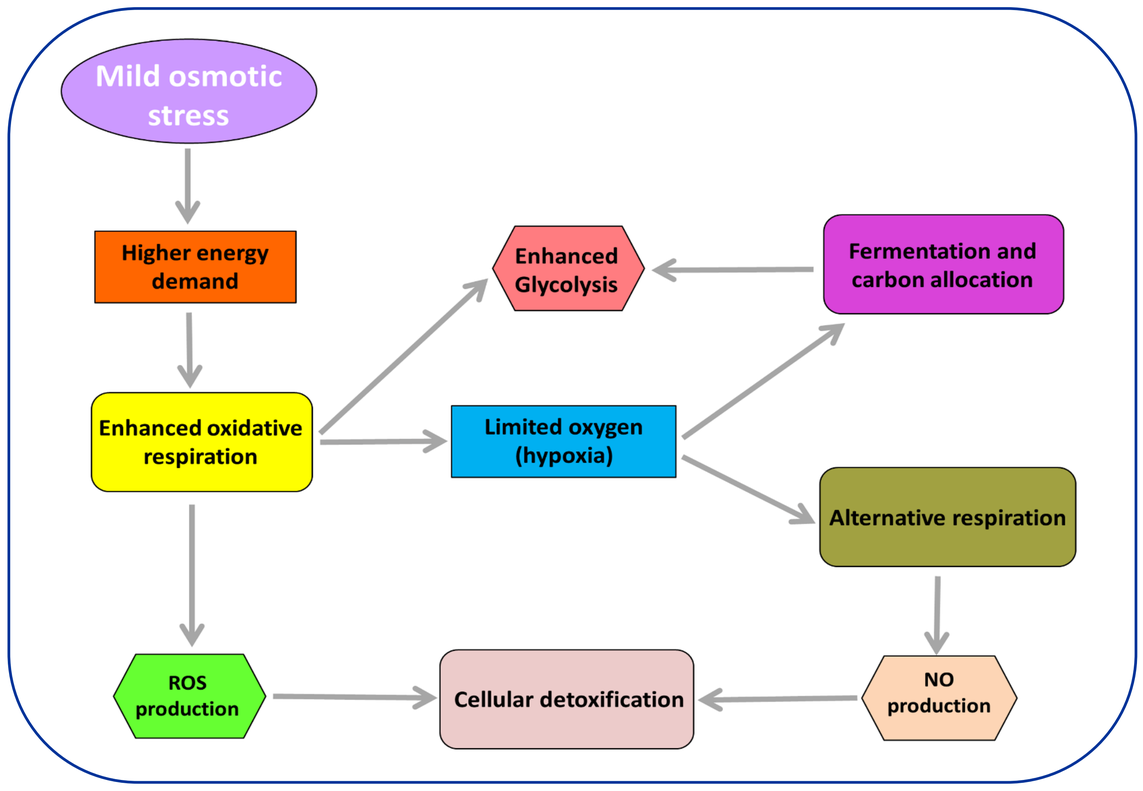

Figure 3. Summary of the main pathways induced during mild osmotic stress in banana root tips.
ROS: reactive oxygen species; NO: nitric oxide.

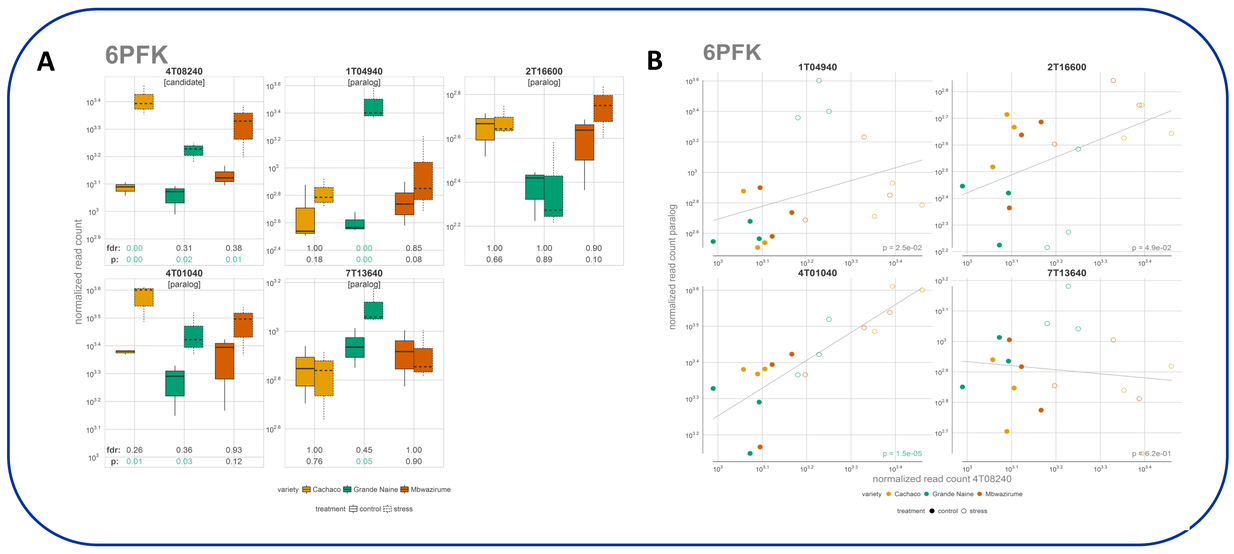

Figure 4. Example of expression patterns for the 6-phosphofructokinase family members in *Musa*.
A) Boxplots showing the expression levels for each cultivar/treatment combination and significance level of the statistical test (edgeR-RLE). Outlier range is indicated as 0.25 - 1.5 IQR and 0.75 + 1.5 IQR. B) Spearman rank correlations between the candidate gene (x-axis) and the corresponding paralogs (y-axis). Number of biological replicates (stress/control): n=3/3. IQR: interquartile range. fdr: false discovery rate. p = p-value. Significant results are highlighted in green. 6PFK: 6-phosphofructokinase.

CONCLUSIONS

- ✓ **Transcriptome profiling in the polyploid non-model crop *Musa* indicated that roots change the broad spectrum of energy metabolism after applying mild osmotic stress.**
- ✓ **Aerobic/anaerobic respiration together with fermentation were induced in banana root tips after 3 days of 5% PEG treatment.**
- ✓ **By validating a subset of genes by RT-qPCR, we confirm the success of RNA-seq for evaluation of a non-model crop.**
- ✓ **Our results highlight the complexity when dealing with polyploid genomes and gene families composed by paralogous copies.**

References

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