Identification of drought responsive genes and promoters in Musa by using RNA-seq.

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Introduction

Bananas and plantains (Musa spp.) are a major staple food with a worldwide production of over 135 million tonnes per year (FAO, 2012). However, water is one of the most limiting abiotic stress factors in the production. Thus, a better understanding of the biodiversity and genetic basis of drought tolerance is needed. In our lab, we have performed an RNA-seq experiment on 3 different banana cultivars known for their contrasted response to mild-drought stress. Read mapping was performed on the double haploid Musa acuminata (AA) reference genome [1] as a template. Since the cultivars used are triploids and have variable genome composition (AAA or ABB), mRNA-seq results had to be analyzed in a special manner. More than 803 million out of 1.2 billion reads were uniquely mapped on the reference genome. Applying various statistical methods, we have identified a set of candidate genes differentially expressed under stress. A number of them are tissue-specific and appropriate for identification and cloning of promoter regions able to drive expression of drought responsive genes. Currently, these candidate genes/promoters are being validated with alternative approaches (qrt-PCR) and in different experiments carried out in the lab, greenhouse and under field conditions.

Results

Characterizing drought-stress reaction in banana

Figure 1. Multivariate Partial Least Square (PLS) analysis. Results indicate that the transcriptome is significantly different after 3 days of osmotic stress in the tissues analyzed. Example Cachaco root (similar results were obtained for the other genotypes and tissues).

Table 1. Selected genes for qRT-PCR validation. List of 21 unique transcripts induced after 3 days of osmotic stress in banana roots. *Abbreviation according to the orthologous gene in Arabidopsis. b KEGG pathway assigned using Blast2GO (http://www.blast2go.com). c involvement in drought or other abiotic stresses based on previous studies.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Gene annotation</th>
<th>KEGG pathway</th>
<th>Identified in abiotic stress</th>
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</table>
| TPH2         | 1-Cyclohexanecarboxyl-CoA dehydratase | Porphyrin and chlorophyll metabolism | O 
| TPH1         | 5-Methyltetrahydropterin reductase | Pteridine metabolism | O 
| TPH2         | -               | O 
| TPH2         | -               | O 
| TPH1         | -               | O 
| TPH2         | -               | O 
| TPH1         | -               | O 
| TPH2         | -               | O 
| TPH1         | -               | O 
| TPH2         | -               | O 
| TPH1         | -               | O 
| TPH2         | -               | O 
| TPH1         | -               | O 
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| TPH2         | -               | O 
| TPH1         | -               | O 
| TPH2         | -               | O 
| TPH1         | -               | O 

Conclusions

This study characterizes the drought-stress reaction in banana using RNA-seq and it proved to be a valid method to select genes significantly altered during stress and with tissue specificity.

The upregulation in the 3 genotypes of 14 (76 %) out of the 21 selected candidates has been verified by qRT-PCR in an independent lab experiment. Further validations in field experiments are needed to confirm their involvement in drought-stress response.

References


The authors would like to thank Hen Do, Elis Thiry, Saskia Windelckx and Edwige André for technical assistance, and Hugues Pannier and Ernici Dubois (MGM-Montpellier) for the RNA-picking and raw data analyses. Financial support from the Bioversity International project ‘1TC characterization’ (research project financed by the Belgian Directorate-General for Development Cooperation (DGDC)) is gratefully acknowledged.