Characterizing MADS-box transcription factor-mediated regulation of fumonisin biosynthesis in *Fusarium verticillioides* with computational subnetwork module analysis

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**Abstract**

*Fusarium verticillioides* is an important maize pathogen, leading to Fumonisin B1 (FB1) in infested kernels. MADS-box transcription factors (TF) were found to modulate polyketide synthase (PKS) gene expression and FB1 production in *F. verticillioides*. With next-generation sequencing of *F. verticillioides* wild type and MADS-box TF mutant cultures, we used a suite of computational network-based tools, e.g., partial correlation, log-likelihood ratio matrix and seed-and-extend approach, to perform a system-oriented transcriptome analysis to predict downstream genetic subnetwork modules associated with FB1 production. Our aim was to identify and characterize system-level changes across correlated genes rather than simply focusing on individual gene expression. The resulting subnetwork modules are predicted to contain hub genes, which likely play a crucial regulatory hub gene in each subnetwork and regulate FB1 biosynthesis.

**Aims**

- Identification of putative hub genes with *in silico* analysis
- Functional characterization
  - Growth and development
  - FB₁ production
- Putative hub impact on network robustness and transcriptional directionality

**In silico analysis**

- PKS genes and other secondary metabolite genes
- Vegetative development genes
- Other genes and signalling pathways under MADS-box TF regulation

**Gene-deletion Confirmation**

- FvNog1 and FvCup9 were confirmed by Southern blot
- FvRsr1, FvCyn3, and FvEfM3 were confirmed by PCR

**Putative hub genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Subnetwork A</th>
<th>Subnetwork B</th>
</tr>
</thead>
<tbody>
<tr>
<td>FvRsr1</td>
<td>😍</td>
<td>😍</td>
</tr>
<tr>
<td>FvCup9</td>
<td>😍</td>
<td>😍</td>
</tr>
<tr>
<td>FvEfM3</td>
<td>😍</td>
<td>😍</td>
</tr>
</tbody>
</table>

**Putative hub genes on neighboring genes**

<table>
<thead>
<tr>
<th>Genes</th>
<th>WT</th>
<th>FvRsr1</th>
<th>FvCup9</th>
<th>FvCyn3</th>
<th>FvEfM3</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVEG_00035</td>
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</tr>
</tbody>
</table>

**Summary**

- Network-based gene association analysis was used to predict two subnetworks downstream of MADS1 important for FB1 regulation
- Five genes were selected for knockout and functional characterization
- All five gene-deletion mutants affected secondary metabolism
- FvRsr1 and FvEfM3 mutations resulted in a significant FB1 reduction
- Subnetwork gene expression was altered in FvRsr1 and FvEfM3
- FvRsr1 – RAS GTPase and FvEfM3 – methyltransferase have a directional impact on transcription in neighboring genes
- FvRsr1 and FvEfM3 likely play hub role in predicted subnetworks

**Method**

- Knockout mutants were constructed to analyze the role of *Mads1* and *Mads2* in secondary metabolism and sexual reproduction in *F. verticillioides*
- *Mads1* produced significantly less FB₁, than the WT
- PKS gene expression levels decreased as time passed, with 14 of the PKS transcripts in *Mads1* at least half of that of the WT, 10-dpi

**Acknowledgements**

This project was supported by the United States Department of Agriculture USDA-NIFA Agriculture and Food Research Initiative Competitive Grants Program under contract 2013-68004-20359.