Systematic network-based computational identification of functional modules associated with pathogenicity and fumonisins in Fusarium verticillioides

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Abstract

Characterizing pathogenicity and fumonisin biosynthesis in Fusarium verticillioides, a major pathogen of maize, is essential to better understand F. verticillioides – maize interactions. Recently, computational identification of biological functional modules using large-scale RNA-seq data emerged as a powerful tool for facilitating functional genomics research. In this study, we performed a systematic network-based comparative analysis of two distinct F. verticillioides – maize transcriptome RNA-seq datasets, where one was inoculated on moderately resistant maize B73 and the other on susceptible hybrid 33K44. For a systematic analysis of the pathogenicity as well as fumonisin biosynthesis, we first inferred the co-expression networks of the fungal pathogen. Subsequently, we predicted functional subnetwork modules in the co-expression networks consisting of interacting genes that display strongly coordinated behavior in the respective datasets. A probabilistic pathway inference method was applied and a computationally efficient branch-out technique was used to identify functional subnetwork modules likely to be involved in the pathogenicity or fumonisin biosynthesis in F. verticillioides. Here, we present four potential subnetwork modules, where the datasets contained several enriched GO terms as well as potential pathogenicity genes that are orthologous to genes known in other plant pathogenic fungi.

1. Introduction

Fusarium verticillioides ( Gibberella moniliforme) is a fungal pathogen responsible for ear rot and fumonisin contamination in maize. While some F. verticillioides pathogenicity genes have been characterized, our understanding of underlying cellular structures and functions regulated by genes and their products is still limited. Here, we performed a systematic comparative analysis with F. verticillioides RNA-seq data to identify functional subnetwork modules associated with maize ear rot pathogenesis and fumonisin biosynthesis.

2. Method

2.1 Sample preparation

Maize inbred B73 and hybrid 33K44 were inoculated with wild-type F. verticillioides and sampled at 5 time points (0 dpi, 2 dpi, 4 dpi, 6 dpi, and 8 dpi). Samples of 3 dpi and 8 dpi were only used in this study (dpi: days post inoculation).

2.2 RNA sequencing & Preprocessing

Samples sequenced with Illumina HiSeq 2500 and sequencer outputs were aligned through TopHat using F. verticillioides strain 7000 reference genome & annotation.

Filtering: 14.7% genes with very low expression levels were filtered out, and thus 12,076 genes remained to be analyzed.

Normalization: i) normalized by the corresponding gene length

ii) normalized with beta-subunit genes (FvEG05512 & FvEG04881)

2.3 Constructing co-expression networks

Partial correlation was applied to measure the strength of association among genes to infer the underlying co-expression networks.

2.4 Conversion into Log-likelihood ratio (LLR) matrix

With genes \( G = (g_1, g_2, \ldots, g_n) \) of a subnetwork module and their expression levels \( X = (x_1, x_2, \ldots, x_n) \), a log-likelihood ratio (LLR) between wild type and the mutant can be computed by

\[
LLR = \sum_{i=1}^{n} \frac{x_i}{\mu_i} \ln \left( \frac{x_i}{\mu_i} \right) + \ln \left( \frac{\sum_{i=1}^{n} x_i}{\sum_{i=1}^{n} \mu_i} \right)
\]

2.5 Subnetwork activity \& Discriminatory power

- Subnetwork activity level can be also computed by

\[
\text{Activity} = \sum_{i=1}^{n} \frac{x_i}{\mu_i} \ln \left( \frac{x_i}{\mu_i} \right)
\]

- Discriminatory power of the given subnetwork based on ftest statistics can be estimated by

\[
\text{Discrim} = \frac{\text{Activity} - \mu}{\sigma}
\]

3. Results

Fig. 1. Identifying potential subnetwork modules

Fig. 2. F. verticillioides subnetwork modules associated with 33K44 against B73

Fig. 3. F. verticillioides subnetwork modules associated with B73 against 33K44

4. Module A is composed of sixteen F. verticillioides genes, with six genes annotated by a GO term (GO:0016027) "integral component of membrane" and suggested as major facilitator proteins. There were genes encoding an oxidase, a resedute, a transcription factor as well as a fungal effector. Three genes with unknown function, but with significant correlation with other genes were found.

5. Module B is comprised eight F. verticillioides genes, where four were annotated by a significant GO term (GO:0055114) "oxidation-reduction process" and suggested to perform metabolite modification. Potential pathogenicity-associated gene was found as well as those involved in carbohydrate/energy utilization. Orthologous genes were identified in plant pathogens Botrytis cinerea and Magnaporthe grisea.

Conclusion

We identified potential F. verticillioides pathogenicity-associated subnetwork modules, where the member genes were harmonously coordinated and significantly differentially activated between the two different maize inbred hybrid background. The modules possessed at least one gene whose orthologs were known as virulence genes. Some genes in the modules were annotated to significant GO terms suggesting a role in ear rot pathogenicity.

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Reference

- "Fusarium coatiene competing project." Ethnic Institute of Harvard and MIT.