



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 comprehend *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 kernel RNA-seq datasets, where one was inoculated on moderately resistant maize inbred 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 activity 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 modules contained several enriched GO terms as well as potential pathogenicity genes that are orthologous to genes known in other plant pathogenic fungi.

Introduction

Fusarium verticillioides (*Gibberella moniliformis*) is a fungal pathogen responsible for ear rots 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.

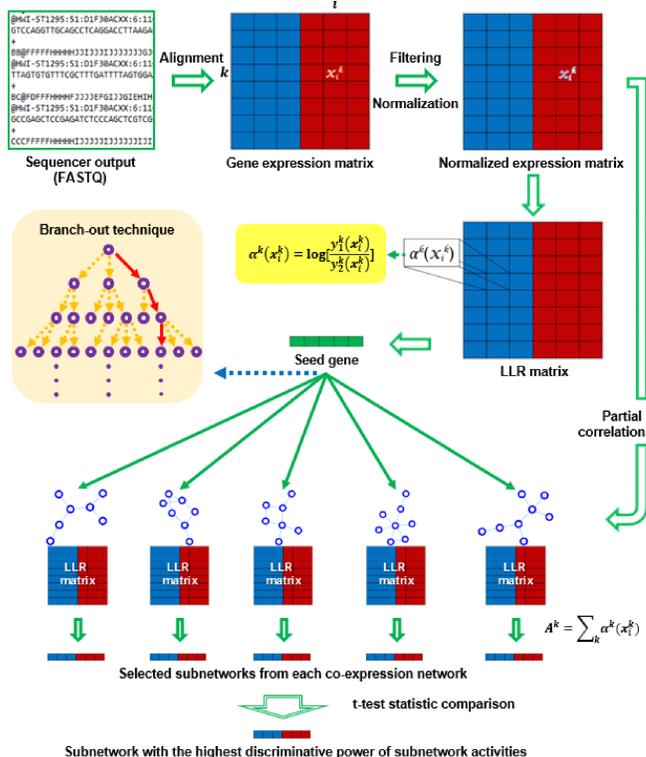


Fig. 1. Identifying potential subnetwork modules

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 **6 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 Tophat2 Using *F. verticillioides* strain 7600 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-tubulin genes (**FVEG_05512 & FVEG_04081**)

	33K44		B73	
	6 dpi	8 dpi	6 dpi	8 dpi
Type of run	paired-end			
Read length	124 (bp)			
Mean # of reads aligned	7155626	13473461	6532043	18301912
Median depth of coverage	505.3	951.4	461.3	1292.4

2.3 Constructing co-expression networks

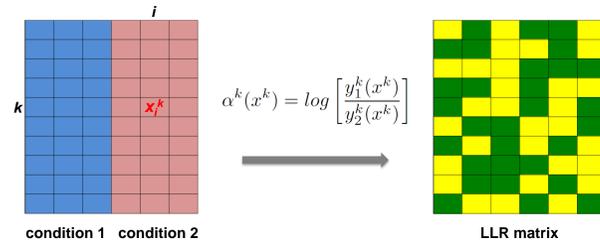
- Partial correlation was applied to measure the strength of association among genes to infer the underlying co-expression networks.

Threshold (cut-off partial correlation)	33K44		B73	
	# of gene	# of interaction	# of gene	# of interaction
0.8	12061	227294	12065	328115
0.775	12072	376826	12075	532898
0.75	12075	589424	12076	815325
0.735	12075	752595	12076	1026786
0.72	12075	945433	12076	1273123
0.705	12075	1171432	12076	1557755

Threshold (cut-off partial correlation)	B73		33K44	
	# of gene	# of interaction	# of gene	# of interaction
0.82	12036	210688	12029	125967
0.795	12067	363720	12066	252986
0.77	12076	582422	12075	403945
0.755	12076	751792	12075	541188
0.74	12076	952199	12075	704821
0.725	12076	1186834	12075	877346

2.4 Conversion into Log-likelihood ratio (LLR) matrix

With genes $G = \{g_1, g_2, g_3, \dots, g_n\}$ of a subnetwork module and their expression levels $X = \{x_1, x_2, x_3, \dots, x_n\}$, $\alpha^k(x^k)$ log-likelihood ratio (LLR) between wild type and the mutant can be computed by



2.4 Subnetwork activity & Discriminative power

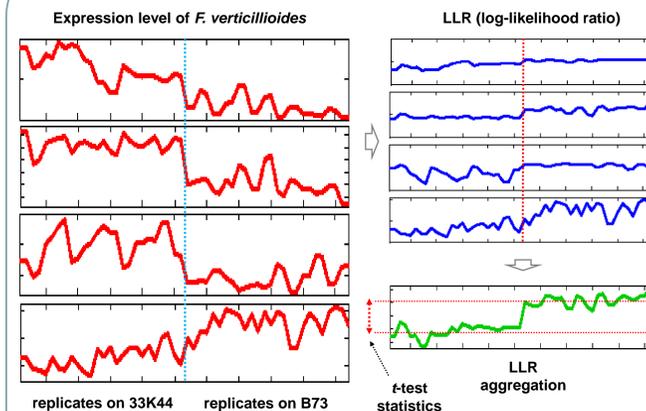
- Subnetwork activity level can be also computed by

$$\eta(x) = \sum_{k=1}^n \alpha^k(x^k)$$

- Discriminative power of the given subnetwork based on t-test statistics can be estimated by

$$t(G) = \frac{\mu_1 - \mu_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

μ : mean, s : variance, n : number of replicates



2.4 Prediction of Pathogenic subnetwork modules

Seed gene: top 1% discriminative power (t-test score) genes at the last time point.

Branch-out: i) discriminative power enhance by at least 10%

ii) consider up to three subnetworks whose discriminative power increases are within 2% of the discriminative power of the best subnetwork

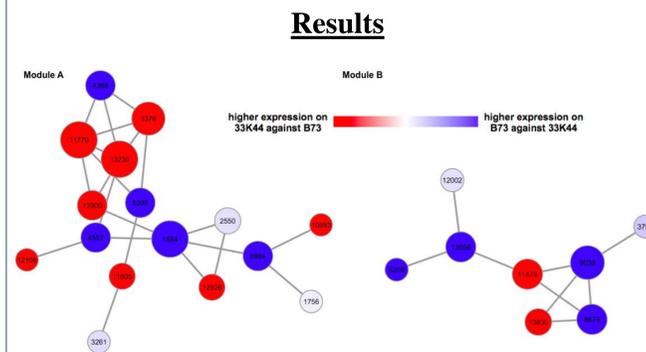
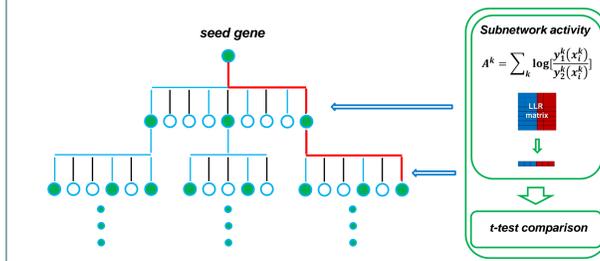


Fig. 2. *F. verticillioides* subnetwork modules associated with 33K44 against B73

i) **Module-A** is composed of sixteen *F. verticillioides* genes, with six genes annotated by a GO term (GO:0016021) "integral component of membrane" and suggested as a major facilitator proteins. There were genes encoding a oxidase, a reductase, a transcription factor as well as a fungal effector. Three genes with unknown function, but with significance correlation with other genes were found.

ii) **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 were found as well as those involved in carbohydrate/nitrogen utilization. Orthologous genes were identified in plant pathogens *Botrytis cinerea* and *Magnaporthe grisea*.

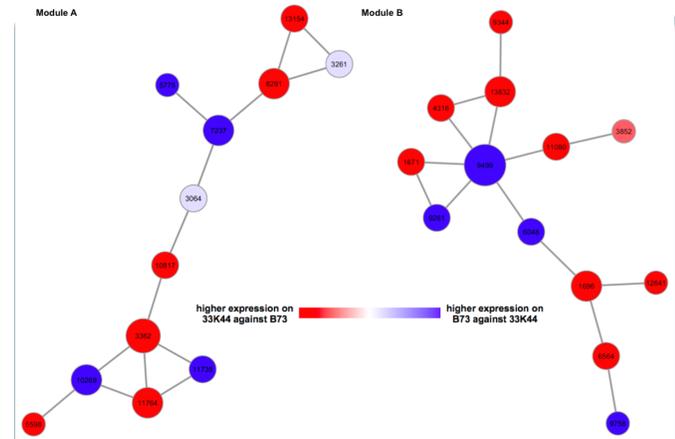


Fig. 3. *F. verticillioides* subnetwork modules associated with B73 against 33K44

Module-A is composed of fourteen *F. verticillioides* genes, where three were annotated by a significant GO term (GO:0022891) "substrate-specific transmembrane transporter activity". Transporters identified in this subnetwork likely work together as negative regulators of ear rot virulence.

Module-B is comprised of thirteen *F. verticillioides* genes, where three were annotated by a significant GO term (GO:0022891) "substrate-specific transmembrane transporter activity". In addition, genes associated with metabolic catabolism and modification were also identified. We also found three genes with no known functional motif but with strong expression correlation to these transporters.

Conclusion

We identified potential *F. verticillioides* pathogenicity-associated subnetwork modules, where the member genes were harmoniously 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.

Acknowledgments

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Reference

- J. Su, B. Yoon, and E. Dougherty, "Accurate and reliable cancer classification based on probabilistic inference of pathway activity," PLoS one, vol. 4, no. 12, pp. e8161–e8161, 2008.
 - W.-B. Shim, U. S. Sagaram, Y.-E. Choi, J. So, H. H. Wilkinson, and Y.-W. Lee, "Fsr1 is essential for virulence and female fertility in fusarium verticillioides and f. graminearum," Molecular plantmicrobe interactions, vol. 19, no. 7, pp. 725–733, 2006.
 - "Fusarium comparative sequencing project," Broad Institute of Harvard and MIT.
 - B. Langmead and S. L. Salzberg, "Fast gapped-read alignment with bowtie 2," Nature methods, vol. 9, no. 4, pp. 357–359, 2012.
 - A. Hero and B. Rajaratnam, "Hub discovery in partial correlation graphs," Information Theory, IEEE Transactions on, vol. 58, no. 9, pp. 6064–6078, 2012.
 - A.-L. Barabasi and Z. N. Oltvai, "Network biology: understanding the cell's functional organization," Nature Reviews Genetics, vol. 5, no. 2, pp. 101–113, 2004.
 - J. Reimand, T. Arak, and J. Vilo, "g:Profiler—a web server for functional interpretation of gene lists (2011 update)," Nucleic acids research, vol. 39, no. suppl 2, pp. W307–W315, 2011.
 - S.-H. Yook, Z. N. Oltvai, and A.-L. Barabasi, "Functional and topological characterization of protein interaction networks," Proteomics, vol. 4, no. 4, pp. 928–942, 2004.
 - R. Winnenburg, T. K. Baldwin, M. Urban, C. Rawlings, J. K'ohler, and K. E. Hammond-Kosack, "Phibase: a new database for pathogen host interactions," Nucleic acids research, vol. 34, 459–464, 2006.