Inference of the Plant Defense Signaling Network Based on Expression Profiles of Arabidopsis Mutants

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Short Abstract — **Expression profiles can be used as detailed descriptions of the states of biological networks. To obtain expression profiles for use in inferring the topology of the plant defense signaling network, an inexpensive and accurate, small-scale microarray technology was developed. The microarray was used to screen Arabidopsis mutants for discovery of new network components. The profiles of newly-identified mutants and known mutants were used to infer the signaling network. Comparison of the bacterial growth *in planta* and the inferred network structure predicts a new mode of defense contributing to resistance against bacterial pathogens.**

Keywords — *Arabidopsis thaliana*, **plant defense response, signaling network, non-linear dimensionality reduction.**

I. PURPOSE

THE purpose of this study is to infer the topology of the signaling network involved in the plant defense response against pathogens. The network inference will be performed based on expression profile changes caused by various genetic perturbations.

II. RESULTS

A. Development of a high performance, small-scale microarray [1]

More than 25% of the protein-coding genes of Arabidopsis undergo significant changes in mRNA levels during infection by an avirulent bacterial strain [2]. For detailed description of a particular network, profiling of genes with non-redundant information is sufficient. We developed a technology to produce an accurate small-scale microarray printed with long oligonucleotide probes, which we call a "miniarray." Special printing patterns permitted use of a linear model for correction of systematic errors, resulting in correlation between technical duplicates of 0.99. A relatively large number of stably expressed genes and a novel normalization scheme enabled accurate array-to-array normalization.

B. Discovery of new network components

Candidate genes for components of the defense signaling network were bioinformatically identified, and plants with loss of function mutations in the candidate genes were screened using expression profiling after infection by an avirulent bacterial strain. Eleven positive lines were identified among 42 lines screened.

C. Inference of the defense signaling network

Since this signaling network contains many non-transcriptional regulatory steps, we used profiles of signaling mutants, in which cause-effect relationships are clear, instead of approaches heavily dependent on time series data. In addition to the profiles of the new mutants, those of 19 Arabidopsis mutants known to affect defense signaling were used for inference. A network model graph was generated based on non-linear dimensionality reduction [3]. This model was consistent with prior knowledge of three well-characterized signaling pathways. It also predicted some unexpected interactions between nodes, one of which was recently demonstrated experimentally [4].

By comparing the network model with the effects of mutations on bacterial growth, we found that while most mutants that allow greater bacterial growth are compromised in salicylic acid-mediated responses, one of the new mutants has defects in different responses, revealing a new mode of defense.

III. CONCLUSION

With a high performance, small-scale microarray, expression profiling can be used as an economical tool to collect a broad spectrum of quantitative data. In combination with specific genetic perturbations, this approach helps rapidly build a network model.

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