



The Fifth International Rice Blast Conference

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Application of Next Generation Sequencing to Study Rice Blast Disease

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In Brazil rice blast fungus caused by *Magnaporthe oryzae* is a major constraint in production and causes losses of up to 100% of the yield depending on cultivar susceptibility, environmental conditions and crop management system. The molecular basis of the defense response to rice blast remains poorly characterized. A thorough understanding of the molecular response mechanisms against rice blast may provide new methods in devising strategies to control rice blast disease. The identification of host genes involved in the early defense responses is one of the most critical steps leading to the elucidation of resistance mechanism in plants. The recent advent of tools enabling the transcriptional profiling of infected plant tissues using next-generation sequencing methods provides an unprecedented depth of analysis permitting application of powerful statistical techniques for discovery of functional relationships among treatments.

We used the Illumina/Solexa technology to study gene expression of rice after infection with *M. oryzae*. This ultra high-throughput sequencing technology produced a digital expression profile with millions of short reads for each treatment. mRNA populations extracted from treatments were subjected to the Genome Analyzer with a total of 16,995,679 signatures being generated reflecting the depth at which the treatments have been sampled. Then individual signatures counting were performed to assign the quantitative variation between treatments to assign the gene expression level. A total of 711,284 distinct signatures were generated, showing a higher number than previously reported. Signatures covered 35% of the total rice genome. Taking advantage of the information content in the 21-bp tag, it was identified signatures from *M. oryzae* messages among the total signatures isolated from blast-infected rice leaves, representing 10% of the analyzed transcript in the blast-infected rice leaves. This opens a possibility to directly study the gene expression of two organisms at the foci of interaction. The signatures that could not be assigned to known transcripts are a rich source of information about the part of the transcriptome that is not yet characterized. Comparison among treatments was performed and then 200 more expressed genes were selected and twenty were used to run qRT-PCR. The comparison of the infection treatments obtained with digital expression profiles (quantification of transcripts) to those obtained with RNA extracted leaves, highlighted the same expressed rate when comparing distinct treatments. Among a subset of 20 these genes, 70% were validated by qRT-PCR. The results presented here are a preliminary analysis of these data. Currently, there are ongoing projects to study and perform detailed functional analysis of the selected candidate genes. Illumina technology promises to be a valuable addition to the repertoire of methodologies for functional genomics.

Reference:

Mehta, A., Brasileiro, A.C., Souza, D.S., Romano, E., Campos, M.A., Grossi-De-Sá, M.F., Silva, M.S., Franco, O.L., Fragoso, R.R., Bevitori, R., Rocha, T.L. 2008. Plant-pathogen interactions: what is proteomics telling us? FEBS J, 15:3731-3746.