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“Development on the margin”

## Finding the Genes and Proving Causality: New Forward and Reverse Genetic Tools and their Application in Rice

NORMAN WARTHMAN<sup>1</sup>, HAO CHEN<sup>2</sup>, STEPHAN OSSOWSKI<sup>1</sup>, PHILIPPE HERVÉ<sup>2</sup>, JÖRG HAGMANN<sup>1</sup>, MARICRIS ZAIDEM<sup>1</sup>, IL-RYONG CHOI<sup>2</sup>, DETLEF WEIGEL<sup>1</sup>

<sup>1</sup>*Max-Planck-Institute for Developmental Biology, Dept. Molecular Biology, Germany*

<sup>2</sup>*International Rice Research Institute (IRRI), Philippines*

### Abstract

The goal of plant breeding is to combine naturally occurring genetic variation between species, strains and varieties to create superior varieties. Being able to locate the genomic region responsible for the trait of interest accelerates this process, because it allows for more direct selection of the breeding material.

Traditionally, standard genetic mapping a trait of interest in crops required established sets of molecular markers able to distinguish the parental lines to genotype the progeny. Marker development and genotyping individual plants is tedious and costly.

We developed a method for genetic mapping by 2nd-generation sequencing, which does not require prior knowledge of molecular markers and will also directly yield the causal polymorphism. It is based on bulked segregant analysis of pools of plants. By sequencing two samples, one lane each on the Illumina Genome Analyzer II, we mapped the causal nucleotide change for a recessive virus resistance in a segregating population of two non-reference indica rice strains within a few weeks. While a reference genome is necessary, sequence information of the parental genomes is not.

The current resolution of genetic mapping projects often only allows one to define a region of the genome responsible for the trait rather than attributing it to a single gene. However, researchers are often able to identify one or several candidate genes in those regions. Specifically silencing these genes *in vivo* and monitor the effect is an effective way of testing the candidates. This can be achieved by post-transcriptional gene silencing (PTGS). Silencing genes with Artificial miRNAs (amiRNAs) – designed to target one or several genes of interest – have been shown to be a highly specific approach in plants.

We devised an amiRNA-based strategy for both japonica and indica type strains of cultivated rice, *Oryza sativa*. The adoption of this technology to rice will be described. Besides candidate gene validation, the technology may be used for comparative functional genomics between different varieties, and for improvement of agronomic performance and nutritional value.

**Keywords:** Genetic mapping, rice