

Transnational Access Report

1. General Information

Project Acronym (ID):	MetaboRice
Project Title	Metabolic changes in rice under drought stress: a genome wide association study
Name of Group Leader	Prof. Harro Bouwmeester
Name of organization	Laboratory of Plant Physiology, Wageningen University, NL

2. Duration of access

Duration of the access refers to the use of the installation only and does not include the preparation of the experiment or data analysis.

Begin of the project 06-10-2014	End of the project 27-02-2015
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3. Project summary (max. 250 words)

Drought is the most important stress for crops in terms of growth, yield and ultimately survival. This is particularly true for the cultivation of rice where periods of limited water availability during sensitive stages such as flowering, are responsible for a strong yield reduction. To cope with drought stress plants evolved many adaptive mechanisms, resulting in a combination of constitutive and drought-induced traits. The aim of these mechanisms is to control an excessive use of water and to confer a higher adaptation and tolerance to the detrimental effects of water-limited conditions. The activation of these mechanisms results into a profound change in the physiology of the plant which is reflected in an altered metabolic profile.

In this project we analyzed rice metabolic changes under control and drought conditions using a metabolomics approach (GC-TOF-MS). The identified metabolites were used in a genome wide association study (GWAS) as independent traits to map for the genetic basis of drought tolerance from a metabolic perspective. The GWAS results linked the observed metabolic differences in a population of 300 rice accessions under drought stress with their genetic differences. We identified many QTL (quantitative trait loci) associated with genes responsible to control metabolite levels in a treatment dependent manner.

4. Description of work (max. 250 words)

We analyzed leaf material from a population of 300 diverse accessions of *Oryza sativa*, subsp. *indica*. Leaf material was collected from a field experiment conducted during the dry season of 2013 at the International Rice Research Institute (IRRI), Los Baños, Philippines. In this experiment the genotypes were synchronized in order to be subjected to drought stress during the flowering stage (50% flowering). The stress consisted of 15 consecutive days of water withholding before re-watering. Collected samples consisted of a pool of 8 top leaves harvested from the main tiller of 8 different plants for every field replicate of every genotype. We collected 2 control and 3 drought replicates of the entire population. All samples were immediately frozen in liquid nitrogen (on field), processed a few days afterwards (ground in liquid nitrogen) and stored at -80°C before being sent to Wageningen University.

For the metabolomics work at the IPK-MP facility we first pooled the 2 control replicates and the 3 drought replicates to produce a total of 600 samples. We extracted the polar phase of every sample that, after in-line derivatization, was injected in the GC-TOF-MS for untargeted metabolite analysis. Around 30 days were needed for injections and runs of all samples. Two additional weeks were required for data processing (peak annotation and peak height extraction) and basic statistical analysis. In total we identified 91 known and 197 unknown metabolites. Every metabolite was then used as an independent trait to run GWAS with a very high resolution haplotype map (212k SNPs) available for this population.