Specific aims of the project

To identify and validate TF involved in root differentiation in rice, we will adopt a three-step approach.

A specific tissue transcriptome analysis for cortex tissues has been performed using Laser Capture Microdissection (LCM) technology to retrieve mRNAs, create and sequence RNAseq libraries before the beginning of the project. We have recently developed this technology (Partner 1) and obtained mRNAs in large quantities and of optimal quality (T. Mounier et al in revision for BMC Molecular and Cellular Biology). Based on these data, we will identify all genes involved in programmed cell death and cell wall modifications that are expressed specifically in cortex using a time course transcriptome experiment for aerenchyma formation. Three developmental time points has been analyzed, meristematic, first sign of cortex differentiation, first sign of aerenchyma formation. Data will been curated with massive transcriptome data available in public databases to build high quality expression data needed to feed gene network reconstruction (WP1)

A gene network will be reconstructed using curated data by partner 2 (WP1) and a database containing all these data, accessible through intuitive and dynamic query interfaces (partner 3), will support the identification of the TFs that regulate the genes involved cortex formation and aerenchyma formation. These TF will be candidate TFs for the identity and differentiation of cortex tissue based on their influence (WP1). A simulation tool will be developed to test in silico hypotheses on the role of each TF by recomputing the gene network by removing them one by one, simulating a set of loss-of-function mutants and their impact in the gene network. An interactive data visualization interface will help the biologist analyze and contrast different states of the network throughout the simulation experiments using Hive Plots to identify the most pertinent gene network potentially involved in aerenchyma formation.

The most promising candidate TFs issued from the WP1 simulation experiments, will then be validated alone, or in combination, in protoplasts, using a gene-network manipulation tool that allows an arbitrary number of genes (WP2) to be simultaneously overexpressed and under expressed. We recently demonstrated the functionality of the system using fluorescent reporter promoter (T. Mounier et al unpublished data). The validation will involve either the transcriptional demonstration of a direct regulation by one or more TFs on effectors genes, by Droplet RT-PCR, or by using molecular markers (vital dyes, anti-wall antibodies) for cortex differentiation. The validation or invalidation results will be re-injected into the gene network database in order to improve progressively the gene network. Finally, the function of 2-3 TF among the most interesting ones, will be analyzed in planta using CRISPR and dynamic multiphoton imaging technologies available from partner 3 in parallel to follow defects at cellular and tissue levels.

The proposed approach is innovative, with an original methodology to identify transcription factors through an inverse genetics approach, by developing a dynamic approach for modeling gene networks that allows the use of validation data in real time to improve this network, and finally by developing a fast, universal and high throughput system for validating and manipulating networks. Demonstration of the efficiency of this approach will come from the identification of new key factors for cortex and aerenchyma formation and differentiation in rice.