Linking the gene regulatory network with the functional physical structure of whole-genome engineered Arabidopsis mutants : an HR-MAS NMR-based metabolomics approach

Augustijn, D.

Summary

Climate change is a challenge for both current and future generations. New biological resources have to be developed in order to meet the demand for energy as well as the demand for food for the rising world population. One way of doing this is to make use of so-called smart crops, as they have improved yields and a small environmental footprint. The development of smart crops can be accelerated by using genome editing methods like zinc finger artificial transcription factor-mediated whole genome interrogation to obtain novel mutant phenotypes. Model plant organisms, such as *Arabidopsis thaliana*, can contribute to the development of smart crops as they have been studied extensively by the research community. In this PhD thesis, two phenotypically engineered Arabidopsis mutants with enhanced growth characteristics have been studied.

The purpose of this PhD thesis is to unravel the pathways leading to the phenotype of *Arabidopsis thaliana* mutants from a systems biology perspective, by using metabolomics. In metabolomics, metabolites are studied both qualitatively and quantitively under specific growth conditions. Since metabolites are the end products of cellular processes, the metabolome is most closely related to the phenotype of plants. To obtain the metabolic profile directly from the leaves of *Arabidopsis*, high-resolution magic angle spinning (HR-MAS) NMR is used. Combined with multivariate analysis, biomarkers, related to the mutant phenotype, are determined. The biological interpretation of the biomarkers will be performed in a systems biology approach by using the available information from the literature to obtain a model to explain the mutant phenotype.

Chapter 1 contains a detailed explanation of metabolomics and the role of metabolomics in systems biology. The most common techniques to obtain metabolic profiles are mass spectrometry and liquid-state NMR. Extraction of metabolites from the sample is necessary for these techniques, which leads to the loss or degradation of metabolites during extraction. The extraction procedure can be eliminated by using HR-MAS NMR which enables the use of intact leaf material. It is, therefore, a powerful tool in plant metabolomics and it has been used for an increasingly wide range of applications. In this chapter, a HR-MAS NMR-based workflow is proposed and described, including used pulse sequences, data pre-processing and multivariate analysis.

Chapter 2 describes the metabolic profile that has been collected directly from the Arabidopsis thaliana leaves by using HR-MAS NMR. Wild-type *Arabidopsis thaliana* accession Columbia-0 (Col-0) has been studied throughout the light/dark cycle to examine primary metabolites and to determine their functional periodicity during the circadian cycle. Both one- and two-dimensional ^{1}H HR-MAS NMR spectra are used to identify and quantify primary metabolites including organic acids, sugars and amino acids. Multivariate analysis revealed modules of primary metabolites for which the concentrations vary during the circadian cycle.

In **Chapter 3**, the HR-MAS NMR-based workflow is used to obtain the metabolic profile

for the *Arabidopsis thaliana* mutant VP16-02-003 and VP16-05-014 with enhanced growth characteristics at a time-point in the middle of the light period. Eighteen identified metabolites showed significant alteration of the metabolite concentration between the mutants and the wild-type Col-0. A biological interpretation of the concentration levels of the metabolites in terms of a reduced ability of the plants to respond against stress is presented. The diminished defence response leads to an altered growth-defence trade-off for both mutants. The results in this chapter contribute to converging evidence that improved biomass yield can be obtained by changing the balance between growth and defence.

Chapter 4 describes how the eighteen biomarkers for the enhanced growth characteristics of both mutants can be studied throughout the light/dark cycle, as the photosynthetic machinery is under control of the circadian clock. The circadian rhythm is robust, since the variation of the metabolite concentrations is very similar for the wild-type Col-0, the VP16-02-003 and the VP16-05-014. In contrast, the overall metabolite concentrations, throughout the whole circadian cycle, are different for the three species. Hence the clock functional periodicity appears independent of the growth-defence trade-off. This indicates that downstream phenotypic engineering of sinks and bottlenecks leading to growth is more effective than altering the circadian clock upstream.

Finally, **Chapter 5** provides a general discussion of this work and a future outlook. In conclusion, HR-MAS NMR metabolomics combined with multivariate analysis is a powerful tool to understand the phenotype of plants and it can be used for *Arabidopsis* mutants or other crops which are interesting to use as biological resources for bioenergy. In fluxomics, the metabolic fluxes, resulting from the interplay of gene expression, protein concentration, protein kinetics and regulation and metabolite levels, can be used to interpreted complex metabolic pathways and the regulation for a better understanding of the phenotype of plants. Thus, when metabolomics is combined with other omics technologies, such as transcriptomics and fluxomics, the complexity of a biological system can be further unravelled.