



**CEPLAS**

Cluster of Excellence on Plant Sciences

Excellent Science for Tomorrow's Plants  
Opportunities for Public-Private Partnerships  
- Update -



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Participating Institutions:





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**CEPLAS**

Cluster of Excellence on Plant Sciences



# General presentation



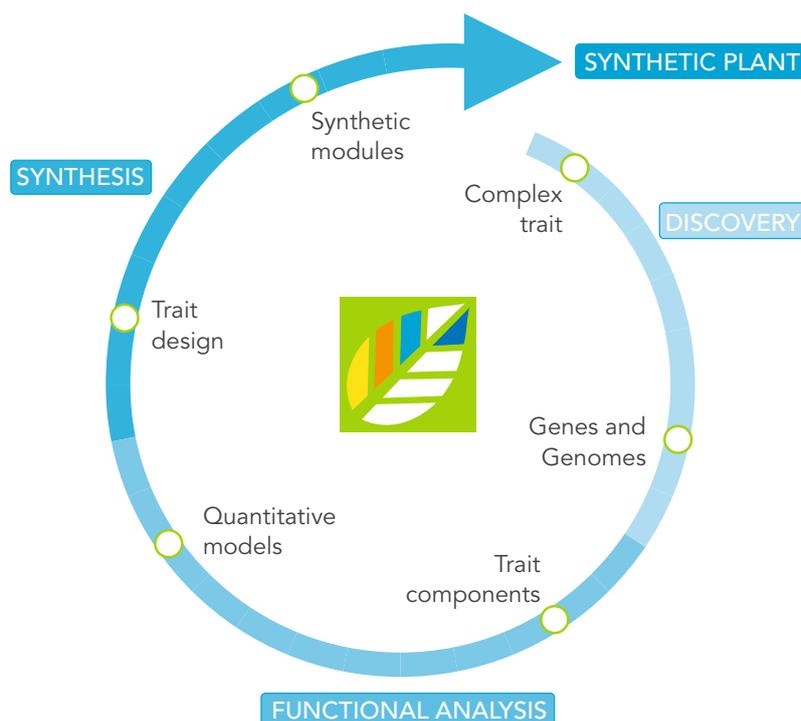
## 1. Summary

Plants are the basis of all human life. They provide food and feed, medical drugs, as well as raw materials for a variety of industrial applications. However, global climate changes are challenging the sustained production of crops and thus the agronomic basis of human civilization.

At the same time, arable land is becoming scarce due to increased erosion and population pressure. Increasing population and altered consumption patterns demand increased crop production for food, feed, and industrial raw materials. To meet the challenges of these developments, it has been estimated that agricultural yields have to double by the year 2050. This will require innovative strategies for crop improvement that aim at enhancing yield without compromising

increases in the use of water, nutrients and soil, or diminished resistance to diseases and pests.

The ultimate goal of CEPLAS is to support the development of crop plants with improved yield potential and yield stability in a sustainable agriculture that protects natural resources. In this context, CEPLAS works on achieving a fundamental understanding of the genetic and molecular mechanisms that determine the yield potential of crops and enable them to adapt to adverse environmental conditions and constraints. The major question behind this is: How is the genetic code translated into a flexible system that can cope with varying environmental conditions? It employs research that is driven by comparative





evolutionary analyses with the goal to provide molecular modules for synthetic biology.

The scientific program addresses the mechanistic basis and genetic architecture of four complex biological processes that have a crucial impact on the yield potential and yield stability of crop plants and, therefore, are of outstanding importance in designing and breeding the crops of the future, as well as in developing novel concepts for fertilization and protection against disease in agricultural production:

- Annual and perennial life patterns
- Photosynthetic carbon conversion efficiency
- Composition and function of the plant microbiome
- Metabolic interactions between plants and microbes

CEPLAS research is divided into four stages: "Discovery", "Functional Analysis", "Synthesis", and "Synthetic Plant". A significant portion of the projects will develop from "Discovery" to "Functional Analysis" and "Synthesis" during the ongoing funding period of CEPLAS (until 2018).

Besides its comprehensive research program for addressing key biological questions in plant sciences, CEPLAS also puts a strong emphasis on education and career development of its young scientists. For this purpose, various training programs are established, including introductions into R&D programs and activities of companies translating results from plant sciences into application for product and process development.

A key goal of CEPLAS also is to pave the way for the translation of its research activities and results into the development of plants, plant varieties and production processes that meet the challenges of the future. For this purpose, cooperations with the private sector are strongly supported, comprising joint research projects, the license-

based exploitation of results, the exchange of know-how and scientific personnel, as well as joint educational programs. In this document, the scientific areas and the opportunities for cooperations within public-private partnerships are illustrated.

## 2. About this document

This document represents a complementation to the previously published compendium "Excellent science for tomorrow's plants – Opportunities for Public-Private Partnerships", that has focused on application relevant aspects of research plans and research results in CEPLAS projects.

Facts and figures about the CEPLAS research organization are updated, and the current status of research plans, research results and lists of publications is presented in new versions of "Project Profiles", considering the time frame between beginning of 2016 and end of 2017. Again, the focus is on application relevant aspects and on opportunities for cooperations between CEPLAS projects and partners from the private sector.

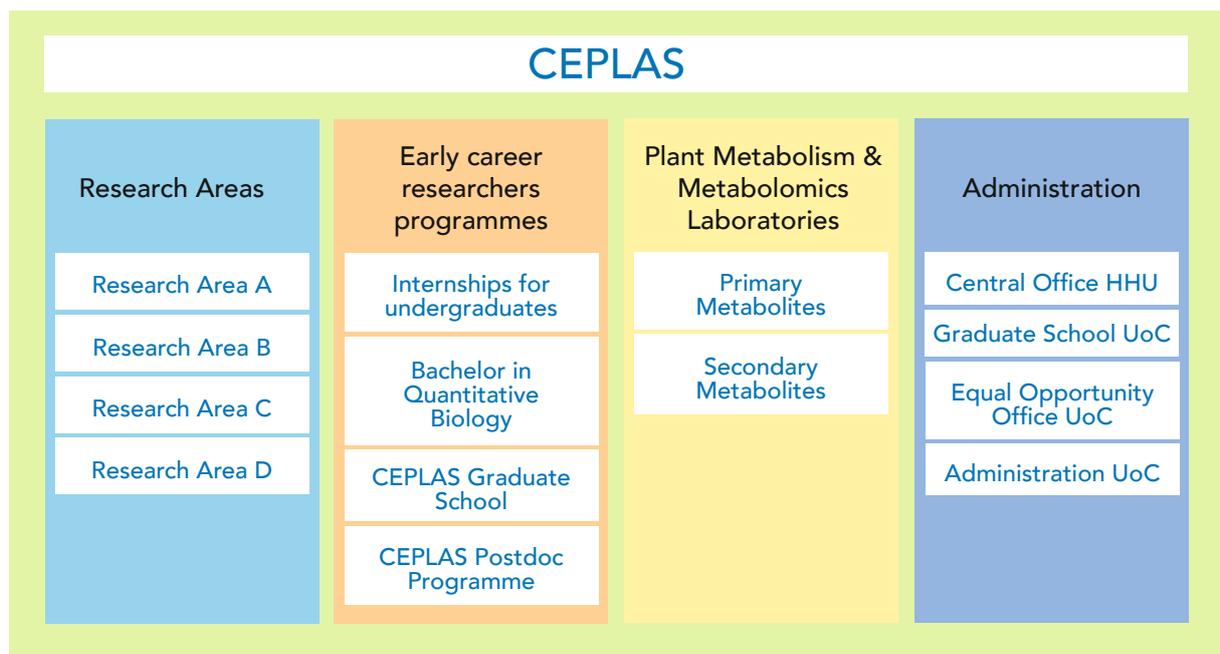
The goal is to show in a condensed form the progress of research projects in the CEPLAS program, to indicate the potential for translation of its results into novel products and processes with economic value and, thereby, also to stimulate the interest of companies in discussions about partnerships with CEPLAS.

Since its beginning, a key element of the CEPLAS program has been to not only provide excellent basic science but also to open up routes for the development of innovative products and processes in related fields of agriculture and biotechnology.

## 3. Structure

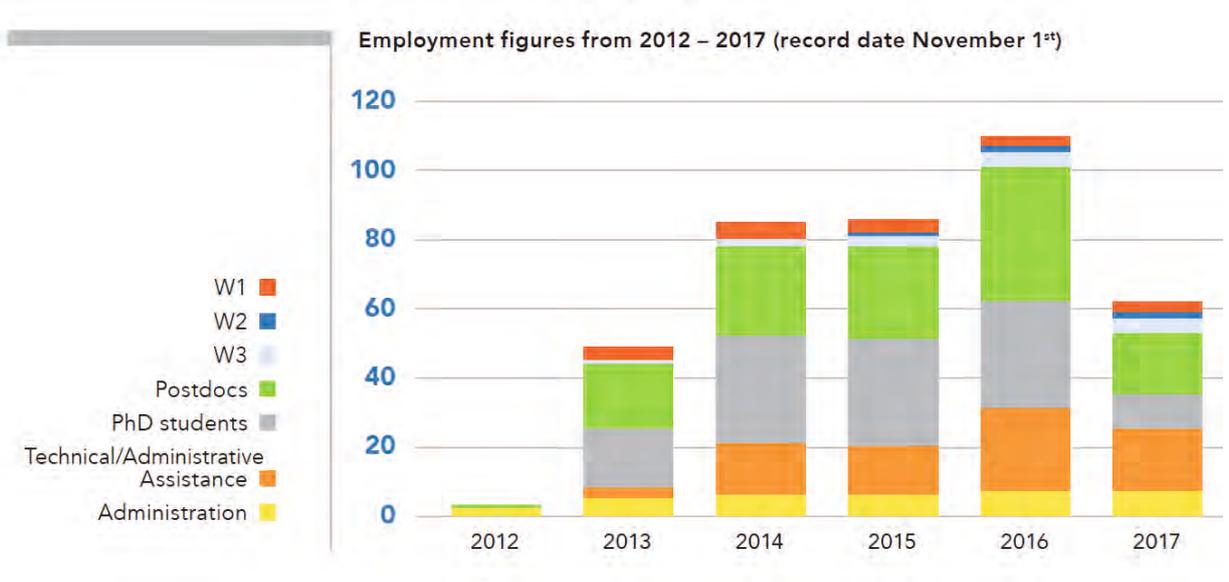
### 3.1 Organization

CEPLAS is a joint initiative of Heinrich Heine University Düsseldorf, University of Cologne, Max Planck Institute for Plant Breeding Research and Forschungszentrum Jülich. CEPLAS comprises four different research areas, one Graduate School, a structured Postdoc Programme, the Plant Metabolism and Metabolomics facilities and an administration unit. All research area coordinators are part of the CEPLAS Steering Committee, together with the cluster speaker and deputy speaker, the equal opportunity representative and one representative of the early career researchers.

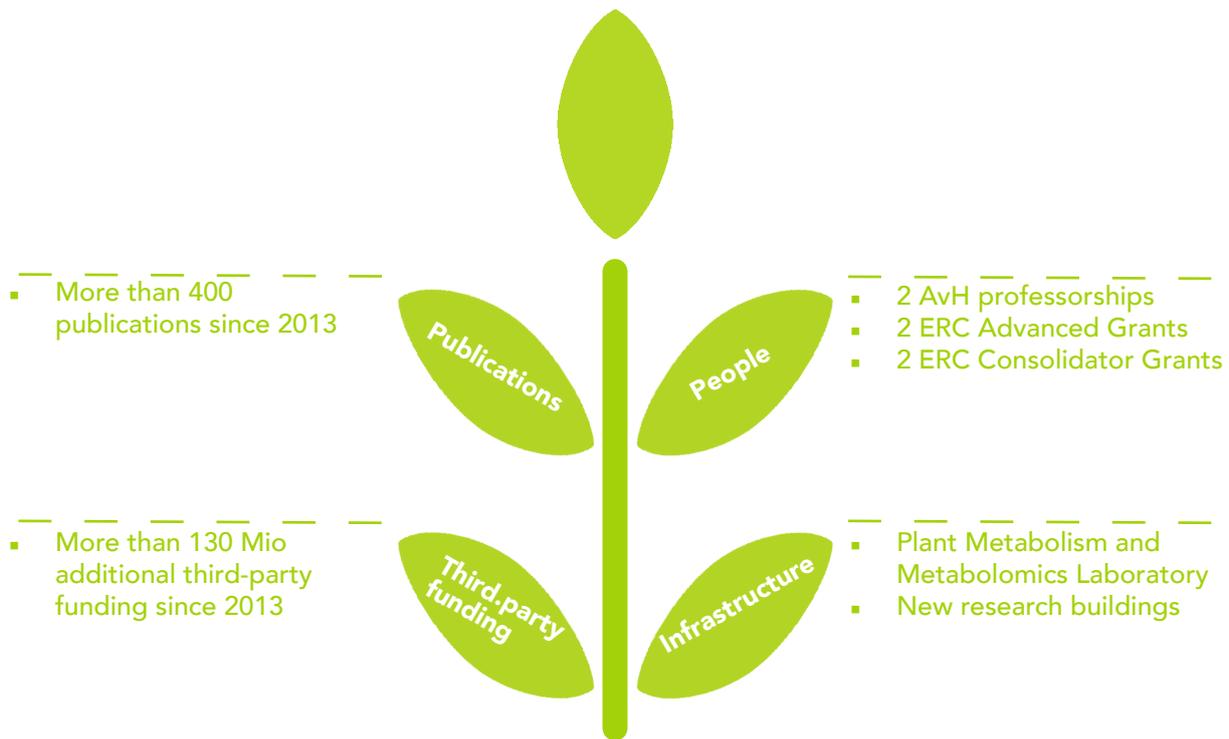


### 3.2 Personnel funded by CEPLAS

In 2017, the number of people directly financed by the cluster slightly decreased due to the official funding end in October 2017. From November 2017 to December 2018, the cluster is financed by a bridge funding from the DFG covering only costs for the maintenance of the cluster, the new faculty and some postdoc and PhD positions. Although the cluster personnel decreased to around 60 people, the number of researchers working in CEPLAS labs on CEPLAS relevant topics remained constant with currently around 200 researchers.



## 4. Overview on Achievements





## 5. CEPLAS Research Areas

### Research Area A

#### Coordinator

George Coupland

#### Co-coordinator

Rüdiger Simon

#### **Elucidation and manipulation of the mechanisms that differentiate annual and perennial life histories:**

#### **Basis for the use of beneficial traits from perennials in annuals**

Plant life history varies widely, even among closely related species. In this Research Area we compare annual and perennial species and aim to explain how key traits diversify during the divergence of these life histories. Annuals evolve from perennial progenitors in response to environmental selective pressures that reduce survival of adult perennials, and this has occurred often in the Angiosperms. Evolution of annuals affects traits such as life span, adaptation to environment, storage and recycling of metabolites, propensity for clonal propagation, timing and duration of flowering as well as number of progeny. Several traits characteristic of perennials would be beneficial in breeding crop plants, but were removed from annual crops in the early stages of domestication.

In this Research Area, we aim to identify regulatory modules that diverged during the evolution of annuals from perennials with the objective of engineering perennial traits in annual species. We focus on characteristic differences in meristem function, flowering behaviour, nutrient recycling, root growth and longevity. The groups within Research Area A concentrate their efforts on two major model systems, which are closely related Brassicaceae species, particularly in the *Arabidopsis* and *Arabis* genera, as well as *Hordeum* species related to barley.

A range of approaches are used including forward genetics, reverse genetics based on CRISPR-Cas9, transcriptomics exploiting RNAseq, comparison of newly acquired genome sequences from phylogenetically closely related species, ChIPseq for interspecies comparison of transcription factor targets and development of algorithms for inferring gene regulatory networks from transcriptome data.

Ongoing projects in the Research Area are summarised in the following sections, so here only a few of the major developments that occurred recently are mentioned.

An improved assembly of the genome of perennial *Arabis alpina* based on long Pac Bio reads was published in 2017. This can now be compared with the Pac Bio assemblies of the genomes of the closely related annuals *Arabis montbretiana* and *Arabis iberica* to identify copy number variants and other rearrangements that arose during the divergence of life history.

By exploiting a population generated by crossing annual *A. montbretiana* and perennial *A. alpina*, we were able to show that introgression of a single chromosomal region of *A. montbretiana* into *A. alpina* was sufficient to generate plants exhibiting a combination of flowering traits, vernalisation requirement and perpetual flowering, not found in either parent.

We have also mapped short segments of the *Arabis montbretiana* genome that when introgressed into perennial *Arabis alpina* alter flowering time, some of this allelic variation might have arisen during evolution of annual life history to confer reproductive assurance.

Furthermore, by developing CRISPR-Cas9 for use in *A. alpina*, we were able to generate null alleles of genes proposed to have a central role in conferring competence to flower in the context of the perennial life cycle, and thereby to test models



of how this trait diverged during the evolution of annuals. We have also extended the range of species used to include *Cardamine resedifolia*, which shows dramatic clonal propagation of shoots from roots, a trait usually associated with perennials. This Brassicaceae species provides a practical model to study this developmental plasticity and an interesting point of comparison with the study of adventitious root formation on the aerial shoots of perennial *Arabis alpina*.

In addition, we have initiated the examination of nutrient recycling and of senescence patterns within the perennial life cycle of *Arabis alpina*, which generates collaborations with Research Area D and extends our analysis beyond developmental traits.

### **Construction of a phylogenetic and genomic framework for the study of divergence of annual and perennial life cycles**

In this research section we apply genomics tools within a phylogenetic framework to generate platforms for studying the divergence of the annual and perennial life cycles. As well as obtaining genomic sequences from model species, this includes RNAseq of staged material and ChIPseq to compare the target genes and regulatory networks associated with key transcription factors. These tools are applied to the study of traits that characteristically differ between annuals and perennials, such as seasonal flowering patterns, senescence patterns and nutrient recycling.

### **Elucidation of regulatory networks that determine formation and identity of meristems**

Annual and perennial plants show marked differences in meristem formation and identity, which contribute to divergence in life history. For example, flowering and vegetative shoots

are maintained on individual perennial plants to ensure that they survive reproduction. Furthermore, perennials more often utilise clonal reproduction, which can involve formation of ectopic meristems and switches in meristem identity. In this work package, we study these processes by examining branching patterns in *A. alpina* and barley, adventitious root formation in *A. alpina* and clonal propagation in *C. resedifolia*.



## Research Area B

**Coordinator**  
Peter Westhoff

**Co-coordinator**  
Martin Lercher

### **Decoding function and development of a C<sub>4</sub> leaf: basis for improving photosynthetic efficiency in C<sub>3</sub> plants**

Research Area B aims at understanding the molecular mechanisms underlying the evolutionary trajectory from C<sub>3</sub> to C<sub>4</sub> photosynthesis to a level of detail that enables the construction of C<sub>4</sub> trait modules and their introduction into C<sub>3</sub> model species.

The majority of our research was and hence still is targeted at dissecting key characteristics of C<sub>4</sub> leaf anatomy and gene expression.

CEPLAS funds were complemented by research funds from the DFG-funded International Research Training Group 1525, the DFG Research Group Promics, the DFG Priority Programme Adaptomics, the Bill & Melinda Gates Foundation (C<sub>4</sub> Rice) and HHU Düsseldorf.

Work conducted in this area in the reporting period focused on four different research topics:

1. Analysis of the transcriptome data sets from C<sub>3</sub> and C<sub>4</sub> Flaveria species to identify putative regulators of C<sub>4</sub> leaf differentiation.
2. Continuation of the forward genetic studies with *Arabidopsis thaliana* for the detection of bundle-sheath differentiation genes, and of the natural variation of this species for the identification of genes affecting general leaf morphology and anatomy.
3. Completion of the experimental

evolution studies with *Arabidopsis* to select directly for traits that should appear on the trajectory towards C<sub>4</sub>.

4. Studies of selected regulatory modules of C<sub>4</sub> metabolism and investigation of the interaction of C<sub>4</sub> photosynthesis with other metabolic pathways.

### **Evolutionary transcriptomics of C<sub>3</sub> and C<sub>4</sub> species pairs**

The presence of closely related C<sub>3</sub> and C<sub>4</sub> species in the genera *Flaveria* and *Cleome* offered the unique opportunity of using comparative transcriptome analyses for the identification of genes that are involved or required for the establishment and/or functioning of the C<sub>4</sub> photosynthetic pathway. By comparing leaf transcriptomes at various developmental stages, i.e. from the primordia up to fully matured leaves, it was particularly aimed at the identifying regulators of C<sub>4</sub> leaf differentiation. While the studies with the *Cleome* species have been finished in 2015 (Külahoglu et al., *Plant Cell*, 2014), those with the *Flaveria* species are still ongoing, but in its final stage.

### **Genetic analyses with *Arabidopsis thaliana* for identifying genes involved in leaf anatomy and morphology**

The forward genetic approaches aiming at identifying genes involved in bundle-sheath differentiation relied on the observation that the bundle-sheath is not a novel invention of C<sub>4</sub> species, but is present in C<sub>3</sub> species and its ontogeny and functional maintenance may therefore be studied in a genetically tractable C<sub>3</sub> model species i.e., *Arabidopsis*.

EMS mutagenesis of *Arabidopsis* lines expressing a bundle-sheath chloroplast targeted reporter GFP resulted in the identification, until currently, of about mutant lines with altered bundle-sheath



and bundle morphology. The affected genomic regions have been located by the SHORE mapping approach and candidate mutant genes identified in these regions are presently being verified by RNAi and/or CRISPR/Cas9 knocking-down approaches. Similar mutant phenotypes – a total of six at present – were obtained by using activation tags containing mesophyll and/or bundle-sheath specific promoters. The affected genes were identified and are presently verified by cell-specific overexpression and their knock-down or knock-out.

In addition to forward genetics, projects assembled in this research section also used natural variation in *Arabidopsis* to identify genes involved in leaf anatomy and morphology.

are essentially known. However, still important details in the composition and regulation of the  $C_4$  cycle are unknown, i.e. which transporters are involved shuttling metabolites between organelles and cytosol or how is regulatory specificity achieved at the molecular level. It is also not well understood how  $C_4$  cycle is coordinated with other metabolic pathways such as sulphur or nitrogen metabolism.

By using the transcriptomic data sets generated from  $C_3/C_4$  species pairs of *Flaveria* and *Cleome*, the knowledge about  $C_4$  enzymes and their regulators as well as methods of structural biology the projects of this research section attempt to fill the gaps in our understanding of the  $C_4$  cycle and its regulation.

### **Experimental evolution towards $C_4$ photosynthesis**

The quantitative evolutionary model of  $C_4$  photosynthesis, developed in the framework of CEPLAS (Heckmann et al., Cell, 2013; Mallmann et al., eLife, 2014), indicates that the transition from  $C_3$  to  $C_4$  photosynthesis proceeded in modules and that each of the individual modules was adaptive. This implies that each step brought a small but detectable advantage in the photosynthetic capacity and suggests that it should be possible to evolve a  $C_4$ - like or, at least, a  $C_3$ - $C_4$  intermediate type of photosynthesis by applying the concept of experimental evolution combined with synthetic biology. Projects combined here either attempt to refine the current model by incorporating not yet considered factors of evolutionary pressure/selection or by using experimental/mutagenic approaches to select for  $C_3$ - $C_4$  like properties by using *Arabidopsis thaliana*.

### **Regulation and metabolic interactions of $C_4$ photosynthesis**

The general make-up of the  $C_4$  cycle and its regulation by metabolic and environmental factors



## Research Area C

**Coordinator**  
Alga Zuccaro

**Co-coordinator**  
Jane Parker

### **Molecular basis of plant-microflora interaction: know-how and tools for designing microbial communities with beneficial effects on plant growth**

Area C has been a springboard for developing tools to define the structure and functions of the plant microbiota, thus revealing that members of the microbiota can contribute elements of fundamental plant traits. In the past years marker gene amplicon sequencing and metagenome data enabled quantitative surveys of microbial assemblages associated with different plant organs, species, genotypes and developmental stages over a range of environmental and experimental conditions. Using different bio-informatics, biochemical and genetics tools we have:

- Generated a wealth of information on the structure, spatio-temporal organisation, function, and ecology of the plant microbiota within and between species of the Brassicaceae (Bulgarelli et al., *Nature*, 2012; Bulgarelli et al., *Plant Cell*, 2013; Schlaeppli et al., *PNAS*, 2014; Bai et al., *Nature*, 2015; Donn et al., *Environ Microbiol*, 2015; Dombrowski et al., *ISME J*, 2017; Kawasaki et al., *PLoS One*, 2016; Ploch et al., *J Euk Microbiol*, 2016).
- Produced new insights to how biotic and abiotic factors affect the composition and activities of plant-associated microbial communities (Rosenberg et al., *ISME J*, 2009; Scherber et al., *Nature*, 2010;

Dombrowski et al., *ISME J*, 2017). Recently, we quantified the relative contributions of factors explaining variation in *A. alpina* root-associated bacterial consortia by analysing high-quality 16S rRNA reads from >200 samples and multiple environmental factors. This revealed the variable compartment as strongest determinant of community variation (18–36%). Additional variables influencing community variation were soil type (11–15%), residence time of plants in soil (7–12%), environmental conditions (8–11%), host species (7–10%) and host genotype (5–12%) (Dombrowski et al., *ISME J*, 2017). We have additionally described the fungal microbiota of *A. alpina*, a plant that thrives in P-limited alpine habitats and lacks the ability to establish an AM symbiosis. We uncovered its association with a beneficial Helotiales fungus F229 capable of promoting plant growth and P uptake, thereby facilitating plant adaptation to low-P environments (Almario et al., *PNAS*, 2017).

- Identified microbial hubs (Kemen et al., *New Phytol*, 2015; Agler et al., *PLoS Biol*, 2016) which play a disproportional role in shaping microbial communities and showed that green algae play an important role in microbiota composition and activities. We are now functionally characterizing these hubs.
- Identified and characterized host and microbial genes that influence the outcome of these beneficial interactions, e.g., genes involved in metabolic functions, plant-microbe communication or in counteracting plant defence (Lahrman et al., *New Phytol*, 2015; Fesel & Zuccaro, *Environ Microbiol*, 2016b; Fesel & Zuccaro, *Fungal Genet Biol*, 2016a; Hacquard et al., *Nat Commun*, 2016; Hiruma et al., 2016; Matei & Doehlemann, *Curr Opin Microbiol*, 2016; Misas-Villamil et al., *New Phytol*, 2016; Frantzeskakis et al., *Mol Plant Microbe Interact*, 2017). With respect



to the plant immune system recent findings suggest that at least one layer of this system is also engaged in cooperative plant-microbe interactions and influences host colonization by beneficial microbial communities. Indeed several reports illustrate how commensal and beneficial plant-associated microbes have evolved the ability to evade PRR recognition directly, through modification of the MAMP epitope, or indirectly, through inhibition of the biosynthesis of MAMP-containing molecules or alteration of microbial cell wall composition e.g. (Wawra et al., Nat Commun, 2016). Thus, the reciprocal interplay between microbiota and the immune system likely plays a critical role in shaping beneficial plant-microbiota combinations and maintaining microbial homeostasis (Hacquard et al., Annu Rev Phytopathol, 2017).

- Critically assessed methods for assembly, taxonomic profiling and binning which are key to interpreting metagenome data. We participated in the Critical Assessment of Metagenome Interpretation (CAMI) challenge that engaged the global developer community to benchmark their programmes on highly complex and realistic data sets, generated from about 700 newly sequenced microorganisms and about 600 novel viruses and plasmids and representing common experimental setups. The CAMI results highlight current challenges but also provide a roadmap for software selection to answer specific research questions (Szyrba et al., Nat Methods, 2017).
- Determined reciprocal effects of interactions on the production of secondary metabolites in hosts and microbes (Jacobi et al., Front Plant Sci, 2017) with a special focus on glucosinolates (Jousset et al., Funct Ecol, 2008; Lahrmann et al., PNAS, 2013; Lahrmann et al., New Phytol, 2015; Bernsdorff et al., Plant Cell, 2016; Hacquard et al., Nat Commun, 2016; Hiruma et al., Cell, 2016;

Stahl et al., Mol Plant, 2016). Recently, we have shown that a functional link between the endoplasmic reticulum body (ER body), an organelle derived from the ER that occurs in only three families of the order Brassicales, and glucosinolate metabolism exist and we provided insights into the diversity and evolutionary processes of glucosinolate/myrosinase systems in the order Brassicales (Nakano et al., Plant J, 2017; Piślewska-Bednarek et al., Plant Physiol, 2018). We are now implementing analyses on novel diterpene phytoalexins pathways in Brassicales and in barley, whose local and systemic microbe-induced biosynthesis can be inferred from split root transcriptome data. Ultimately, their role in multispecies root interactions will be evaluated (cooperation with Area D).

- Started to construct synthetic symbioses to test hypotheses on the nature and activities of endophytic microbes, towards enhancing plant performance (Bai et al., Nature, 2015).

The important finding that endophytic microbial communities of plants are composed of bacteria, fungi and protists (Sapp et al., Environ Microbiol, 2018), demands an interdisciplinary approach to dissect the interplay of organisms of different phyla and kingdoms. Progress achieved in Area C allows us now to characterize the mechanisms underlying microbe-plant symbioses in a community context and thus achieve a step change in understanding the functional interconnections between soil, microbiota and plants.

## Research Area D

**Coordinator**  
Markus Pauly

**Co-coordinator**  
Karl-Erich Jaeger

### **Plant metabolism: from biotic challenges to synthetic biology**

Plants produce a great variety of biochemically unexplored secondary metabolites with plethora of functions. The chemical diversity of plant secondary metabolites can be shaped by environmental stressors as well as in response to microbial interactions.

Plants produce a great variety of secondary metabolites with plethora of functions that biochemically remain to be explored. The chemical diversity of plant secondary metabolites is created by environmental cues in particular responses to the microbiome. Our aim is the identification and functional analysis of plant secondary metabolites, which are decisive for the interaction of plants with the root microbiome (cooperation with Research Area C).

Research Area D focuses on three main interconnected themes:

Firstly, plant-microbe interactions are studied in the context of plant nutrition and defense reactions.

In this approach regulatory networks leading to the synthesis of glucosinolates and other tryptophan-derived indolic compounds are investigated. These secondary metabolites play an important role in plant defense including systemic acquired resistance. In addition, *Arabidopsis* ecotypes and a genome-wide association study were used to identify genes responsible for the observed

genotype-specific differences in the rhizosphere microbiome. For the plant-microbe interaction studies a microbe collection provided by RA C that covers an entire range of root-derived and sequenced bacteria strains will be used.

Secondly, signalling molecules involved in plant-microbe interactions are identified and characterized.

This approach intends to understand reciprocal metabolic signalling in plant-microbe interactions. Interactions strongly depend on the plant variety and microbial strain and thus an exuded metabolic cocktail by one partner and its recognition by the other interaction partner. The metabolomics analysis of such exudates derived from various *Arabidopsis* strains and mutants probed with defined microbes clearly demonstrated the impact of the interaction partners on the exudate.

Thirdly, key compounds in synthetic microbial communities are produced.

Plant biosynthetic modules such as the biosynthesis of terpenoids have been placed in synthetic microbes such as *Rhodobacter capsulatus*, cyanobacteria, and *E. coli* leading to the successful biosynthesis of these important secondary metabolites. This milestone will have a significant impact on developing next generation agricultural products and may also lead to biotechnological and pharmaceutical innovations.

### **Plant-microbe interactions in the context of plant nutrition and defense reactions**

The impact of glucosinolates and tryptophan-derived indolic compounds on plant-microbe interaction are studied. Predictive mathematical models to simulate secondary metabolite biosynthetic pathways, e.g., the biosynthesis of methionine-derived glucosinolates have successfully been established. The model could correctly predict the glucosinolate composition of various *Arabidopsis* accession varying in their



glucosinolate substituents. Moreover, it could be demonstrated that glucosinolate derived products boosts the production of plant defense molecules such as phytoalexins.

Using *Arabidopsis* accessions and specific microbes plant genes have been identified, which are involved in the ability to shape the microbiome in response to nutrient deficiency. As a result, candidate genes including sulfatases, cytochrome P450s and various transporters are currently further investigated to assign mechanistic functions. In the future their impact on plant-microbe interactions will be studied. Moreover, multiple components have been identified in root exudates of *Arabidopsis* accessions and mutants grown under different nutritional conditions and co-cultivated with a selection of defined root-derived bacteria.

In addition, transporters potentially involved in terpenoid export were successfully expressed and their substrate specificity determined.

Moreover, cytochrome P450s acting on terpenoids were successfully expressed in *E. coli* leading in the future to a structural diversification of terpenoids. The produced terpenoids (up to a titer of 0.3 mg/liter) will then be functionally tested in plant-microbe interactions.

### **Signalling molecules and metabolic modules**

It is hypothesised that plant-associated bacteria (provided by RA C) use signal molecules to modulate plant growth via signalling peptides. Plant triterpenes (e.g., marneral and thalianol) act downstream of these signalling peptides. The aim of this approach is to explore the triterpene pathway in plants and to identify bacteria acting on this pathway. To date, further components of regulatory modules controlling the biosynthesis and transport of triterpenes in roots and in the rhizosphere have been characterized.

### **Production of key secondary metabolites in orthogonal systems**

This project aims at developing and testing synthetic modules for the production of terpenoids (sesquiterpenes, triterpenes and products derived thereof) in orthogonal systems. By introducing plant and myxobacterial genes into the cyanobacteria *Synechocystis* or *Rhodobacter* terpenoids were successfully produced.

## 6. Core Competencies

Based on long lasting, proven experience complemented by the integration of new expertise, the core scientific competencies of CEPLAS are seen in:

**Plant Development:** Genetic and molecular basis of annual/ perennial life cycles; mechanisms mediating annual/perennial life switches; regulatory networks controlling organ development (root, leaf, tillering, flowering); ecological and agricultural advantages of traits related to perennialism.

**Photosynthesis:** Genetic, molecular and physiological basis of processes involved in the evolutionary development of  $C_4$  plants from  $C_3$  plants; genetic, molecular, cellular and metabolic components determining the efficiency of converting light energy into chemical energy.

**Plant-Microbe Interactions:** Genetic and molecular characterization of the plant microflora; biotic and abiotic factors controlling the composition and activities of microbial communities associated with roots and leaves; molecular and metabolic processes determining the type and outcome of plant-microbe interactions.

**Metabolic Plasticity:** Plant secondary metabolites and biosynthetic pathways mediating the communication between plants and microbial endophytes; regulatory modules controlling the biosynthesis of these metabolites.

**Theoretical and Quantitative Biology:** Mathematical description and simulation of molecular and metabolic processes determining plant life and performance.

**Synthetic Biology:** Models and molecular modules for the engineering of plants with novel features, in particular with respect to plant development, photosynthesis and plant-microbe interactions.

## 7. Perspectives for Application

With respect to the translation of research results into application, CEPLAS provides a variety of perspectives:

- Molecular engineering of perennial plants from annuals by integrating the genetic determinants of perennialism
- Transfer of favourable agronomic traits from perennials into annuals: abiotic stress tolerance, nutrient-use efficiency
- Design of synthetic  $C_4$  plants by transferring the genetic/molecular determinants of  $C_4$  photosynthesis into  $C_3$  plants
- Molecular engineering of optimized  $C_3$  and  $C_4$  photosynthesis by targeting the rate limiting steps in the conversion of light energy to chemical energy
- Development of “biologicals” for plant protection and plant nutrition, on the basis of understanding the composition and function of the plant microbiome
- Design of novel chemical components for plant protection and plant nutrition, on the basis of understanding the biochemical processes of “communication” between plants and their microbiome
- Molecular breeding and engineering of plants with improved disease resistance using the molecular components that determine the type and outcome of plant-microbe interactions.
- Modelling of approaches for molecular engineering of improved plant traits
- Development of tool boxes for molecular engineering of improved plant traits

## 8. Opportunities for Cooperation

CEPLAS offers the licensing of IPR-protected know-how and research results and joint, co-funded research activities leading to new IPR and rights to economically use such IPR by partner companies (preferred, co-exclusive or exclusive). However, opportunities for partnerships go far beyond these “classical” ways of translation of know-how and research results into economic exploitation, comprising the following key elements:

- 1. Establishing, sponsoring and steering of additional Graduate Schools in areas of common interest**
  - partner companies to participate in the planning, design and steering of the program, and in the selection of students
- 2. Funding of additional Postdoctoral Fellows within the CEPLAS program, in research areas of common interest**
  - opportunity to partly or fulltime work in CEPLAS laboratories and in laboratories of partner companies
  - co-supervision by representatives from CEPLAS and partner companies
  - preferred rights to use IPR generated by the work of such Postdoctoral Fellows by supporting companies
- 3. Sponsoring of Junior Professorships in areas of mutual interest**
  - partner companies to participate in the decision of the research areas and in the structural design of programs
  - partner companies to participate in Search and Steering Committees
- 4. Mentoring of Postdoctoral Fellows working in research areas of interest for partner companies**
  - regular discussions with Postdoctoral

Fellows on their research work, and also coaching them in their professional career planning

- participation of company mentors in CEPLAS internal presentations of these Postdoctoral Fellows

### 5. Internships

- CEPLAS scientists at partner companies, or partner company scientists at CEPLAS
- partner companies obtaining preferred rights to use IPR generated in such exchange programs

### 6. Invitation of partner company scientists to scientific presentations and seminars of CEPLAS

### 7. Support/participation of partner companies in the foundation of Start-up businesses, on the basis of CEPLAS research results.

You are invited to learn more details about the status of research in the CEPLAS program, the potential for economic exploitation of existing and expected research results, and about opportunities for cooperation with CEPLAS, at any level indicated above. If you are interested please contact:

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**CEPLAS**

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# Project profiles





## Research Area A

### Project Title

Control of flowering

### Project Leader

George Coupland, Max Planck Institute for Plant Breeding Research, Cologne

### Project Goals

The scientific aim of the project is to describe the diversification of flowering control in annual and perennial plants, at the genetic and molecular level, and thereby isolate the molecular regulators for this process. Deciphering how these regulators are controlled by environmental cues and confer seasonal patterns of flowering is of particular interest. This provides insight into how flowering is controlled independently on different branches of the plant and is related to the age of the plant. It also provides an increased knowledge of flowering control in annuals and the know-how to transfer agriculturally useful traits from perennial to annual plants, such as increasing the number of flowers and seeds produced, the seed yield of axillary branches, manipulating the duration of flowering and increasing biomass production by delaying flowering and senescence.

### Activities

In different accessions of the phylogenetically related model plants *Arabidopsis thaliana* (annual) and *Arabis alpina* (perennial), and under different environmental conditions, the genetic determinants for the diversity in vernalization requirement, time of flowering, duration of flowering and bud opening are identified. In particular, the impact of microRNAs on flowering has been recently studied. For these purposes, QTL mapping, genotyping by sequencing, EMS mutagenesis and comparing the expression levels of candidate regulatory genes are applied. For

functional characterization of identified candidate genes, novel approaches for genome editing or classical transgenic approaches are used.

### Results

Ongoing research has led to the identification and isolation of various regulatory genes (transcription factors) and their targets controlling vernalization requirement, the duration of flowering, photoperiod response and the length of juvenility. Specifically, a transcription factor that represses flowering until the plant is exposed to winter cold was shown to exhibit different patterns of transcription in annual and perennial plants, and to contribute to the larger number of flowers produced by annuals. In addition, the age at which plants become old enough to respond to vernalization (adult) was shown to be controlled by a microRNA and its target transcription factor, demonstrating how manipulating sensitivity to particular microRNAs can influence the age at which plants become sensitive to environmental cues.

### Application relevant Aspects

The research results of this group are considered a decisive basis for molecular engineering of traits relevant for agricultural cultivation of crops and for efficient breeding methodology:

- Number of flowers and seeds
- Timing of flowering and flowering behavior of different branches
- Adaptation of flowering to environmental changes
- Synchronization of flowering



### **Opportunities for Cooperation**

The group is interested in research co-operations on topics of flowering control, in which both sides contribute specific materials and/or scientific and technological expertise. In this context, the transfer of results from model plants to agriculturally relevant crop plants has

a high priority. In addition, the group would be interested in collaborating to study the basis of the juvenile to adult transition or environmentally-controlled flowering responses in cultivars or breeding material. Also the exchange of scientific personnel for know-how transfer is considered an attractive form of cooperation.

### **Relevant publications (since 2016)**

- Fernandez V, Takahashi Y, Le Gourrierc J, Coupland G (2016) Photoperiodic and thermosensory pathways interact through CONSTANS to promote flowering at high temperature under short days. *Plant J* 86(5):426-440.
- Heidel AJ, Kiefer C, Coupland G, Rose LE (2016) Pinpointing genes underlying annual/perennial transitions with comparative genomics. *BMC Genomics* 17(1):921.
- Hyun Y, Richter R, Vincent C, Martinez-Gallegos R, Porri A, Coupland G (2016) Multi-layered Regulation of SPL15 and Cooperation with SOC1 Integrate Endogenous Flowering Pathways at the Arabidopsis Shoot Meristem. *Dev Cell* 37(3):254-266.
- Jung C, Pillen K, Staiger D, Coupland G, von Korff M (2016) Editorial: Recent Advances in Flowering Time Control. *Front Plant Sci* 7(2011):2011.
- Almario J, Jeena G, Wunder J, Langen G, Zuccaro A, Coupland G, Bucher M (2017) Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition. *Proc Natl Acad Sci U S A* 114(44):E9403-E9412.
- Dombrowski N, Schlaeppi K, Agler MT, Hacquard S, Kemen E, Garrido-Oter R, Wunder J, Coupland G, Schulze-Lefert P (2017) Root microbiota dynamics of perennial *Arabis alpina* are dependent on soil residence time but independent of flowering time. *ISME J* 11(1):43-55.
- Kiefer C, Severing E, Karl R, Bergonzi S, Koch M, Tresch A, Coupland G (2017) Divergence of annual and perennial species in the Brassicaceae and the contribution of cis-acting variation at FLC orthologues. *Mol Ecol* 26(13):3437-3457.



## Research Area A

### Project Title

Control of plant architecture and growth

### Project Leader

Rüdiger Simon, Heinrich Heine University  
Düsseldorf

### Project Goals

The major goal of the project is to analyze how meristem growth and activity is controlled, at the molecular level. Thereby, molecular targets for the adaptation and optimization of root and shoot development and function in different genetic backgrounds and/or under different environmental conditions should be identified.

### Activities

We are using *Arabidopsis thaliana* and *Hordeum vulgare* (barley) to analyse plant architecture, root and shoot growth and seed number as our main traits of interest. Within these plant species, regulatory genes and signaling pathways determining the fate of stem cells in root and shoot meristems are identified and functionally characterized, leading the way to finding components that cause different developmental patterns in roots and shoots of model and crop plants. A specific focus is put on describing the dynamics of effector/receptor interactions in signaling cascades and the impact of environmental parameters.

### Results

Various key factors for the development of root and shoot meristems and their functional role in signaling cascades have been described, in the past (see publications). On the basis of this know-how and experience, commonalities

and differences in shoot and root development between model plants and crop plants have been identified. Investigations on the genetic factors that determine the number of seeds that are formed by plants are in progress.

### Application relevant aspects

The project identifies and functionally characterizes molecular targets for a potential optimization of root and shoot development in plants. The already known factors controlling meristem function in root and shoot development have also been shown to be involved in conferring nematode resistance and to regulate nodule formation. Therefore, the results of this project could build a basis for optimization of a variety of components determining yield potential and stability. In addition, a high-throughput test system for the function of peptide effectors in signaling cascades that are involved in root and shoot development is available. This test system allows the screening of small molecule libraries for their potential effects on plant growth, and is applicable for model plant species as well as for crop plant species. Knowledge generated previously on model plant species such as *Arabidopsis* is now being applied to generate a fundamental framework to investigate the regulation of root development in the crop species barley.

### Opportunities for Cooperation

The project offers R&D cooperations in the fields of identification and functional characterization of regulators of root, shoot and seed development in model or crop plants, and in the screening of compounds with potential effects on root and shoot growth.



### Relevant publications (since 2016)

- Breiden M, Simon R (2016) Q&A: How does peptide signaling direct plant development? *BMC Biol* 14:58.
- Somssich M, Bleckmann A, Simon R (2016) Shared and distinct functions of the pseudokinase CORYNE (CRN) in shoot and root stem cell maintenance of Arabidopsis. *J Exp Bot* 67(16):4901-4915.
- Somssich M, Je BI, Simon R, Jackson D (2016) CLAVATA-WUSCHEL signaling in the shoot meristem. *Development* 143(18):3238-3248.
- Hanemian M, Barlet X, Sorin C, Yadeta KA, Keller H, Favery B, Simon R, Thomma BP, Hartmann C, Crespi M, Marco Y, Tremousaygue D, Deslandes L (2016) Arabidopsis CLAVATA1 and CLAVATA2 receptors contribute to *Ralstonia solanacearum* pathogenicity through a miR169-dependent pathway. *New Phytol* 211(2):502-515.
- Brambilla V, Martignago D, Goretti D, Cerise M, Somssich M, de Rosa M, Galbiati F, Shrestha R, Lazzaro F, Simon R, Fornara F (2017) Antagonistic Transcription Factor Complexes Modulate the Floral Transition in Rice. *The Plant Cell* 29(11):2801-2816.
- Kirschner GK, Stahl Y, Von Korff M, Simon R (2017) Unique and Conserved Features of the Barley Root Meristem. *Front Plant Sci* 8:1240.
- Long Y, Stahl Y, Weidtkamp-Peters S, Postma M, Zhou W, Goedhart J, Sanchez-Perez MI, Gadella TWJ, Simon R, Scheres B, Bliou I (2017) In vivo FRET-FLIM reveals cell-type-specific protein interactions in Arabidopsis roots. *Nature* 548(7665):97-102.
- Rast-Somssich MI, Žádníková P, Schmid S, Kieffer M, Kepinski S, Simon R (2017) The Arabidopsis JAGGED LATERAL ORGANS (JLO) gene sensitizes plants to auxin. *J Exp Bot* 68(11):2741-2755.
- Somssich M, Simon R (2017) Studying Protein-Protein Interactions In Planta Using Advanced Fluorescence Microscopy. *Methods Mol Biol* 1610:267-285.



## Research Area A

### Project Title

Competition for light in the canopy of perennial plants

### Project Leader

Ute Höcker, University of Cologne

### Project Goals

On the one hand, the project aims at understanding why perennial plants exert less responsiveness to reduced light intensity than annual plants, at the genetic and molecular level. This should provide insights into the regulation of growth length of plants, as an important parameter determining yield. On the other hand, the molecular basis for regulation of flowering by day length is addressed.

### Activities

Comparisons between *Arabis alpina* (perennial) and *Arabidopsis thaliana* (annual) as well as within phenotypically divergent *Arabis alpina* accessions are used to describe the functional involvement of previously determined candidate genes in regulating responsiveness of growth length to light intensity, and dependence of flowering time on day length. Finally, the effect of a transfer of such regulatory genes from *Arabis alpina* to *Arabidopsis thaliana* will be tested. The potential transfer of know-how to crop plants will require the cooperation with third parties from plant breeding business.

### Results

Phenotypic diversity in the dependence of flowering on day length could be proven in *Arabis alpina*: mostly, flowering is induced by long-day conditions (16 h light); one accession flowers at the same time also under short-day conditions (8 h light). A potentially new regulatory mechanism

for this phenomenon could be found at the expression level by detailed transcriptomics.

### Application relevant Aspects

Molecular components regulating the responsiveness to light intensity (competition for light in the canopy) and the dependence of flowering time on day length are considered as tools for molecular breeding of crop varieties with improved yield potential.

### Opportunities for Cooperation

The group is interested and provides scientific expertise in research cooperations on the light regulation of plant growth and metabolism. In addition, the transfer of know-how to crop plants (especially corn) is in the focus of cooperation interests.



### Relevant publications (since 2016)

- Chen S, Wirthmueller L, Stauber J, Lory N, Holtkotte X, Leson L, Schenkel C, Ahmad M, Hoecker U (2016) The functional divergence between SPA1 and SPA2 in Arabidopsis photomorphogenesis maps primarily to the respective N-terminal kinase-like domain. *BMC Plant Biol* 16(1):165.
- Holtkotte X, Dieterle S, Kokkelink L, Artz O, Leson L, Fittinghoff K, Hayama R, Ahmad M, Hoecker U (2016) Mutations in the N-terminal kinase-like domain of the repressor of photomorphogenesis SPA1 severely impair SPA1 function but not light responsiveness in Arabidopsis. *Plant J* 88(2):205-218.
- Jaegle B, Uroic MK, Holtkotte X, Lucas C, Termath AO, Schmalz HG, Bucher M, Hoecker U, Hülskamp M, Schrader A (2016) A fast and simple LC-MS-based characterization of the flavonoid biosynthesis pathway for few seed(ling)s. *BMC Plant Biol* 16(1):190
- Arthaut LD, Jourdan N, Mteyrek A, Procopio M, El-Esawi M, d'Harlingue A, Bouchet PE, Witczak J, Ritz T, Klarsfeld A, Birman S, Usselman RJ, Hoecker U, Martino CF, Ahmad M (2017) Blue-light induced accumulation of reactive oxygen species is a consequence of the Drosophila cryptochrome photocycle. *PLoS One* 12(3):e0171836.
- Balcerowicz M, Kerner K, Schenkel C, Hoecker U (2017) SPA Proteins Affect the Subcellular Localization of COP1 in the COP1/SPA Ubiquitin Ligase Complex during Photomorphogenesis. *Plant Physiol* 174(3):1314-1321.
- Hoecker U (2017) The activities of the E3 ubiquitin ligase COP1/SPA, a key repressor in light signaling. *Curr Opin Plant Biol* 37:63-69.
- Holtkotte X, Ponnu J, Ahmad M, Hoecker U (2017) The blue light-induced interaction of cryptochrome 1 with COP1 requires SPA proteins during Arabidopsis light signaling. *PLoS Genet* 13(10):e1007044.
- Ordonez-Herrera N, Trimborn L, Menje M, Henschel M, Robers L, Kaufholdt D, Hansch R, Adrian J, Ponnu J, Hoecker U (2018) The Transcription Factor COL12 Is a Substrate of the COP1/SPA E3 Ligase and Regulates Flowering Time and Plant Architecture. *Plant Physiol* 176(2):1327-1340.



## Research Area A

### Project Title

Balance between sexual and asexual reproduction in perennial plants

### Project Leader

Maria Albani, University of Cologne

### Project Goals

The project aims at describing the molecular mechanisms of competition and switches between vegetative and generative phases in the life cycle of perennial plants, in particular the regulatory components controlling adventitious root and flower formation. These processes have a major impact on clonal amplification, seed yield and seed quality in perennials and, thereby, affect their agricultural and horticultural use.

### Activities

Research activities comprise the identification of regulatory genes determining adventitious rooting, inflorescence formation and seed quality in the perennial plant *Arabis alpina*. For this purpose, genes regulating these traits are analyzed in *Arabis alpina* and compared to the annual *Arabidopsis thaliana*.

### Results

In the course of the project, the group could show that PEP1 determines floral fate and causes floral reversion after insufficient vernalization. Furthermore, results of the group have proven that the formation of adventitious roots in *Arabis alpina* is induced by flowering, providing significant insights into the regulation of clonal amplification of this perennial plant. In addition, the mechanism with which PEP1 ensures the return of vegetative development and therefore the perennial life cycle has been deciphered.

### Application relevant Aspects

Research results from this group are relevant in the context of breeding perennials with optimized forms of clonal amplification and with improved seed yield and seed viability.

### Opportunities for Cooperation

The group is interested in any kind of cooperations addressing the transfer of its results from the model plant *Arabis alpina* to crop plants in horticulture and agriculture. This includes joint research projects and consulting.



**Relevant publications (since 2016)**

- Lazaro A, Obeng-Hinneh E, Albani MC (2018) Extended vernalization regulates inflorescence fate in *Arabidopsis alpestris* by stably silencing PERPETUAL FLOWERING 1. *Plant Physiol.* doi 10.1104/pp.17.01754



## Research Area A

### **Project Title**

Genetic control of tillering and spike development in barley

### **Project Leader**

Maria von Korff Schmising, Heinrich Heine University Düsseldorf

### **Project Goals**

The goal of the project is to understand the genetic control of tillering and spike development in barley, by isolating key components and characterizing molecular networks controlling these processes.

Understanding the genetic and physiological correlations between tillering and spike development and characterizing the genetic differences that determine annual and perennial growth are the major focus of research.

In addition, the developmental processes addressed here have a proven relationship to the adaptation of barley to abiotic stress. In summary, the group finally aims at providing plant material and molecular components for yield improvement in plants.

### **Activities**

So far, the group has carried out detailed phenotypic analyses of barley tillering mutants and conducted RNA sequencing in developing shoot apical meristems to characterize molecular changes linked to distinct developmental changes. The analysis of tillering and row-type mutants has revealed genes and gene networks that control spikelet development and, consequently, the number of seeds per spike.

### **Results**

Data from a detailed phenotyping of barley mutant populations are available. The molecular

changes in shoot apical meristem during development have been described. Similarities as well as differences in development of annual and perennial barley genotypes have been identified and provide the basis for further understanding the genetic and molecular control of different life strategies in the Hordeum and Triticeae tribes.

### **Application relevant Aspects**

The group develops detailed know-how on molecular components that control decisive developmental steps during plant growth. These components are made accessible for the improvement of yield in cereal breeding.

### **Opportunities for Cooperation**

The interest in cooperations focuses on joint research projects in the field of barley shoot and spike development. An extension of the work to wheat is conceivable, based on existing scientific expertise and available plant material. Also the exchange and testing of plant material is of high value for the research of the group.



### Relevant publications (since 2016)

- Digel B, Tavakol E, Verderio G, Tondelli A, Xu X, Cattivelli L, Rossini L, von Korff M (2016) Photoperiod-H1 (Ppd-H1) Controls Leaf Size. *Plant Physiol* 172(1):405-415.
- Jung C, Pillen K, Staiger D, Coupland G, von Korff M (2016) Editorial: Recent Advances in Flowering Time Control. *Front Plant Sci* 7(2011):2011.
- Mulki MA, von Korff M (2016) CONSTANS Controls Floral Repression by Up-Regulating VERNALIZATION2 (VRN-H2) in Barley. *Plant Physiol* 170(1):325-337.
- Ejaz M, von Korff M (2017) The Genetic Control of Reproductive Development under High Ambient Temperature. *Plant Physiol* 173(1):294-306.
- Gol L, Tomé F, von Korff M (2017) Floral transitions in wheat and barley: interactions between photoperiod, abiotic stresses, and nutrient status. *J Exp Bot* 68(7):1399-1410.
- Kirschner GK, Stahl Y, Von Korff M, Simon R (2017) Unique and Conserved Features of the Barley Root Meristem. *Front Plant Sci* 8:1240.
- Liller CB, Walla A, Boer MP, Hedley P, Macaulay M, Effgen S, von Korff M, van Esse GW, Koornneef M (2017) Fine mapping of a major QTL for awn length in barley using a multiparent mapping population. *Theor Appl Genet* 130(2):269-281.
- Pankin A, von Korff M (2017) Co-evolution of methods and thoughts in cereal domestication studies: a tale of barley (*Hordeum vulgare*). *Curr Opin Plant Biol* 36:15-21.
- Templer SE, Ammon A, Pscheidt D, Ciobotea O, Schuy C, McCollum C, Sonnewald U, Hanemann A, Forster J, Ordon F, von Korff M, Voll LM (2017) Metabolite profiling of barley flag leaves under drought and combined heat and drought stress reveals metabolic QTLs for metabolites associated with antioxidant defense. *J Exp Bot* 68(7):1697-1713.
- van Esse GW, Walla A, Finke A, Koornneef M, Pecinka A, von Korff M (2017) Six-Rowed Spike3 (VRS3) Is a Histone Demethylase That Controls Lateral Spikelet Development in Barley. *Plant Physiol* 174(4):2397-2408.
- Pankin A, Altmüller J, Becker C, von Korff M (2018) Targeted resequencing reveals genomic signatures of barley domestication. *New Phytol*. doi: 10.1111/nph.15077.



## Research Area A

### Project Title

Comparative leaf development: Impact of leaf shape on photosynthetic efficiency

### Project Leader

Miltos Tsiantis, Max Planck Institute for Plant Breeding Research, Cologne

### Project Goals

Photosynthesis has been proposed to be a novel key route to increase yield of major crops. In this respect, one possible but underexplored target for increasing yield is tinkering with aspects of leaf development and anatomy. With the help of understanding the genetic and molecular basis of leaf development, anatomy and its causal relationship to photosynthetic efficiency, the group aims to provide a basis for novel breeding approaches that are directed towards increasing yield.

### Activities

The group uses comparative genetic analyses to describe the genetic basis for variation of leaf morphology between and within species. In this context, it addresses the key question of how variation in leaf shape is causally related to both photosynthetic efficiency and seed yield. As a first step, regulatory genes and their functional networks determining leaf shape in various genetic backgrounds are being identified. *Arabidopsis thaliana* and *Cardamine hirsuta* have been chosen as model plant species for these investigations.

### Results

In the past two years, the causal molecular basis for different leaf morphology has been identified that is of direct relevance for photosynthetic efficiency and seed biomass has been identified. Specific

DNA sequences in a transcription factor gene from *Cardamine hirsuta* have been causally correlated with leaf shape development, also exerting their regulatory function in *Arabidopsis thaliana*. These regulatory elements controlling leaf shape development and seed biomass can now be exploited for molecular breeding. In addition, first insights were gained into the trade-off between seed size and seed number.

### Application relevant Aspects

The research of the group provides insights into the molecular genetic basis of leaf development and leaf anatomy and its causal relationship with photosynthetic efficiency. The regulatory components of these processes are potential targets for designing new molecular breeding approaches that aim at increasing crop yield.

### Opportunities for Cooperation

The group seeks cooperation with partners that are interested in testing the possibility of transferring the research results found with model plants into crop plants. In this context, the major goal will be to test the phenotypic effects of candidate genes for modification of leaf anatomy and improving photosynthetic efficiency in crop plants. Such cooperation can include joint research activities as well as the exchange of scientific personnel in order to mutually extend the know-how in this field. Biochemical analysis of relevant germplasm is also of interest.



#### Relevant publications (since 2016)

- Gan X, Hay A, Kwantes M, Haberer G, Hallab A, Ioio RD, Hoffhuis H, Pieper B, Cartolano M, Neumann U, Nikolov LA, Song B, Hajheidari M, Briskine R, Kougioumoutzi E, Vlad D, Broholm S, Hein J, Meksem K, Lightfoot D, Shimizu KK, Shimizu-Inatsugi R, Imprialou M, Kudrna D, Wing R, Sato S, Huijser P, Filatov D, Mayer KF, Mott R, Tsiantis M (2016) The *Cardamine hirsuta* genome offers insight into the evolution of morphological diversity. *Nat Plants* 2(11):16167.
- Hay A, Tsiantis M (2016) *Cardamine hirsuta*: a comparative view. *Curr Opin Genet Dev* 39:1-7.
- Vuolo F, Mentink RA, Hajheidari M, Bailey CD, Filatov DA, Tsiantis M (2016) Coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. *Genes Dev* 30(21):2370-2375.
- Monniaux M, McKim SM, Cartolano M, Thevenon E, Parcy F, Tsiantis M, Hay A (2017) Conservation vs divergence in *LEAFY* and *APETALA1* functions between *Arabidopsis thaliana* and *Cardamine hirsuta*. *New Phytol* 216(2):549-561.
- Runions A, Tsiantis M, Prusinkiewicz P (2017) A common developmental program can produce diverse leaf shapes. *New Phytol* 216(2):401-418.



## Research Area A

### Project Title

Develop and characterize a resource for high resolution joint linkage and association mapping in barley

### Project Leader

Benjamin Stich, Heinrich Heine University Düsseldorf

### Project Goals

Barley is one of the model species for the cereals. Many technologies are nowadays available for this species such as the creation of double haploids, a genome sequence, the untargeted and targeted creation of mutants via tilling or CRISPR/Cas. However, despite the high genotypic and phenotypic diversity of barley, no resource for high resolution joint linkage and association mapping is available that would facilitate forward genetic approaches using natural genetic variation.

The current project aims to develop such a resource using a double round robin crossing design based on 23 spring barley inbreds that represent the world-wide diversity. Furthermore, we are interested to characterize the presence-absence variation as well as DNA methylation variation in the barley genome and quantify their contribution to the phenotypic variation of agronomically relevant traits.

### Activities

We developed 45 segregating populations derived from systematic crosses between 23 spring barley inbreds. More than 4000 F6 genotypes (DRR population) are available that were genotyped with a 50k iSelect SNP Array. The progenies are evaluated together with the parental inbreds in multi-location field experiments for various traits. For a subset, we perform in 2018 a grain and straw yield assessment. The parental inbreds are furthermore characterized by multi-tissue RNAseq

and Methylseq for presence-absence variation as well as the presence of differentially methylated regions.

### Technologies/Know-how

In the past two years, we have put emphasis on the development of expertise, technology, and workflows for efficient medium scale field experimentation as well as sequence based bioinformatics.

Furthermore, our group has long-standing expertise on quantitative and statistical genetics.

### Results

Major achievements of the past two years are:

- Pipelines for reference based and *de novo* DNA and RNA assembly have been established.
- The DRR population was evaluated for various traits in a field experiment in 2017.

### Opportunities for Cooperation

The group is interested in research cooperations on the genetic and molecular mechanisms influencing agronomically relevant traits in barley and maize.

In more general terms, the group wishes to jointly address biological questions for breeding-relevant topics, and is open to extend the field of research.



### Relevant publications (since 2016)

- Clarke WE, Higgins EE, Plieske J, Wieseke R, Sidebottom C, Khedikar Y, Batley J, Edwards D, Meng J, Li R, Lawley CT, Pauquet J, Laga B, Cheung W, Iniguez-Luy F, Dyrszka E, Rae S, Stich B, Snowdon RJ, Sharpe AG, Ganai MW, Parkin IA (2016) A high-density SNP genotyping array for *Brassica napus* and its ancestral diploid species based on optimised selection of single-locus markers in the allotetraploid genome. *Theor Appl Genet* 129(10):1887-1899.
- Li J, Bus A, Spamer V, Stich B (2016) Comparison of statistical models for nested association mapping in rapeseed (*Brassica napus* L.) through computer simulations. *BMC Plant Biol* 16:26.
- Körber N, Bus A, Li J, Parkin IA, Wittkop B, Snowdon RJ, Stich B (2016) Agronomic and Seed Quality Traits Dissected by Genome-Wide Association Mapping in *Brassica napus*. *Front Plant Sci* 7:386.
- Frey FP, Presterl T, Lecoq P, Orlik A, Stich B (2016) First steps to understand heat tolerance of temperate maize at adult stage: identification of QTL across multiple environments with connected segregating populations. *Theor Appl Genet* 129(5):945-961.
- Stich B, Van Inghelandt D (2018) Prospects and Potential Uses of Genomic Prediction of Key Performance Traits in Tetraploid Potato. *Front Plant Sci* 9:159.doi: 10.3389/fpls.2018.00159.



## Research Area B

### Project Title

The impact of leaf anatomy on photosynthetic efficiency

### Project Leader

Peter Westhoff, Heinrich Heine University Düsseldorf

### Project Goals

The first goal of the project is to identify and isolate key regulatory genes determining leaf anatomy, with a focus on genes that control the development of leaf structures characteristic for the Kranz anatomy of  $C_4$  plants and are causally related to photosynthetic efficiency. Finally, this should provide the basis for designing  $C_3$  plants with altered leaf anatomies typical for  $C_4$  plants and, thereby, increasing photosynthetic efficiency in  $C_3$  plants.

### Activities

Research in this group comprises the development of a test system for the identification and molecular isolation of regulatory genes involved in the development of leaf structures and functions characteristic for  $C_4$  plants. This test system is applied in *Arabidopsis thaliana* to create mutants in bundle sheath cell structure and function and, thereby, isolate the corresponding regulatory genes. The test system is presently also applied to obtain mutants with altered mesophyll structure in  $C_3$  leaves aiming to increase their photosynthetic output. Function of candidate genes are verified by testing the effect of their over-expression or knock out in transgenic plants. The final proof of concept is to demonstrate the impact of such candidate genes on photosynthetic efficiency.

### Results

As a first step, promoters specific for activity in bundle sheath cells (in  $C_4$  as well as in  $C_3$  plants) and the chlorenchymatic tissues of  $C_3$  leaves have been identified. These promoters were used for activation tagging mutagenesis in the  $C_3$  plant *Arabidopsis thaliana* resulting, up to date (September 2017), in the identification of seven genes that affect leaf anatomy, as confirmed by the reconstruction of the mutational event in wild type *Arabidopsis*. Some of the isolated genes could be assigned to known gene/protein classes (transcription factors or transporters), but the putative functions of several genes remained elusive. The analysis of the impact of these genes on leaf physiology is in progress, the search for further candidate genes is continued.

### Application relevant aspects

The working hypothesis is that regulatory genes of plant leaf anatomy and function are causally related to photosynthetic efficiency and photosynthetic output. Thereby, these genes potentially provide a basis for the improvement of yield in  $C_3$  (and  $C_4$ ) plants by molecular breeding.

### Opportunities for Cooperation

The group is interested in research cooperations (also including the exchange of scientific personnel and scientific know-how) addressing the development of concepts for a change of leaf anatomy, as a way to improve photosynthetic efficiency.



#### **Relevant publications (since 2016)**

- Flügge U, Westhoff P, Leister D (2016) Recent advances in understanding photosynthesis [version 1; referees: 3 approved]. *F1000Research* 2016 5(2890).
- Lauterbach M, Billakurthi K, Kadereit G, Ludwig M, Westhoff P, Gowik U (2017) C3 cotyledons are followed by C4 leaves: intra-individual transcriptome analysis of *Salsola soda* (Chenopodiaceae). *J Exp Bot* 68(2):161-176.
- Lauterbach M, Schmidt H, Billakurthi K, Hankeln T, Westhoff P, Gowik U, Kadereit G (2017) De novo Transcriptome Assembly and Comparison of C3, C3-C4, and C4 Species of Tribe Salsoleae (Chenopodiaceae). *Front Plant Sci* 8:1939.
- Schuler ML, Sedelnikova OV, Walker BJ, Westhoff P, Langdale JA (2018) SHORTROOT-Mediated Increase in Stomatal Density Has No Impact on Photosynthetic Efficiency. *Plant Physiol* 176(1):757-772.



## Research Area B

### Project Title

Genetic mechanisms underpinning photosynthetic efficiency and the evolution of  $C_4$  photosynthesis from  $C_3$  ancestors

### Project Leader

Andreas Weber, Heinrich Heine University Düsseldorf

### Project Goals

The project aims at defining the genetic underpinnings of sub-traits of  $C_4$  photosynthesis, such as Kranz leaf anatomy and the metabolic steps in  $C_4$  carbon fixation, as well as variation in photosynthetic performance and efficiency. Thereby, regulatory and functional modules (sets of genes) determining typical features of  $C_4$  plants will be identified and become available for increasing photosynthetic efficiency in  $C_3$  plants (transfer of  $C_4$  traits into the genetic background of  $C_3$  plants). Finally, know-how generated in this project provides the basis for modelling of evolutionary steps in the development of  $C_4$  photosynthesis, and functionally characterized regulatory modules should become tools for molecular breeding or engineering of crop plants with improved photosynthetic efficiency, as an element for yield increase.

### Activities

The research employs two complementary approaches. On the one hand, a systems biological approach in which phylogenetically and genetically well characterized intermediates in the evolution from  $C_3$  to  $C_4$  plants within the order of Brassicales are used to identify candidate genes mediating sub-traits of  $C_4$  photosynthesis. The functional verification of such candidate genes is approached in transgenic model plants. On the other hand, quantitative genetics using segregating populations from crosses between various intermediates in  $C_4$  evolution is applied to

localize and identify candidate genes. In addition, these segregating populations are also used for comparative transcriptomics, as an additional source for the identification of candidate genes.

### Technologies/Know-how

In the past few years, strong emphasis has been put on the establishment and use of CRISPR/Cas mediated genome editing technology for the functional analysis of candidate regulatory genes involved in controlling photosynthetic efficiency and evolution from  $C_3$  to  $C_4$  plants. Likewise, quantitative genetic identification of such regulatory genes has gained growing importance.

### Results

Major achievements of the past two years are:

- Reflection spectrometry has been established as a reliable tool to measure parameters indicating photosynthetic efficiency, such as gas exchange and C/N-content in plants.
- CRISPR/cas9 has been established and successfully used for the editing of alleles and the generation of deletions in regulatory genes, such as the glabrous (gl) gene, encoding a transcription factor involved in trichome development.
- Interspecific wide crosses between  $C_3$  and  $C_4$  plants have been generated, leading to offspring plants with intermediate  $CO_2$  compensation points; these experiments serve as a basis to analyze the genetic development of  $C_4$ -like traits in plants, to map the corresponding regulatory genes and correlate them with results from transcriptomics and genome sequencing of  $C_3$ ,  $C_4$  and  $C_3/C_4$  intermediate plants.

### Application relevant Aspects

The results from research of the group provides know-how on the genetic determinants and molecular processes for the evolution of  $C_4$  plants



from C<sub>3</sub> plants. Thereby, molecular modules can be designed and synthesized for pre-breeding towards an increase of photosynthetic efficiency as a contribution to yield improvement in crop plants.

### Opportunities for Cooperation

The group is interested in research cooperations on the genetic and molecular mechanisms determining sub-traits of C<sub>4</sub> photosynthesis, as

an approach for the development of molecular modules that can be used for improving photosynthetic efficiency and, consequently, yield of crop plants. In this context, partners are specifically welcome that address and support the transition of know-how from the model plants used in the research to crop plants. In more general terms, the group wishes to jointly address biological questions for breeding-relevant topics, and is open to extend the field of research.

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## Research Area B

### Project Title

Impact of light intensity on leaf anatomy

### Project Leader

Ute Höcker, University of Cologne

### Project Goals

Based on the different leaf anatomy of  $C_3$  and  $C_4$  plants, research in this project aims at the understanding of molecular mechanisms regulating leaf thickness and density of leaf veins, as parameters for potential yield improvement.

### Activities

Research activities cover the identification, isolation and functional characterization of regulatory genes controlling leaf thickness and density of leaf veins, in the model system *Arabidopsis thaliana*. The natural variability of these characteristics in the model plant species is used for QTL-cloning of such regulatory genes. In addition, transcription profiling with leaves from plants grown under different light intensities (leading to differences in leaf thickness and leaf vein density) is applied for the search of candidate genes. Further functional characterization of candidate genes will comprise testing their effect in transgenics, at the phenotypic and molecular level. The potential transfer of know-how generated in this research to crop plants will require the cooperation with third parties from plant breeding business.

### Results

More than 100 genotypes have been screened in a QTL analysis. In this approach, various QTLs for vein density could be identified. However, no QTLs for leaf thickness were found as this feature

does not show sufficient phenotypic variation for such an approach. Molecular cloning of genes encoded in the QTLs for leaf vein density is ongoing. In parallel, transcriptome analysis is used to study the expression profiles and regulatory networks associated with variability in leaf vein density and its dependence on light intensity.

### Application relevant Aspects

Molecular components regulating leaf anatomy and its responsiveness to light intensity are considered as tools for molecular breeding of crop varieties with improved yield potential.

### Opportunities for Cooperation

The group is interested in research cooperation addressing the molecular regulation of leaf anatomy and its dependence on light intensity. In addition, the transfer of know-how to crop plants (especially corn) is in the focus of cooperation interests.



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## Research Area B

### Project Title

*In silico* exploration of paths towards  $C_4$  metabolism

### Project Leader

Martin Lercher, Heinrich Heine University Düsseldorf

### Project Goals

First, the project aims at establishing a mechanistic model of photosynthesis that encompasses  $C_3$  and  $C_4$  metabolic systems and explicitly considers environmental factors, such as temperature, light intensity, and external  $CO_2$  and  $O_2$  pressures. This model is used to understand the evolution from  $C_3$  photosynthesis to  $C_3$ - $C_4$  intermediates and to  $C_4$  photosynthesis, and to predict likely evolutionary paths in the future. Finally, this model is used to design promising strategies for  $C_3$  to  $C_4$  conversions that combine genome engineering (for plant features that are sufficiently understood at the genetic and molecular basis) with artificial selection (for plant features with unclear genetic basis).

### Activities

The current understanding of the biochemical and anatomical basis of  $C_3$ ,  $C_3$ - $C_4$  intermediate, and  $C_4$  photosynthesis forms the foundation for metabolic modeling approaches, including whole genome metabolic modeling, such as Flux Balance Analysis, and mechanistic models of sub-systems, such as carbon fixation in photosynthesis. In our model, the effect of genetic and phenotypic changes (e.g., expression changes of key enzymes) can be simulated mathematically. This model also allows to predict the evolutionary response to environmental pressure (e.g.,  $CO_2/O_2$  concentrations, Temperature) of candidate genes for the development of  $C_4$  photosynthetic metabolism.

### Results

Using a detailed mathematical model allows to better understand how plants allocate resources in terms of nitrogen and energy and how this is affected by various environmental factors. The photosynthetic characteristics of the  $C_4$  species *Flaveria bidentis* are successfully explained by a resource allocation that is optimal under the environment that dominated the lineage's recent evolutionary history. Conversely, the identification of the environment that best explains the measured data allows to add quantitative information to the environment previously inferred from  $C_3$ - $C_4$  habitat comparisons.

Modeling the effect of environmental factors on the performance of the different photosynthetic pathways allows us to analyze the evolvability from  $C_3$  to  $C_4$  metabolism and its potential reversibility. The results indicate that the reversion of  $C_4$  metabolism to a full  $C_3$  pathway is highly unlikely in all relevant environments.

### Application relevant Aspects

The detailed understanding of effects on carbon fixation generated by specific mutations allows the rational design of strategies to move  $C_3$  crops towards  $C_4$  photosynthesis, thereby providing a potential approach for improving yield in molecular plant breeding.

### Opportunities for Cooperation

The group is interested in research cooperations (also including the exchange of scientific personnel and scientific know-how) addressing the development and application of metabolic models for improving photosynthetic efficiency.



### Relevant publications (since 2016)

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## Research Area B

### Project Title

Mathematical models of plant energy metabolism

### Project Leader

Oliver Ebenhöf, Heinrich Heine University Düsseldorf

### Project Goals

The overall goal of this project is to develop mathematical models which describe the dynamic processes in plants governing photosynthetic efficiency and metabolite production. A first aim is to understand how the photosynthetic electron transport chain adapts to fluctuations in its environment and how these responses influence metabolism and the production of compounds of nutritional or economic value. Thereby, this project contributes to generating the basis for molecular engineering of optimized photosynthesis.

### Activities

The mathematical models are developed on the basis of experimental data from collaborating groups within CEPLAS. Various methods are used, including the description of the dynamics by differential equation systems, the analysis of the regulation by metabolic control analysis, the description of stationary fluxes by flux balance analysis models, and the development of completely new concepts, such as the description of polymer biochemical systems in the language of statistical thermodynamics. The models of the group are applied to reproduce experimental data, thereby supporting the general assumptions made about the molecular mechanisms, and to put forward new predictions which then are tested in the experimental laboratories. In this collaborative cycle of theoretical and experimental studies, new insights into the principles governing the

control of photosynthesis and plant metabolism is produced.

### Results

The group has successfully created a dynamic mathematical model of the photosynthetic electron transport chain, which realistically reflects short-term acclimation mechanisms and allows understanding under which conditions excess energy dissipation is triggered. This model will be useful to develop optimal growth strategies and identify putative gene targets to optimize plant and algal growth by minimizing energy loss. Furthermore, existing models of the Calvin-Benson-Bessham cycle, in which carbon dioxide is reduced to sugars, were implemented and improved and are now used to study the supply-demand regulation in plant energy metabolism. A novel model version including isotope labeling patterns allows explanation of the observed asymmetric incorporation of labeled carbons and provides a novel modeling framework in which dynamic labeling experiments can be interpreted in a highly quantitative way. Models were created to study secondary metabolite production, focusing on glucosinolates. These models provide a mechanistic link between genotype, environment and phenotypic properties and are currently used to relate genetic differences in plants to different performance parameters. In the long term, such models will help to unravel the genotype-phenotype map and help developing predictive genetic intervention strategies to generate optimally performing plants.

### Application relevant Aspects

Mathematical models provide a theoretical framework in which experimental results can be analyzed and interpreted. As such, they form the workbench of theoretical biologists. They provide the unique opportunity to identify underlying principles behind the structure and design of biological systems. Therefore, they can produce novel predictions and help to



optimize the experimental strategies to pursue a particular goal, which includes the optimization of photosynthetic efficiency or the design of new strains to produce a desired combination of valuable and biotechnologically interesting compounds.

#### **Opportunities for Cooperation**

The group is interested in research cooperation (also including the exchange of scientific personnel and scientific know-how) addressing all aspects of plant and algal biotechnology that aim to exploit their unique and diverse metabolism.

#### **Relevant publications (since 2016)**

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## Research Area B

### Project Title

Evolution of phosphoenolpyruvate carboxylase regulation in the development of  $C_4$  photosynthesis

### Project Leader

Georg Groth, Heinrich Heine University Düsseldorf

### Project Goals

The goal of the project is to understand the regulation of PEP carboxylase (PEPC) enzyme activity in the development from  $C_3$  to  $C_4$  photosynthesis. On the one hand, results from this research should provide a crucial know-how basis for the engineering of  $C_3$  plants into  $C_4$  plants. On the other hand, this research should also open-up the way for designing novel herbicides specifically directed against  $C_4$  weeds.

### Activities

Two different levels of PEP carboxylase regulation are analyzed in  $C_3$ ,  $C_3$ - $C_4$  and  $C_4$  *Flaveria* species: phosphorylation by specific isoforms of phosphoenolpyruvate carboxylase kinase (PPCK) and binding of potential ligands (inhibitors) of enzyme activity. Isoforms of PPCK show specific and preferred interaction with either the  $C_3$  or the  $C_4$  isoform of PEP carboxylase thereby controlling phosphorylation and in turn activity of PEPC. The isoforms of PEPC and PPCK are synthesized in heterologous expression systems; functional folding of the purified recombinant proteins is verified by CD-spectroscopy, followed by activity assays and protein-protein as well as protein-ligand interaction studies. Isolated proteins and protein-protein complexes are finally applied to systematic X-ray crystallography.

### Results

As a first step, various PEPC and PPCK isoforms from *Flaveria* have been expressed in *E. coli* and purified. Verification of functional folding of the recombinant proteins and X-ray crystallography of isolated proteins and protein-protein complexes are in progress. In addition, various inhibitors have been identified specifically targeting PEPC from  $C_4$  plants, thereby, representing potential  $C_4$ -specific herbicides.

### Application relevant Aspects

The working hypothesis is that  $C_4$  key enzymes such as PEPC or PPDK are tightly regulated by phosphorylation affecting their catalytic efficiency and photosynthetic output. Understanding the molecular determinants controlling the interaction of PEPC/PPDK with their related regulatory proteins provides an important basis for successful engineering  $C_4$  characteristics into  $C_3$  enzymes. Inhibitors specifically affecting the activity of  $C_3$  or  $C_4$  isoforms of PEPC are candidates for  $C_3$ - or  $C_4$ -specific herbicides.

### Opportunities for Cooperation

The group is interested in research cooperation (also including the exchange of scientific personnel and scientific know-how) addressing structural evolution of photosynthetic proteins and determinants of molecular specificity in protein-protein or protein-ligand interactions as a way to improve photosynthetic efficiency. In addition, the group looks for cooperations with industry partners in the area of developing  $C_3$ - and  $C_4$ -specific herbicides (formulation, chemical optimization of active substances, field testing).

### Existing Cooperations with Partners from Industry

Cooperation with an industry partner for further development of a  $C_4$ -specific herbicide is under negotiation, on the basis of specific inhibitors of



C<sub>4</sub>-isoforms of PEPC, previously identified by the group. In this context, the focus is put on finding the most effective and most efficient formulation and release concept for the active ingredient, allowing minimal amounts of the active ingredient in field application.

#### **Relevant publications (since 2016)**

- Minges A, Ciupka D, Winkler C, Hoppner A, Gohlke H, Groth G (2017) Structural intermediates and directionality of the swiveling motion of Pyruvate Phosphate Dikinase. *Sci Rep* 7:45389.
- Minges A, Groth G (2017) Small-molecule inhibition of pyruvate phosphate dikinase targeting the nucleotide binding site. *PLoS One* 12(7):e0181139.
- Minges A, Hoppner A, Groth G (2017) Trapped intermediate state of plant pyruvate phosphate dikinase indicates substeps in catalytic swiveling domain mechanism. *Protein Sci* 26(8):1667-1673.

#### **Patent applications / Patents**

- Georg GROTH, Judith Katharina PAULUS, Daniel SCHLIEPER, Peter WESTHOFF, Selective inhibition of phosphoenolpyruvate carboxylases of C<sub>4</sub> plants, PCT/EP2012/076648 (CN104114024A, EP2797417A1, US20150216167).
- Georg GROTH, Judith Katharina PAULUS, Daniel SCHLIEPER, Peter WESTHOFF, Selective inhibition of C<sub>4</sub>-PEP carboxylases, PCT/EP2014/050766 (CN105101804A, US20150366206, WO2014111448A1)



## Research Area B

### Project Title

Differentiation of vascular strains in mesenchymal tissue

### Project Leader

Wolfgang Werr, University of Cologne

### Project Goals

Research of this group addresses the molecular basis of early leaf primordia differentiation. A specific goal is to understand the activity of marginal or plate meristem with respect to vascularisation and to elucidate on the temporal series of events and signals between the mesenchymal cell niche and the vascular cambium during the earliest phase of leaf development. According to genetic data this interplay relates to the density of vascular veins during leaf lamina expansion and will be put into the context of Kranz leaf anatomy that in  $C_4$  plants typically develops at the interface of mesenchyme and vascular strands.

### Activities

As a first step, the expression patterns of two WUSCHEL-RELATED-HOMEOBOX (WOX) genes will be elaborated: WOX3 enables lamina expansion by promoting stem cell identity in the mesenchymal niche, whereas WOX4 transcription has been related to the fascicular cambium, development of the interfascicular cambium and secondary thickening growth (Suer et al., 2011). However, the WOX4 pattern possibly comprises a partial view as the WOX4<sub>short</sub> promoter lacks distal promoter elements that are evident via phylogenetic shadowing. The project intends to include missing WOX4 promoter elements and to establish the complete WOX4<sub>long</sub> activity pattern via live-imaging in comparison to WOX3 activity in the mesenchymal niche. Transgenic marker lines will show spatial and temporal constraints

at the cellular expression level from the earliest stages of primordia initiation, through the leaf maturation program until terminal differentiation. Based on the characterised WOX3 and WOX4 promoters, stable and inducible driver lines will be constructed that will allow reproducible expression of single or multiple transgenes in a spatially and temporally controlled cell-type-specific pattern in early leaf primordia. Resulting transgenic lines can then be analysed for histological or physiological consequences due to ectopic transgene activity; a primary research focus will be the gain of  $C_4$  features in  $C_3$  models such as *Arabidopsis* or rice, and transgenes will be chosen based on data elaborated in CEPLAS.

### Results

During development of the *Arabidopsis* leaf, the project has outlined a spatial and temporal concert of WOX3 and WOX4 activity

- The initiation of a new primordium starts with mesenchymal identity marked by WOX3 activity in marginal and plate meristems
- There is a delay of at least 12 hours before markers like AtHB8 or WOX4<sub>short</sub>/WOX4<sub>long</sub> are detectable in the cambium of vascular cells in a complementary pattern to WOX3 promoter activity, which is now restricted to plate meristems.
- However, WOX4<sub>long</sub> promoter activity is not confined to the vascular cambium and in the stem, WOX4 activity marks a sub-cortical cylindrical domain of cells, which starts in the ground tissue of the shoot apex and connects the fascicular cambium through the interfascicular region throughout the stem.
- In the nodes, WOX4<sub>long</sub>-expressing cells connect this cylindrical domain of the stem to vascular system of lateral branches.
- In young leaf petioles or floral pedicels, WOX4<sub>long</sub> promoter activity remains detectable in the sub-epidermal layer and extends into the superficial layers of the leaf blade, with a



- preference for the adaxial side.
- In differentiated vascular strands the *WOX4* long promoter is transcribed in the xylem parenchyma cells
  - *WOX4* long activity apparently marks a pluripotent or parenchymal ground state that allows various cellular decisions during stem, axillary branch and leaf development.
  - The layering during the initial phase of leaf blade development is presently analysed in *WOX4*<sup>long</sup>::*CERULEAN* *WOX3*::*GFP* double transgenic plants. However, a transient superficial parenchymal layer marked by *WOX4* expression subtended by a mesenchymal layer determined by medial *WOX3* activity (Nakata et al., 2012) suggests analogy to the stem, where *WOX4*<sup>long</sup>::*CERULEAN* activity in the subcortical layer of the interfascicular regions prepatterns the interfascicular cambium and where *WOX4* is essential for cambium development (Suer et al., 2011).

#### **Application relevant Aspects**

The research of the group provides insights into cambium development in leaf primordia from an initially mesenchymal ground state and relates to the vascular density achieved during lamina

expansion. The finding that *WOX4*<sup>long</sup> expression correlates with a parenchymal ground state and transient promoter activity as the layering during the initial phase of lamina expansion defines a most interesting developmental window, where crosstalk between parenchymal and mesenchymal cells possibly relates to vein density and is of key importance for the formation of bundle-sheath-like cells enclosing vascular strands in  $C_3$  and  $C_4$  plants that in the latter acquired the typical Kranz anatomy accompanied by physiological adaptations.

#### **Opportunities for Cooperation**

The group is interested in cooperative research projects addressing the molecular basis of leaf development. An especially valuable resource in this context with respect to  $C_4$  evolution would be annotated genome sequences of extant  $C_3$ , transitory and  $C_4$  species within a plant family enabling comparative access to true gene orthologies and expressional, promoter or chromatin changes that accompany the  $C_4$  invention.

#### **Relevant publications (since 2016)**

- Nardmann J, Chandler JW, Werr W (2016) Stem Cell Fate versus Differentiation: the Missing Link. *Trends Plant Sci* 21(9):725-727



## Research Area C

### Project Title

Structure and functions of the root microbiota in plant growth and health

### Project Leader

Paul Schulze-Lefert, Max-Planck-Institute for Plant Breeding Research, Cologne

### Project Goals

A major goal of this project is a quantitative survey of the bacterial and fungal assemblages colonizing roots of healthy plants grown in natural environments using culture-independent microbial community profiling techniques. In parallel, systematic culture-dependent bacterial and fungal isolation efforts aim at the establishment of comprehensive DNA sequence-indexed culture collections of root-associated microorganisms. In a third part, the project aims at developing multiple gnotobiotic plant systems for microbiota reconstitution experiments with synthetic microbial communities in laboratory environments to dissect plant microbiota functions in indirect pathogen protection, mobilization of soil nutrients and abiotic stress tolerance.

### Activities

Key activities of the research group involve the development of bacterial and fungal high-throughput isolation methods as well as computational methods for the establishment of DNA sequence-indexed culture collections of the *Arabidopsis thaliana* root microbiota. On the basis of these efforts, a collection of isolates representing the majority of bacterial species colonizing *A. thaliana* could be established and is now available for further investigation (Bai et al., Nature 2015). In addition, the majority of abundant *A. thaliana* fungal root endophytes has been isolated (unpublished data). Physiologically relevant reconstitution of the plant microbiota

is dependent on the availability of germ-free plant systems in which the plants are grown in a simplified inert matrix or in sterilized soil together with defined (=synthetic) microbial communities (Bai et al., Nature 2015). By this means it is possible to control nutrient availability and mimic defined environments for plant growth. A large number of rationally designed synthetic communities is then tested for their capacity to confer specific microbiota traits such as plant protection against soil-borne pathogenic bacteria, fungi and nematodes or nutrient mobilization from soil for plant growth.

### Results

The group has succeeded in the isolation of the majority of bacterial species colonizing *A. thaliana* roots and has been able to reconstitute complex synthetic communities on germ-free plants that resemble the taxonomic structure of the root microbiota of plants grown in natural soil (Bai et al., Nature 2015). This provides a proof-of-principle of our reductionist approaches to study plant microbiota functions. Furthermore, a root-colonizing beneficial fungal endophyte of *A. thaliana*, called *Colletotrichum tofieldiae*, with biological activities resembling taxonomically unrelated mycorrhiza fungi has been isolated recently. This novel fungal root endophyte mobilizes insoluble forms of phosphate which is translocated via fungal hyphae to the plant root and shoot for plant growth. The fungus promotes *A. thaliana* growth and increases plant fertility under phosphate-limiting conditions. This is a proof-of-principle that the plant microbiota is a rich source for the isolation of novel microbes that promote plant growth under adverse environmental conditions (Hiruma et al., Cell, 2016).

### Application-relevant Aspects

The large-scale microbial isolation and functional characterization of the plant microbiota provides lead strains that can be used for further rational design of biologicals for crop plants in agricultural settings, to improve plant health and productivity.

### Opportunities for Cooperation

The group is interested in research cooperation (including the exchange of scientific personnel and scientific know-how) towards the development of robust biologicals in agricultural settings.

### Relevant publications (since 2016)

- Hacquard S, Kracher B, Hiruma K, Munch PC, Garrido-Oter R, Thon MR, Weimann A, Damm U, Dallery JF, Hainaut M, Henrissat B, Lespinet O, Sacristan S, Ver Loren van Themaat E, Kemen E, McHardy AC, Schulze-Lefert P, O'Connell RJ (2016) Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi. *Nat Commun* 7:11362.
- Hiruma K, Gerlach N, Sacristan S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramirez D, Bucher M, O'Connell RJ, Schulze-Lefert P (2016) Root Endophyte *Colletotrichum tofieldiae* Confers Plant Fitness Benefits that Are Phosphate Status Dependent. *Cell* 165(2):464-474.
- Zgadzaj R, Garrido-Oter R, Jensen DB, Koprivova A, Schulze-Lefert P, Radutoiu S (2016) Root nodule symbiosis in *Lotus japonicus* drives the establishment of distinctive rhizosphere, root, and nodule bacterial communities. *Proc Natl Acad Sci U S A* 113(49):E7996-E8005.
- Dombrowski N, Schlaeppli K, Agler MT, Hacquard S, Kemen E, Garrido-Oter R, Wunder J, Coupland G, Schulze-Lefert P (2017) Root microbiota dynamics of perennial *Arabis alpina* are dependent on soil residence time but independent of flowering time. *ISME J* 11(1):43-55.
- Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P (2017) Interplay Between Innate Immunity and the Plant Microbiota. *Annu Rev Phytopathol* 55:565-589.
- Nakano RT, Pislewska-Bednarek M, Yamada K, Edger PP, Miyahara M, Kondo M, Bottcher C, Mori M, Nishimura M, Schulze-Lefert P, Hara-Nishimura I, Bednarek P (2017) PYK10 myrosinase reveals a functional coordination between endoplasmic reticulum bodies and glucosinolates in *Arabidopsis thaliana*. *Plant J* 89(2):204-220.
- Szczyrba A, Hofmann P, Belmann P, Koslicki D, Janssen S, Droge J, Gregor I, Majda S, Fiedler J, Dahms E, Bremges A, Fritz A, Garrido-Oter R, Jorgensen TS, Shapiro N, Blood PD, Gurevich A, Bai Y, Turaev D, DeMaere MZ, Chikhi R, Nagarajan N, Quince C, Meyer F, Balvociute M, Hansen LH, Sorensen SJ, Chia BKH, Denis B, Froula JL, Wang Z, Egan R, Don Kang D, Cook JJ, Dettel C, Beckstette M, Lemaitre C, Peterlongo P, Rizk G, Lavenier D, Wu YW, Singer SW, Jain C, Strous M, Klingenberg H, Meinicke P, Barton MD, Lingner T, Lin HH, Liao YC, Silva GGZ, Cuevas DA, Edwards RA, Saha S, Piro VC, Renard BY, Pop M, Klenk HP, Goker M, Kyrpides NC, Woyke T, Vorholt JA, Schulze-Lefert P, Rubin EM, Darling AE, Rattei T, McHardy AC (2017) Critical Assessment of Metagenome Interpretation—a benchmark of metagenomics software. *m Nat Methods* 14(11):1063-1071.
- Pislewska-Bednarek M, Nakano RT, Hiruma K, Pastorczyk M, Sanchez-Vallet A, Singkaravanit-Ogawa S, Ciesiolka D, Takano Y, Molina A, Schulze-Lefert P, Bednarek P (2018) Glutathione Transferase U13 Functions in Pathogen-Triggered Glucosinolate Metabolism. *Plant Physiol* 176(1):538-551.
- Robbins C, Thiergart T, Hacquard S, Garrido-Oter R, Gans W, Peiter E, Schulze-Lefert P, Spaepen S (2017) Root-Associated Bacterial and Fungal Community Profiles of *Arabidopsis thaliana* Are Robust Across Contrasting Soil P Levels. *Phytobiomes:PBIOMES-09-17-0042-R*.



## Research Area C

### Project Title

Linking microbial diversity and emergence of plant pathogens

### Project Leader

Eric Kemen, Max-Planck-Institute for Plant Breeding Research, Cologne

### Project Goals

The goal of this project is to identify the evolutionary and mechanistic link between plant pathogens and the microbial diversity in the phyllosphere of plants. This will provide a knowledge basis for the development of novel concepts for supporting plant health via biocontrol (knowledge-based design of biocontrol agents), molecular engineering of crop plants or new agrochemicals.

### Activities

Research in this group comprises the development and application of methods to profile and characterize microbial diversity in the phyllosphere of plants. For this purpose, the group uses a combination of high throughput amplicon sequencing, metagenomics, proteomics and metabolomics. Studies are performed on the model plant *Arabidopsis thaliana* and other Brassicaceae and comprises sampling of wild populations, field experiments and lab re-construction experiments. The group has established a significant collection of microbes, in particular fungi (incl. yeasts) and oomycetes, and an expertise in their cultivation. Gnotobiotic systems have been developed and are used to identify and functionally characterize the regulatory genes controlling microbe-microbe and plant-microbe communication (inter-kingdom communications), and finally to determine phenotypic effects of candidate genes on interactions between microbes and microbes and plant (disease development).

### Results

The group has identified that leaf associated microbial communities communicate with their host plants via "microbial hubs". These hubs are microbes that are able to colonize the plant and, at the same time, regulate the structure of the microbial community. Microbial hubs are able to communicate across kingdoms and lead to a stabilization of diversity and microbial composition. On that basis, the group works on the hypothesis that tolerating "hub" microbes benefits host and microbial community and explains why plants tolerate certain pathogens without evolving resistances while other pathogens lead to evolution of massive resistance gene clusters.

Various regulatory genes controlling the microbiome composition in the phyllosphere have been identified; the functional characterization of these genes is ongoing. Furthermore, secreted surface proteins of oomycetes have been found and structurally and functionally characterized that are relevant for the interaction of these oomycetes with their host plants and other microbes, for the establishment of their function as a "hub", and for the colonization of the host plant by microbes. In this context, also specific inhibitors for the function of these surface proteins could be identified. Finally, signal molecules of these oomycetes were found and characterized which restrict the apoplastic access for bacteria in leaves.

### Application relevant Aspects

Results from the investigation of regulatory processes in microbe-microbe and plant-microbe interactions and, thereby, controlling the effect of these interactions on plant health are seen as a basis to design a beneficial microbial environment for plants. One application perspective is seen in the design and molecular engineering of crop plants that select "hub" microorganisms from the natural environment with beneficial effect on plant health and performance. Other potential applications of results from this group are the



development of specific novel biologicals or agrochemicals controlling the composition and function of the microbiome in the phyllosphere of plants.

### **Opportunities for Cooperation**

The group is interested in research cooperation (also including the exchange of scientific personnel and scientific know-how) addressing pathogenicity related diversity studies of microbial communities. A special focus is put on any kind of approaches for the translation of know-how gained with model plants to crop plants. Specifically, the

research fields for cooperation are the molecular engineering or molecular breeding of plants with improved pathogen resistance, the development of concepts for biocontrol and the development of agrochemicals mediating pathogen resistance or enhanced plant health via controlling the composition of the plant microbiome.

### **Existing Cooperations with Industry Partners**

Negotiations with industry partners, on the basis of research results of the group and their potential translation into novel, economically relevant products and/or processes are ongoing.

### **Relevant publications (since 2016)**

- Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM (2016) Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLoS Biol* 14(1):e1002352.
- Hacquard S, Kracher B, Hiruma K, Munch PC, Garrido-Oter R, Thon MR, Weimann A, Damm U, Dallery JF, Hainaut M, Henrissat B, Lespinet O, Sacristan S, Ver Loren van Themaat E, Kemen E, McHardy AC, Schulze-Lefert P, O'Connell RJ (2016) Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi. *Nat Commun* 7:11362.
- Ruhe J, Agler MT, Placzek A, Kramer K, Finkemeier I, Kemen EM (2016) Obligate Biotroph Pathogens of the Genus *Albugo* Are Better Adapted to Active Host Defense Compared to Niche Competitors. *Front Plant Sci* 7:820.
- Dombrowski N, Schlaeppli K, Agler MT, Hacquard S, Kemen E, Garrido-Oter R, Wunder J, Coupland G, Schulze-Lefert P (2017) Root microbiota dynamics of perennial *Arabidopsis thaliana* are dependent on soil residence time but independent of flowering time. *ISME J* 11(1):43-55.
- Kruse J, Doehlemann G, Kemen E, Thines M (2017) Asexual and sexual morphs of *Moesziomyces* revisited. *IMA Fungus* 8(1):117-129.

### **Patent Applications / Patents**

- Submission of a patent application on research results of the group is in progress.



## Research Area C

### Project Title

Mechanistic basis of beneficial root/microbe interactions

### Project Leader

Marcel Bucher, University of Cologne

### Project Goals

The scientific goal of the research group is to understand the regulatory processes controlling the growth of plants on nutrient-limited soil in dependence on the interaction with microorganisms in roots and in the rhizosphere, at the molecular level. This also addresses the mechanisms for cellular re-programming in the establishment of beneficial plant/microbe interactions and its differentiation from pathogenic plant/microbe interactions. Finally, this will build the knowledge basis for the design of synthetic communities of microorganisms that can function as biologicals for strengthening plant fitness, and for breeding crop plants optimized in their interaction with beneficial microbes while fighting pathogens.

### Activities

Research activities address the variation of microbiomes in the rhizosphere of plants under natural and controlled soil conditions, by "omics" approaches such as metagenomics, transcriptomics, metabolomics, and multi-elemental profiling (ionomics). Thereby, regulatory genes controlling the development and outcome of the interaction are identified from both sides, the host plants and their microbial partners. The function of candidate regulatory genes in establishing beneficial plant/microbe interactions is proven by mutant analysis (transposon insertional mutants, T-DNA knock-out, RNAi knock-down). The objects of investigation

comprise the formation of beneficial interactions between *Lotus japonicus*, maize and various Brassicaceae species with arbuscular mycorrhizal fungi and a variety of other soil microbes. A specific focus is put on the investigation and improvement of phosphate nutrition of plants by modification/optimization of the microbiome in roots and in the rhizosphere.

### Results

A transcription factor, CBX1, has been identified and characterized as a central regulator for the reciprocal exchange of carbon and phosphate and other nutrients in the symbiotic interaction between host plants and mycorrhizal fungi (transport of carbon from the host plant to the fungi and of phosphate and other nutrients from the fungi to host plants, mediated by proton ATPases, nutrient transporters, and key enzymes in the synthesis of fatty acids). In addition, it could be shown that under conditions of reduced symbiotic functionality the accumulation of pathogenic fungi in the roots of the host plant increases. Moreover, through investigation of the root microbiome of plants growing in soils with very low P availability, a novel beneficial fungus capable of transferring phosphate to its host was identified.

### Application relevant Aspects

Results of this research project will contribute to building the basis for the re-construction of mutualistic symbiotic communities, i.e. the design of biologicals for supporting plant growth and health. Furthermore, the know-how generated in this project provides a basis for the breeding of crop plants that are improved in their interaction with beneficial soil microorganisms while exerting increased resistance to pathogenic microorganisms (for example by stimulating symbiotic processes and plant defense). A collection of more than 500 fungal isolates from soil has been brought into cultivation on artificial media and can serve as a source for the



identification of novel metabolites with economic value.

### **Opportunities for Cooperation**

The group is interested in joint research with third parties engaged in investigating the regulation and function of plant/microbiome interactions, the design of artificial microbial communities with beneficial effect on plant growth and plant health

(biologicals), and in designing crop plants with improved plant/microbe interaction features. Any step in the translation of know-how generated in this research into novel products and processes is of high interest for the research group, specifically - but not exclusively - transformation/editing of plants, introgression of novel traits into breeding material, field testing, biostatistics and formulation of biologicals.

### **Relevant publications (since 2016)**

- Bombarely A, Moser M, Amrad A, Bao M, Bapaume L, Barry CS, Bliet M, Boersma MR, Borghi L, Bruggmann R, Bucher M, D'Agostino N, Davies K, Druege U, Dudareva N, Egea-Cortines M, Delledonne M, Fernandez-Pozo N, Franken P, Grandont L, Heslop-Harrison JS, Hintzsche J, Johns M, Koes R, Lv X, Lyons E, Malla D, Martinoia E, Mattson NS, Morel P, Mueller LA, Muhlemann J, Nouri E, Passeri V, Pezzotti M, Qi Q, Reinhardt D, Rich M, Richert-Poggeler KR, Robbins TP, Schatz MC, Schranz ME, Schuurink RC, Schwarzacher T, Spelt K, Tang H, Urbanus SL, Vandenbussche M, Vijverberg K, Villarino GH, Warner RM, Weiss J, Yue Z, Zethof J, Quattrocchio F, Sims TL, Kuhlemeier C (2016) Insight into the evolution of the Solanaceae from the parental genomes of *Petunia hybrida*. *Nat Plants* 2(6):16074.
- Bucher M, Fabianska I (2016) Long-Sought Vacuolar Phosphate Transporters Identified. *Trends Plant Sci* 21(6):463-466.
- Hiruma K, Gerlach N, Sacristan S, Nakano RT, Hacquard S, Kracher B, Neumann U, Ramirez D, Bucher M, O'Connell RJ, Schulze-Lefert P (2016) Root Endophyte *Colletotrichum tofieldiae* Confers Plant Fitness Benefits that Are Phosphate Status Dependent. *Cell* 165(2):464-474.
- Jaegle B, Uroic MK, Holtkotte X, Lucas C, Termath AO, Schmalz HG, Bucher M, Hoecker U, Hülskamp M, Schrader A (2016) A fast and simple LC-MS-based characterization of the flavonoid biosynthesis pathway for few seed(ling)s. *BMC Plant Biol* 16(1):190.
- Vijayakumar V, Liebisch G, Buer B, Xue L, Gerlach N, Blau S, Schmitz J, Bucher M (2016) Integrated multi-omics analysis supports role of lysophosphatidylcholine and related glycerophospholipids in the *Lotus japonicus*-*Glomus intraradices* mycorrhizal symbiosis. *Plant Cell Environ* 39(2):393-415.
- Almario J, Jeena G, Wunder J, Langen G, Zuccaro A, Coupland G, Bucher M (2017) Root-associated fungal microbiota of nonmycorrhizal *Arabidopsis thaliana* and its contribution to plant phosphorus nutrition. *Proc Natl Acad Sci U S A* 114(44):E9403-E9412.



## Research Area C

### Project Title

Functional effector biology in plant microbe interactions

### Project Leader

Gunther Döhlemann, University of Cologne

### Project Goals

Research of this group aims at identifying and understanding molecular mechanisms of microbe-plant interactions, with a focus on effector proteins of biotrophic microbes which interfere with host immunity and metabolism. In this context, also the interplay between various microbes in their effect on host plants is addressed. This is considered a basis to develop new concepts for crop protection via molecular engineering of resistant crops or the development of biological or chemical protectants.

### Activities

Using the interaction between the pathogenic fungus *Ustilago maydis* and maize as a model system, key research activities comprise:

- Identification of effector molecules from the pathogenic fungus (mediating virulence) and their target molecules from the host plants.
- Identification of genes encoding these effectors and targets.
- Functional characterization of the interaction between effectors and targets; identification of molecular mechanisms leading to modifications of this interaction and, thereby, also to modifications of the outcome of pathogen/host interactions (disease or resistance).
- Identification of line specific, quantitative virulence factors and their targets that contribute to quantitative resistance. Effectors as tools to identify QTLs.

More specifically, the role of plant cysteine proteases in the development of immunity, and the mechanisms of their modulation by microbial effectors is analyzed. Organ-specific factors of both plant and colonizing microbes are identified contributing to the outcome of the interaction.

### Results

A peroxidase inhibitor could be identified as an effector of Ustilaginales; this effector is conserved as essential virulence factor in all smut fungi analyzed so far. Cysteine proteases have been shown to play a crucial role in signal transduction during the establishment of systemic acquired resistance in maize and effectors that interfere with their activity have been characterized. Furthermore, virulence functions of effectors have been found to be tissue- and cell-type specific.

### Application relevant Aspects

Inhibiting the action of effectors of pathogenic microbes is considered a target for the development of novel compounds for plant protection (agrochemicals or biologicals). Pathogen effectors are versatile tools to identify novel plant genes contributing to resistance, for example in quantitative resistance. Beyond this approach, the molecular engineering of host plant targets/receptors for insensitivity to pathogenic effectors offers new ways for improving resistance in crop plants.

### Opportunities for Cooperation

The group is interested in research cooperation in which the scientific and technological expertise and/or the availability of biological material from the partners complement each other for studying the molecular basis of effector/receptor interactions in host/pathogen systems, with the goal to finally design and test novel concepts for crop plant protection. In the field of finding novel agrochemicals or biologicals, especially the



establishment of high-throughput test systems is considered a relevant contribution from third parties. Cooperation may include the exchange of scientific personnel between the partners.

### Relevant publications (since 2016)

- Brych A, Mascarenhas J, Jaeger E, Charkiewicz E, Pokorny R, Bolker M, Doehlemann G, Batschauer A (2016) White collar 1-induced photolyase expression contributes to UV-tolerance of *Ustilago maydis*. *Microbiologyopen* 5(2):224-243.
- Hampel M, Jakobi M, Schmitz L, Meyer U, Finkernagel F, Doehlemann G, Heimel K (2016) Unfolded Protein Response (UPR) Regulator Cib1 Controls Expression of Genes Encoding Secreted Virulence Factors in *Ustilago maydis*. *PLoS One* 11(4):e0153861.
- Matei A, Doehlemann G (2016) Cell biology of corn smut disease-*Ustilago maydis* as a model for biotrophic interactions. *Curr Opin Microbiol* 34:60-66.
- Misas-Villamil JC, van der Hoorn RA, Doehlemann G (2016) Papain-like cysteine proteases as hubs in plant immunity. *New Phytol* 212(4):902-907.
- Ökmen B, Doehlemann G (2016) Clash between the borders: spotlight on apoplastic processes in plant-microbe interactions. *New Phytol* 212(4):799-801.
- Redkar A, Doehlemann G (2016) EdU Based DNA Synthesis and Cell Proliferation Assay in Maize Infected by the Smut Fungus *Ustilago maydis*. *Bio-protocol* 6(6):e1761.
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- Doehlemann G, Ökmen B, Zhu W, Sharon A (2017) Plant Pathogenic Fungi. *Microbiol Spectr* 5(1).
- Kruse J, Doehlemann G, Kemen E, Thines M (2017) Asexual and sexual morphs of *Moesziomyces* revisited. *IMA Fungus* 8(1):117-129.
- Redkar A, Matei A, Doehlemann G (2017) Insights into Host Cell Modulation and Induction of New Cells by the Corn Smut *Ustilago maydis*. *Front Plant Sci* 8:899.
- Matei A, Ernst C, Gunl M, Thiele B, Altmüller J, Walbot V, Usadel B, Doehlemann G (2018) How to make a tumour: cell type specific dissection of *Ustilago maydis*-induced tumour development in maize leaves. *New Phytol* 217(4):1681-1695.
- Van Der Linde K, Timofejeva L, Egger RL, Ilau B, Hammond R, Teng C, Meyers BC, Doehlemann G, Walbot V (2018) Pathogen Trojan horse delivers bioactive host protein to alter maize (*Zea mays*) anther cell behavior in situ. *Plant Cell*. doi: 10.1105/tpc.17.00238
- Ziemann S, van der Linde K, Lahrmann U, Acar B, Kaschani F, Colby T, Kaiser M, Ding Y, Schmelz E, Huffaker A, Holton N, Zipfel C, Doehlemann G (2018) An apoplastic peptide activates salicylic acid signalling in maize. *Nat Plants* 4(3):172-180.



## Research Area C

### Project Title

Characterization and functional assay of oomycete and protist communities in plants

### Project Leaders

Laura Rose, Heinrich Heine University  
Düsseldorf;  
Michael Bonkowski, Universität of Cologne

### Project Goals

The project has finished its phase of identification and taxonomic characterization of asymptomatic protists and oomycetes building the microbiome in the rhizosphere and phyllosphere of plants. Currently, the major goal of the project is to elucidate the effect of varying microbial compositions (protozoa and bacteria) on plant growth. Finally, these investigations will provide a crucial basis for establishing artificial communities of microorganisms with controlled impact on plant performance.

### Activities

Research activities focus on quantifying the effect of protozoa on the composition of bacterial communities in the microbiome and on plant growth. The project also investigates how plants interfere with the establishment of such communities of microorganisms via specific signal molecules. *Arabidopsis thaliana* and *Arabis alpina* have been chosen as model host plant species. Cercozoa are the group of protists under investigation.

### Results

Specific taxa of asymptomatic protozoa and oomycetes were identified in the rhizosphere and phyllosphere of various plant species. Although the taxonomic composition of the rhizosphere and the phyllosphere differs, there is less variation in taxonomic composition across different plant

species. Protozoa were shown to feed on the bacteria in their micro-environment and, thereby, influence the composition of bacterial communities in the rhizosphere and phyllosphere. Similarly, the composition of bacterial communities influences the establishment of protozoan communities.

### Application relevant Aspects

The project will provide insights into the complexity of interactions between various microorganisms in the rhizosphere and phyllosphere of plants, and also into the effect of these complex communities on plant performance (nutrient requirements, disease and pest resistance). Results are a necessary basis to artificially design growth stimulating microbial communities, as novel concepts for plant fertilization and plant protection.

### Opportunities for Cooperation

The group is interested in research cooperations on the variability of interactions between plants and their associated microbes, with potential beneficial effects on host plants. In such cooperations, investigations on the interaction of protozoa and oomycetes with crop plants and the transfer of know-how from model plants to crop plants are considered as topics of high relevance. Cooperations can also extend to the exchange of scientific personnel.



### Relevant publications (since 2016)

- Ploch S, Rose LE, Bass D, Bonkowski M (2016) High Diversity Revealed in Leaf-Associated Protists (Rhizaria: Cercozoa) of Brassicaceae. *J Eukaryot Microbiol* 63(5):635-641.
- Dumack K, Flues S, Hermanns K, Bonkowski M (2017) Rhogostomidae (Cercozoa) from soils, roots and plant leaves (*Arabidopsis thaliana*): Description of *Rhogostoma epiphylla* sp. nov. and *R. cylindrica* sp. nov. *Eur J Protistol* 60:76-86.
- Sapp M, Ploch S, Fiore-Donno AM, Bonkowski M, Rose LE (2018) Protists are an integral part of the *Arabidopsis thaliana* microbiome. *Environ Microbiol* 20(1):30-43.
- Fiore-Donno AM, Rixen C, Rippin M, Glaser K, Samolov E, Karsten U, Becker B, Bonkowski M (2018) New barcoded primers for efficient retrieval of cercozoan sequences in high-throughput environmental diversity surveys, with emphasis on worldwide biological soil crusts. *Mol Ecol Resour* 18(2):229-239.
- Flues S, Blokker M, Dumack K, Bonkowski M (2018) Diversity of Cercomonad Species in the Phyllosphere and Rhizosphere of Different Plant Species with a Description of *Neocercomonas epiphylla* (Cercozoa, Rhizaria) a Leaf-Associated Protist. *J Eukaryot Microbiol*. doi: 10.1111/jeu.12503
- Geisen S, Mitchell EAD, Adl S, Bonkowski M, Dunthorn M, Ekelund F, Fernandez LD, Jousset A, Krashevskaya V, Singer D, Spiegel FW, Walochnik J, Lara E (2018) Soil protists: a fertile frontier in soil biology research. *FEMS Microbiol Rev*. doi: 10.1093/femsre/fuy006
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## Research Area C

### Project Title

Molecular mechanisms for establishing beneficial host/microbe interactions

### Project Leader

Alga Zuccaro, University of Cologne

### Project Goals

Research in this group aims at obtaining a deep and more predictive understanding of molecular mechanisms governing the establishment of compatible plant/fungus interactions in the rhizosphere. Using interactions of *Arabidopsis* and barley with the beneficial sebacinoid fungi *Serendipita indica* and *S. vermifera*, and with the pathogenic root rot fungus *Bipolaris sorokiniana*, host-specific and conserved (core) fungal effectors involved in suppression of plant immunity and manipulation of host metabolism are characterized. Thereby, the group addresses the question how host-microbiota interactions in natural soil shape and are shaped by local and systemic responses to beneficial and pathogenic root associated fungi. The goal is to dissect multispecies chemical communication and its consequences for plant health and yield.

### Activities

Comparative studies on the mechanism behind the beneficial effects of the sebacinoid fungi and the deleterious effects of root-rot pathogens will lead to a better understanding of the colonization dynamics in root-fungal interactions and will give hints as to the question of what makes a fungus beneficial or pathogenic. In order to dissect the molecular basis underpinning fungal pathogenic and beneficial effects in roots, a reductionist approach has recently been established which takes advantage of a gnotobiotic natural soil-based split root system to identify plant- and

microbe-derived transcripts, proteins and metabolites that locally and systemically affect multispecies interactions. This allows addressing the molecular mechanisms with which generalistic fungi cope with distinct plant responses upon infection, and the decisive steps differentiating between beneficial effects on host plants and disease development. The work comprises the identification of protein effectors from the different fungi, the finding of their corresponding targets in the host plant and describing the mechanisms and effects of their interaction. For identifying effector molecules from the fungus, expression profiling during the process of infection, the isolation of proteins with signal peptide sequences, and/or the screening of apoplastic fluids is used. Such effector molecules are then used to identify their corresponding targets in the host plants, with the help of various classical and tailored biochemical binding assays.

### Results

In the course of the project, significant insights into the molecular mechanisms of establishing the host/fungus interaction in the two model systems could be provided, also indicating an essential step for the prevention of defense reactions of the host plants upon infection by the fungus:

A set of common core genes is induced by sebacinoid fungi upon infection of both plant species, *Arabidopsis thaliana* and *Hordeum vulgare*; at the same time, sets of different genes are induced in the two host plant species.

- A lectin protein functions as a fungal effector, mediating the process of interaction; short chain glucans are the target of this effector, generated from fungal long chain glucans by the action of host plant glucanases; binding of the effector to the short chain glucans masks their function as an elicitor of defense reactions in the host plants.
- The fungal effector out-competes the host plant receptor for glucan elicitors.
- Mycotrophy in a broad sense (including



mycoparasitism) may play a role in broad-spectrum beneficial effects of the *Sebacinales* to their plant hosts.

- Apoplastic ATP plays an important role during fungal root colonization.

#### **Application relevant Aspects**

Know-how on effectors responsible for the establishment of fungus/plant interactions and on the mechanisms of this process are considered a basis for molecular engineering and/or improving of such beneficial effects in crop plants. Furthermore, such know-how can also be used for designing novel growth promoting agrochemicals (for example by using the target pathways in the

host plant induced by the effectors as a test system for measuring the effect of novel compounds).

#### **Opportunities for Cooperation**

The group is especially interested in any kind of cooperation with third parties on translating the know-how on the function of biological effectors, gained in the model systems, into molecular engineering of crop plants with improved pathogen resistance or otherwise improved growth features, or into concepts for developing growth promoting agents.

#### **Relevant publications (since 2016)**

- Fesel PH, Zuccaro A (2016) beta-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants. *Fungal Genet Biol* 90:53-60.
- Fesel PH, Zuccaro A (2016) Dissecting endophytic lifestyle along the parasitism/mutualism continuum in *Arabidopsis*. *Curr Opin Microbiol* 32:103-112.
- Rövenich H, Zuccaro A, Thomma BP (2016) Convergent evolution of filamentous microbes towards evasion of glycan-triggered immunity. *New Phytol* 212(4):896-901.
- Wawra S, Fesel P, Widmer H, Timm M, Seibel J, Leson L, Kessler L, Nostadt R, Hilbert M, Langen G, Zuccaro A (2016) The fungal-specific  $\beta$ -glucan-binding lectin FGB1 alters cell-wall composition and suppresses glucan-triggered immunity in plants. *Nat Commun* 7:13188.
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- Almario J, Jeena G, Wunder J, Langen G, Zuccaro A, Coupland G, Bucher M (2017) Root-associated fungal microbiota of nonmycorrhizal *Arabidopsis alpina* and its contribution to plant phosphorus nutrition. *Proc Natl Acad Sci U S A* 114(44):E9403-E9412.



## Research Area C

### Project Title

Exploitation of endophytic fungi for novel concepts of plant protection

### Project Leaders

Vera Göhre, Michael Feldbrügge, Heinrich Heine University Düsseldorf

### Project Goals

The overall scientific goal of the project is to understand the molecular basis of communication between host plants and their microbial environment, allowing the development of novel concepts for plant protection.

### Activities

The interaction between *Thecaphora thlaspeos* and various Brassicaceae is used as a model system for characterizing the interaction of smut fungi with host and non-host plants. In this context, the life cycle of the fungus on its host is described, the genomic constitution of the fungus deciphered, and tools for its axenic cultivation and for the functional characterization of molecules involved in plant/fungus communication are developed. This know-how and these tools are then used to analyze the molecular mechanisms of disease and resistance development in the test system, and the impact of the fungus on the plant microbiome (cooperation with Eric Kemen, Max-Planck-Institut für Pflanzenzüchtungsforschung, Köln)

### Results

The following results were obtained, since 2016:

- The life cycle of *Thecaphora thlaspeos* was described, in detail.
- *Arabidopsis thaliana* was infected with *Thecaphora thlaspeos*. There are no macroscopic symptoms detectable when the fungus spreads along the vasculature throughout

the plant. Only in the siliques, the fungus deposits its spores.

- RNA sequencing was used to describe the transcriptome during the infection process, in both the host plant and the fungus. On this basis and on the basis of existing structural information, effector genes for the communication between the host plant and the fungus were predicted.

Conserved smut fungal effectors were identified and their function was proven in the model smut fungus *Ustilago maydis*; the virulence function of new effector genes was proven in *Pseudomonas syringae* in its interaction with *Arabidopsis thaliana*.

On the plant side the transcriptome shows massive induction of the defense machinery, which contrasts the complete absence of macroscopic symptoms during fungal infection.

A transformation protocol for *Thecaphora thlaspeos* is being developed.

### Application relevant Aspects

Results of this research have the potential for application in various forms: Novel concepts for resistance breeding in Brassicaceae by development of varieties with reduced levels of signal molecules inducing the germination of pathogenic fungi, or varieties that block host/pathogen communication; design of biologicals with beneficial effects on plant health; development of agrochemicals that interfere with the host/pathogen communication necessary for infection.

### Opportunities for Cooperation

The group is interested in research cooperation that address the identification and isolation of signaling molecules involved in the molecular communication between crop plants and fungal pathogens, with the aim to create a know-how basis for novel concepts of plant disease resistance. Integrating specific know-how or



material from partners into such cooperation is considered as an attractive asset.

#### **Existing cooperations with industry partners**

A cooperation with a leading German plant breeding company has been established on identifying and characterizing signal molecules from *Brassica napus* mediating non-host resistance to *Thecaphora thlaspeos*.

#### **Relevant publications (since 2016)**

- Cardenas-Monroy CA, Pohlmann T, Pinon-Zarate G, Matus-Ortega G, Guerra G, Feldbrügge M, Pardo JP (2017) The mitochondrial alternative oxidase Aox1 is needed to cope with respiratory stress but dispensable for pathogenic development in *Ustilago maydis*. PLoS One 12(3):e0173389.
- Frantzeskakis L, Courville KJ, Plucker L, Kellner R, Kruse J, Brachmann A, Feldbrügge M, Gohre V (2017) The Plant-Dependent Life Cycle of *Thecaphora thlaspeos*: A Smut Fungus Adapted to Brassicaceae. Mol Plant Microbe Interact 30(4):271-282.
- Haag C, Pohlmann T, Feldbrügge M (2017) The ESCRT regulator Did2 maintains the balance between long-distance endosomal transport and endocytic trafficking. PLoS Genet 13(4):e1006734.
- Terfrüchte M, Reindl M, Jankowski S, Sarkari P, Feldbrügge M, Schipper K (2017) Applying Unconventional Secretion in *Ustilago maydis* for the Export of Functional Nanobodies. Int J Mol Sci 18(5).
- Zander S, Muntjes K, Feldbrügge M (2018) RNA Live Imaging in the Model Microorganism *Ustilago maydis*. Methods Mol Biol 1649:319-335.
- Zhou L, Obhof T, Schneider K, Feldbrügge M, Nienhaus GU, Kamper J (2018) Cytoplasmic Transport Machinery of the SPF27 Homologue Num1 in *Ustilago maydis*. Sci Rep 8(1):3611.
- Bösch K, Frantzeskakis L, Vranes M, Kamper J, Schipper K, Göhre V (2016) Genetic Manipulation of the Plant Pathogen *Ustilago maydis* to Study Fungal Biology and Plant Microbe Interactions. J Vis Exp (115).
- Kellner R, Göhre V (2017) *Thecaphora thlaspeos*—ein Brandpilz spezialisiert auf Modellpflanzen. BIOSpektrum 23(5):498-500.
- Niessing D, Jansen RP, Pohlmann T, Feldbrügge M (2018) mRNA transport in fungal top models. Wiley Interdisciplinary Reviews: RNA 9(1):e1453.

## Research Area D

### Project Title

Mineral nutrition of plants and its interdependence with microbes in the rhizosphere

### Project Leader

Stanislav Kopriva, University of Cologne

### Project Goals

The overall goal of the research group is to understand the regulation of uptake and assimilation of mineral nutrients in plants, and the role of microbes in the rhizosphere in this process. Finally, this should lead to concepts for optimizing resource efficiency in plant cultivation under changing environmental conditions.

### Activities

Currently, research focuses on sulfur nutrition/metabolism and its interdependence on the interaction with microbes in the rhizosphere, as plants rely on essential metabolic processes contributed by microbes for mineralization of sulfur. Natural variation in *Arabidopsis* accessions is used to identify genes that affect the interaction of plants and bacteria for sulfur mineralization and, thereby, also shape the microbiome of plants. Candidate genes from plants involved in this process are identified by quantitative genetic approaches with plant populations expressing phenotypic and genotypic variation (GWAS), as well as by transcriptome and metabolome analyses. Measuring enzymatic activities, metabolite fluxes and determining the effect of mutations in candidate genes finally lead to their functional characterization and to the description of functional relationships within metabolic networks. As a new research focus, also specific aspects of mineral nutrition in  $C_4$  plants are under investigation.

### Results

Since 2016, the following results have been achieved:

- It could be shown that the composition of plant exudates is substantially changing in response to the nutritional status of plants (availability of sulfur, nitrogen and phosphate nutrients). Exudates show a high chemical variability.
- Various candidate genes involved in the regulation of secondary metabolism were identified, affecting the interaction between plants and microorganisms in their rhizosphere; the increase of plant biomass could be correlated with the activation of specific metabolic enzymes.
- Mutants, transcription patterns and metabolic profiles were found that lead the way to the identification of compounds with growth stimulating effect in plants and, potentially, useful in chemical complementation of plant growth under different environmental conditions.
- Evidence for differences in mineral composition between  $C_3$  and  $C_4$  plants was found, indicating that a "fine tuning" mineral metabolism is required in the transformation of  $C_3$  plants into  $C_4$  plants.

### Application relevant Aspects

The understanding of regulatory processes in the uptake and assimilation of mineral nutrients, and the identification, isolation and functional characterization of genes involved in these processes will lead to novel concepts for resource efficient crop cultivation (i.e. molecular engineering of crops with high yield under low-input conditions), and also for the improvement of food quality (synthesis of beneficial ingredients in field crops and vegetables; reduction of detrimental compounds). This will also be achieved by better mechanistic understanding of plant/microbe interactions in the rhizosphere depending on sophisticated communication via



signal molecules from both partners. On this basis, novel plant growth stimulating metabolites for fertilization of plants adapted to various nutritional states and environmental conditions are potentially identified.

### **Opportunities for Cooperation**

The group is interested in research cooperation on the improvement of nutrient-use efficiency

in crop plants, and on the optimization of the food quality of products harvested from these plants (synthesis of novel beneficial compounds, reduction of detrimental compounds). In this context, a special focus also lies in the transfer of research results from model plants to crop plants. Research cooperation may include the exchange of scientific personnel.

### **Relevant publications (since 2016)**

- Calderwood A, Kopriva S, Morris RJ (2016) Transcript Abundance Explains mRNA Mobility Data in *Arabidopsis thaliana*. *Plant Cell* 28(3):610-615.
- Huang XY, Chao DY, Koprivova A, Danku J, Wirtz M, Müller S, Sandoval FJ, Bauwe H, Roje S, Dilkes B, Hell R, Kopriva S, Salt DE (2016) Nuclear Localised MORE SULPHUR ACCUMULATION1 Epigenetically Regulates Sulphur Homeostasis in *Arabidopsis thaliana*. *PLoS Genet* 12(9):e1006298.
- Kopriva S, Gigolashvili T (2016) Chapter Five - Glucosinolate Synthesis in the Context of Plant Metabolism. *Adv Bot Res*, ed Stanislav K (Academic Press), Vol Volume 80, pp 99-124.
- Koprivova A, Kopriva S (2016) Sulfation pathways in plants. *Chem Biol Interact* 259(Pt A):23-30.
- Koprivova A, Kopriva S (2016) Sulfur metabolism and its manipulation in crops. *J Genet Genomics* 43(11):623-629.
- Koprivova A, Kopriva S (2016) Hormonal control of sulfate uptake and assimilation. *Plant Mol Biol* 91(6):617-627.
- Maillard A, Sorin E, Etienne P, Diquelou S, Koprivova A, Kopriva S, Arkoun M, Gallardo K, Turner M, Cruz F, Yvin JC, Ourry A (2016) Non-Specific Root Transport of Nutrient Gives Access to an Early Nutritional Indicator: The Case of Sulfate and Molybdate. *PLoS One* 11(11):e0166910.
- Jacoby R, Peukert M, Succurro A, Koprivova A, Kopriva S (2017) The Role of Soil Microorganisms in Plant Mineral Nutrition-Current Knowledge and Future Directions. *Front Plant Sci* 8:1617.
- Loeschcke A, Dienst D, Wewer V, Hage-Hülsmann J, Dietsch M, Kranz-Finger S, Hüren V, Metzger S, Urlacher VB, Gigolashvili T, Kopriva S, Axmann IM, Drepper T, Jaeger KE (2017) The photosynthetic bacteria *Rhodobacter capsulatus* and *Synechocystis* sp. PCC 6803 as new hosts for cyclic plant triterpene biosynthesis. *PLoS One* 12(12):e0189816.
- Jacoby RP, Martyn A, Kopriva S (2018) Exometabolomic profiling of bacterial strains cultivated using *Arabidopsis* root extract as the sole carbon source. *Mol Plant Microbe Interact*. doi: 10.1094/MPMI-10-17-0253-R.

## Research Area D

### Project Title

Dynamics of regulatory protein complexes in a changing environment – by the example of plant glucosinolate biosynthesis

### Project Leader

Tamara Gigolashvili, University of Cologne

### Project Goals

The scientific goal of this project is to understand how the information coded by DNA is transformed into specific organismal responses and to study the molecular mechanisms underlying the formation of transcription factor complexes in response to specific signals. This research will lead to a molecular description of processes determining the dynamics of protein-DNA, protein-protein, and protein-metabolite interactions, including the assembly/disassembly of the complexes regulating the transcription of genes for glucosinolate biosynthesis in a changing environment. Results derived from this project will provide important information regarding novel approaches and methods to design artificial transcription factors and custom-made regulatory modules to control gene expression.

### Activities

The central players of combinatorial gene control studied here are proteins belonging to the MYB-bHLH regulatory complex as well as metabolic signals modulating the activity of these regulatory proteins during glucosinolate biosynthesis in *Arabidopsis*. Research activities comprise the following experimental approaches:

- Identification and functional characterization of binding sites for MYB and bHLH proteins in genes for glucosinolate biosynthesis (ChIP-exo).
- Sequence-specific DNA-binding of the MYB domain in structurally similar R2R3-MYBs;

generation of synthetic (chimeric or randomly mutagenised) proteins with novel DNA-binding abilities.

- Understanding molecular mechanisms of transcriptional regulation by the MYB-bHLH complex: Identification of proteins involved in chromatin modification and mediator proteins.
- Metabolite sensing - as new level of regulation in glucosinolate biosynthesis: identification of signalling molecules and sensor proteins

### Results

In previous work, six R2R3-MYBs and four bHLH proteins from *Arabidopsis* involved in the regulation of glucosinolate biosynthesis have been identified and molecularly characterized. These transcription factors mediate control of glucosinolate biosynthesis in response to jasmonic acid, salicylic acid, abscisic acid, brassinosteroids, ethylene, glucose and wounding, and are involved in negative feedback control mechanisms of glucosinolate biosynthesis. In addition, it has been shown that these transcription factors are involved in controlling the accumulation of glucosinolates upon infection of plants by bacterial pathogens as well as in the establishment of resistance to generalist herbivores and some fungi.

### Application relevant aspects

Results from this research will contribute to creating a know-how basis for the design of synthetic transcription factors with custom-made DNA binding activities. This will be an essential element in the molecular engineering of stress response pathways in plants.



### **Opportunities for Cooperation**

The group is interested in research cooperation, including the exchange of scientific personnel, in the field of biochemical pathway engineering in plants, via design of synthetic transcription factors with specific regulatory functions. An intended focus in such cooperation is the translation of results from model plants to crop plants.

### **Relevant publications (since 2016)**

- Aarabi F, Kusajima M, Tohge T, Konishi T, Gigolashvili T, Takamune M, Sasazaki Y, Watanabe M, Nakashita H, Fernie AR, Saito K, Takahashi H, Hubberten HM, Hoefgen R, Maruyama-Nakashita A (2016) Sulfur deficiency-induced repressor proteins optimize glucosinolate biosynthesis in plants. *Sci Adv* 2(10):e1601087.
- Chan KX, Mabbitt PD, Phua SY, Mueller JW, Nisar N, Gigolashvili T, Stroeher E, Grassl J, Arlt W, Estavillo GM, Jackson CJ, Pogson BJ (2016) Sensing and signaling of oxidative stress in chloroplasts by inactivation of the SAL1 phosphoadenosine phosphatase. *Proc Natl Acad Sci U S A* 113(31):E4567-4576.
- Frerigmann H, Pislewska-Bednarek M, Sanchez-Vallet A, Molina A, Glawischnig E, Gigolashvili T, Bednarek P (2016) Regulation of Pathogen-Triggered Tryptophan Metabolism in *Arabidopsis thaliana* by MYB Transcription Factors and Indole Glucosinolate Conversion Products. *Mol Plant* 9(5):682-695.
- Kopriva S, Gigolashvili T (2016) Chapter Five - Glucosinolate Synthesis in the Context of Plant Metabolism. *Adv Bot Res*, ed Stanislav K (Academic Press), Vol Volume 80, pp 99-124.
- Loeschcke A, Dienst D, Wewer V, Hage-Hülsmann J, Dietsch M, Kranz-Finger S, Hüren V, Metzger S, Urlacher VB, Gigolashvili T, Kopriva S, Axmann IM, Drepper T, Jaeger KE (2017) The photosynthetic bacteria *Rhodobacter capsulatus* and *Synechocystis* sp. PCC 6803 as new hosts for cyclic plant triterpene biosynthesis. *PLoS One* 12(12):e0189816.

## Research Area D

### Project Title

Metabolic regulation of the plant immune response

### Project Leader

Jürgen Zeier, Heinrich Heine University Düsseldorf

### Project Goals

The project aims at the description of the metabolic network involved in the regulation of plant immune responses, and at the identification and molecular characterization of components therein. Finally, this should become a basis for the design of new sustainable approaches in resistance breeding and the development of protecting agrochemicals.

### Activities

The research work covers the comparative metabolite profiling of infected and non-infected plants, including mutants defective in pathogen responses. Candidate metabolites with an indicative accumulation pattern under the test conditions are isolated and chemically characterized. This is then followed by complementation assays in which the biological function of such metabolites is tested in mutant plants. Finally, genes controlling the synthesis and function of the metabolites are identified and isolated. The interaction of *Arabidopsis thaliana* with *Pseudomonas syringae* is used as a model system.

### Results

Pipecolic acid has been identified as a novel regulator of plant systemic acquired resistance. It has been shown that this compound is necessary and sufficient for resistance priming, a process that is responsible for increased pathogen

defense reactions in plants, providing effective protection against pathogen infection. It was also revealed that pipecolic acid acts synergistically with the phenolic immune regulator salicylic acid in resistance induction. Enzymes and genes involved in the synthesis and downstream signaling of pipecolic acid have been identified, isolated and biochemically characterized. It was demonstrated that pipecolic acid is synthesized in a two-step enzymatic process from L-lysine that involves consecutive transamination, cyclization, and isomerization steps. In addition, further components and processes regulating plant immunity downstream of pipecolic acid accumulation have been identified and biochemically characterized.

### Application relevant Aspects

Results of this research will provide a basis for the design of new sustainable approaches in resistance breeding, and for the development of new agrochemicals protecting plants against a broad spectrum of pathogenic bacteria and fungi.

### Opportunities for Cooperation

The group is seeking research cooperations for the translation of basic know-how on resistance priming in plants into the development of novel agrochemicals that act as resistance activators or into novel resistance breeding approaches. In this context, support in the establishment of further technical capacities for metabolite profiling and metabolite characterization, in high-throughput testing of new substances or in field testing of new compounds and improved crop varieties are of high interest and relevance. Cooperations may also include the exchange of scientific personnel, leading to an exchange of know-how.



#### **Relevant publications (since 2016)**

- Hartmann M, Kim D, Bernsdorff F, Ajami-Rashidi Z, Scholten N, Schreiber S, Zeier T, Schuck S, Reichel-Deland V, Zeier J (2017) Biochemical Principles and Functional Aspects of Pipecolic Acid Biosynthesis in Plant Immunity. *Plant Physiol* 174(1):124-153.
- Stahl E, Bellwon P, Huber S, Schlaeppli K, Bernsdorff F, Vallat-Michel A, Mauch F, Zeier J (2016) Regulatory and Functional Aspects of Indolic Metabolism in Plant Systemic Acquired Resistance. *Mol Plant* 9(5):662-681.
- Bernsdorff F, Döring AC, Gruner K, Schuck S, Bräutigam A, Zeier J (2016) Pipecolic Acid Orchestrates Plant Systemic Acquired Resistance and Defense Priming via Salicylic Acid-Dependent and -Independent Pathways. *Plant Cell* 28(1):102-129.
- Hartmann M, Zeier T, Bernsdorff F, Reichel-Deland V, Kim D, Hohmann M, Scholten N, Schuck S, Bräutigam A, Holzel T, Ganter C, Zeier J (2018) Flavin Monooxygenase-Generated N-Hydroxypipecolic Acid Is a Critical Element of Plant Systemic Immunity. *Cell*. doi: 10.1016/j.cell.2018.02.049

#### **Patents / Patent Applications**

- A patent application on the effect of pipecolic acid derivatives on resistance priming in plants is in preparation.

## Research Area D

### Project Title

Secondary metabolite spectrum of plant ABC-transporters

### Project Leader

Lutz Schmitt, Heinrich Heine University  
Düsseldorf

### Project Goals

The project aims at providing plant plasma membrane transporters as essential modules for the large-scale production of plant secondary metabolites in microbes. As secondary metabolites are known to exert a variety of biological functions in nature, for example the communication of plants with microorganisms, the corresponding transporters are also potential targets for the search of novel agrochemicals.

### Activities

As a first step, the research activities enclose the cloning, expression and purification of ABC-transporters for plant secondary metabolites in bacteria or yeast. In the next step, the specific substrates for these transporters are identified with a specifically designed test system in yeast that uses the functional comparison of mutant and non-mutant transporter protein complexes. At the moment, the focus is on transporters for plant secondary metabolites from the groups of glucoronides and triterpenes, as members of these chemical groups have been shown to be involved in plant-microbe communication.

### Results

So far, three transmembrane ABC-transporter proteins from plants could be cloned and over-expressed in yeast cells. For one of the three transporters, activity assays demonstrated that precursors of suberine are the

substrates of this particular transporter.

### Application relevant Aspects

Plasma membrane transporters are considered essential modules for synthetic biology in microorganisms. Here, transporters for the export of specific plant secondary metabolites are isolated and functionally characterized and, thereby, made accessible as modules for the production of the corresponding compounds in microorganisms. In addition, the genes for transporters that play a role in plant/microbe communication are potential targets for breeding crop varieties with improved disease resistance and for the design of novel fungicides.

### Opportunities for Cooperation

The group has a strong interest in cooperation with external partners that address overlapping scientific questions or intend to transfer technological know-how and/or results from the functional characterization of plant ABC-transporters into their own R&D programs. This can be based on joint research projects as well as on the exchange of scientific personnel.



**Relevant publications (since 2016)**

- Reimann S, Poschmann G, Kanonenberg K, Stuhler K, Smits SH, Schmitt L (2016) Interdomain regulation of the ATPase activity of the ABC transporter haemolysin B from *Escherichia coli*. *Biochem J* 473(16):2471-2483.

## Research Area D

### Project Title

Plant cell wall structure and biosynthesis

### Project Leader

Markus Pauly, Heinrich Heine University  
Düsseldorf

### Project Goals

The dominant carbon sequestration system and thus removal of carbon dioxide from the atmosphere on this planet is the formation of durable cell wall composite materials by the plant. Such a wall surrounds all cells and consists of the polysaccharide classes cellulose, hemicelluloses, pectic polysaccharides, and the polyaromatic substance lignin and plays an important role not only in the mechanical stability of the plant, but also its response to abiotic and biotic stresses. Complicating matters, the 35 or so different tissues in a given higher plant exhibit differences in their wall polymer composition. However, the path from carbon to the different cell wall components and its regulation is poorly understood. Therefore, the aim of this project is to elucidate molecular mechanisms regulating carbon fixation in cell wall components and providing a basis to engineer cell wall composition and structure.

### Activities

The group uses genetic and genomic approaches to identify components of the biosynthetic machinery necessary for the synthesis of the wall polymers with particular emphasis on the hemicelluloses.

Once such enzymes have been identified their genetic manipulation in the plant is utilized to alter the wall to study not only its function in plant growth and development but also its material attributes for industrial processes such as the conversion of agricultural residues (wood or straw)

for the conversion to green chemicals to establish a bioeconomy.

The group also develops methodologies to study the chemistry of the wall. Current research aims at obtaining a cellular definition of the wall composition by e.g. mass imaging.

### Results

Key components of the synthesis of the hemicellulose xyloglucan and mannan have been identified, allowing for the first time to reconstitute the synthesis of such a polymer heterologously in a microbe. It became clear that the substitution of the wall polymer impact the polymers solubility and interaction with other polymers, but the type of substitution e.g., different glycosyl- or non-glycosyl substituents are functionally equivalent.

### Application relevant Aspects

The research of the group leads to plants with altered, sometimes tailored, cell wall (lignocellulosic) properties, which can enhance e.g. product yields derived from plant biomass.

### Opportunities for Cooperation

The group seeks cooperation with partners that are interested in testing the possibility of transferring the research results found with model plants into crop plants. In this context, the major goal will be to test the phenotypic effects of candidate genes for modification of changes in wall material composition into crop plants. Such cooperation can include joint research activities as well as the exchange of scientific personnel in order to mutually extend the know-how in this field. Material testing of the altered plant materials are also of interest.



#### **Relevant publications (since 2016)**

- Møller SR, Yi X, Velasquez SM, Gille S, Hansen PL, Poulsen CP, Olsen CE, Rejzek M, Parsons H, Yang Z, Wandall HH, Clausen H, Field RA, Pauly M, Estevez JM, Harholt J, Ulvskov P, Petersen BL (2017) Identification and evolution of a plant cell wall specific glycoprotein glycosyl transferase, ExAD. *Sci Rep* 7:45341.

## Research Area D

### Project Title

Synthetic microbes for the production of plant secondary metabolites

### Project Leader

Karl-Erich Jaeger, Thomas Drepper, Heinrich Heine University Düsseldorf

### Project Goals

Plant secondary metabolites comprise a large variety of structurally divergent compounds that serve e.g. as signaling molecules or as protecting agents against microbial pathogens, herbivorous attacks or plant competitors. Among these secondary metabolites, terpenoids including the cyclic plant-type triterpenes constitute one of the largest and most diverse group exhibiting important functions in plant physiology and development, however, the underlying mechanisms are largely unknown. This project aims to develop synthetic modules for the production of plant terpenes in microbes allowing for a detailed analysis and functional screening of physiological effects in plants to be carried out within CEPLAS with partners from Research Areas C and D.

### Activities

The Institute of Molecular Enzyme Technology (IMET) has long lasting and outstanding experience in research with enzymes and proteins from non-pathogenic as well as pathogenic bacteria. Current research foci include (i) the identification of novel genes using metagenomics methodology, (ii) functional expression of genes and gene cluster, folding and secretion of enzymes, as well as (iii) their purification, (iv) biochemical characterization, and (v) elucidation of structure-function relationships. Another major topic at the IMET is the development of new synthetic biology tools for transplanting complete

secondary metabolite pathways as well as light-dependent control and monitoring of bio(techno)logical processes. In this context, novel microbial chassis organisms such as *Pseudomonas putida*, *Burkholderia glumae* and *Rhodobacter capsulatus* are established and perpetually optimized.

### Results

Within CEPLAS, we have successfully applied novel molecular biological tools allowing (i) easy construction of synthetic operons, (ii) transfer and expression of entire recombinant pathways in bacteria and (iii) functional connection of plant pathways with intrinsic metabolic processes of the microbial hosts. For example, on the basis of the bacterial carotenoid pathway, production of 2,3 oxidosqualene was implemented in the photosynthetic host *R. capsulatus* and subsequently combined with different cyclisation reactions catalyzed by the representative oxidosqualene cyclases CAS1 (cycloartenol synthase), LUP1 (lupeol synthase), THAS1 (thalianol synthase) and MRN1 (marneral synthase) derived from model plant *Arabidopsis thaliana*. (Loeschcke et al. 2017).

### Application relevant Aspects

The characterization of terpene mechanisms of action on plants can provide a basis to develop novel strategies to improve plant growth as well as resistance to biotic and abiotic stresses. In addition, the newly developed synthetic biology tools and expression platforms can be used for the production of (i) proteins for bioeconomical and biomedical applications and (ii) high value chemicals, e.g. pharmaceuticals.

### Opportunities for Cooperation

The group is interested in collaborations with groups addressing the identification, analysis, characterization and production of natural products as well as unraveling and manipulating



plant/microbe interactions. These cooperations can comprise joint research activities as well as the exchange of scientific personnel.

#### **Relevant publications (since 2016)**

- Kaschner M, Loeschcke A, Krause J, Minh BQ, Heck A, Endres S, Svensson V, Wirtz A, von Haeseler A, Jaeger KE, Drepper T, Krauss U (2014) Discovery of the first light-dependent protochlorophyllide oxidoreductase in anoxygenic phototrophic bacteria. *Mol Microbiol* 93(5):1066-1078.
- Liebl W, Angelov A, Juergensen J, Chow J, Loeschcke A, Drepper T, Classen T, Pietruszka J, Ehrenreich A, Streit WR, Jaeger KE (2014) Alternative hosts for functional (meta)genome analysis. *Appl Microbiol Biotechnol* 98(19):8099-8109.
- Domröse A, Klein AS, Hage-Hülsmann J, Thies S, Svensson V, Classen T, Pietruszka J, Jaeger KE, Drepper T, Loeschcke A (2015) Efficient recombinant production of prodigiosin in *Pseudomonas putida*. *Front Microbiol* 6:972.
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## Research Area D

### Project Title

Synthesis of signal compounds involved in plant/microbe interactions

### Project Leader

Ilka Axmann, Heinrich Heine University Düsseldorf

### Project Goals

The goal of the project is to provide signal compounds mediating the communication between plants and pathogenic or non-pathogenic microbes in sufficient amounts for functional investigations. In this context, the group focuses on the synthesis of specific triterpenes for which previous investigations have indicated their role in plant/microbe interactions.

### Activities

Candidate genes from the biosynthetic pathway of selected triterpenes in plant are used for the synthesis of these compounds in cyanobacteria. This requires the optimization of cloning procedures and of expression of candidate genes in cyanobacteria (regulation, coding sequences).

### Results

The group has evaluated the photosynthetic bacterium *Synechocystis* sp. PCC 6803 as alternative triterpene production host. Codon optimized genes from *Arabidopsis thaliana* encoding squalene epoxidase SQE1 as well as four different oxidosqualene cyclase (OSC) enzymes, namely cycloartenol synthase CAS1, lupeol synthase LUP1, thalianol synthase THAS1, and marnerial synthase MRN1 have been implemented. A native squalene accumulation was already established in a *delta\_shc* mutant of *Synechocystis* (provided by Pia Lindberg, Uppsala University, Sweden, Englund et al., 2014).

Successful production of the common key precursor for cyclic triterpenoids, 2,3-oxidosqualene, was detected by LC-MS analysis (CEPLAS MS platform Cologne). OSC expression resulted in differential product patterns. While CAS1 catalyzed conversion to cycloartenol, expression of LUP1 yielded lupeol and a triterpenoid, matching an oxidation product of lupeol. In contrast, THAS1 expression did not lead to cyclic product formation, but MRN1-dependent production of marnerial and hydroxymarnerial was observed in *Synechocystis*. Thus, a novel photosynthetic prokaryotic platform for the heterologous biosynthesis of cyclic plant triterpenes was established.

### Application relevant Aspects

The application of research results from the group is seen in the metabolic engineering of complex biosynthetic pathways leading to signal molecules involved in plant/pathogen interactions. With the help of such know-how also other compounds with bioeconomical value can be engineered, such as pharmaceuticals and cosmetics.

### Opportunities for Cooperation

The group is interested in research cooperation targeting the optimization of production systems for complex secondary metabolites in Cyanobacteria, especially also considering the industrial up-scaling of such systems.



#### **Relevant publications (since 2016)**

- Heilmann B, Hakkila K, Georg J, Tyystjarvi T, Hess WR, Axmann IM, Dienst D (2017) 6S RNA plays a role in recovery from nitrogen depletion in *Synechocystis* sp. PCC 6803. *Postdoc BMC Microbiol* 17(1):229.
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# CEPLAS

Cluster of Excellence on Plant Sciences

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