CEPLAS
Cluster of Excellence on Plant Sciences

CEPLAS ANNUAL REPORT 2017

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Content

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General Presentation

CEPLAS at a glance

The global demand for plant products is increasing with unprecedented pace. It has been estimated that agricultural yields will have to double by the year 2050. However, global change and changing consumption patterns are challenging the adequate production of crops and thus the agronomic base of human civilisation. Further, arable land is becoming scarce due to increased erosion and population pressure. The continuously increasing demand must be met with innovative strategies for crop improvement that aim at enhancing yield, while at the same time reducing consumption of precious resources, such as water, nutrients, and soil, and increasing resistance to pests.
CEPLAS aims at achieving a fundamental understanding of the genetic mechanisms that enable plants to adapt to adverse environmental conditions and constraints. CEPLAS employs a research strategy that is driven by comparative evolutionary analyses in combination with modern synthetic biology.
Specifically, the CEPLAS researchers investigate the mechanistic basis and genetic architecture of selected complex traits that have a crucial impact on adaption to limited resources and yield and are therefore of outstanding importance in designing and breeding the crops of the future:

- Annual and perennial life histories
- C_4 photosynthesis (photosynthetic carbon conversion efficiency)
- Plant-microbe interactions
- Metabolic interactions

Research
- Interdisciplinary consortium of experimental and theoretical groups
- Characterisation of four complex plant traits that affect yield and the usage of resources

People
- About 50 research groups
- 9 new faculty appointments
- About 70 PhDs and Postdocs
- Equal Opportunity Programme

Structure
- 4 participating institutions
- 4 Research Areas
- Plant Metabolism and Metabolomics Laboratory
- New research building (projected relocation 07/18), 34 Mio. € financial volume

Training & Career Development
- CEPLAS@School
- Research internships for undergraduates
- Bachelor in Quantitative Biology
- CEPLAS Graduate School
- CEPLAS Postdoc Programme

Achievements

In April 2017, the CEPLAS consortium submitted a draft proposal for a seven-year renewal of CEPLAS. The draft proposal was successful and submission of a full proposal was invited, which will be submitted in February 2018.
The research concept of CEPLAS II builds on the comprehensive work and experiences of CEPLAS I and aims to map the genetic mechanisms that control complex plant traits, thereby enabling the prediction of crop trait performance in given environmental conditions and facilitating rational crop trait (re-)design.

In 2017, 7 doctoral researchers of the CEPLAS Graduate School finished their PhD degrees. Ruben Garrido-Oter received the award for the best PhD thesis of the Faculty of Mathematics and Natural Sciences at HHU. For the postdocs, we performed a detailed project assessment, selecting the most promising projects for extended funding until Dec 2018.
The CEPLAS Homepage was completely restructured beginning of 2017 to provide a better user-experience and improved representation of the CEPLAS programmes and offers. In addition, the appearance in social media was strengthened by setting up a presence on Twitter. For internal communication, an online Newsletter was established, informing cluster members about ongoing activities, publications, and events. For lay audiences, an image movie was produced that is now available via the CEPLAS YouTube channel. In spring, the first CEPLAS Summer School on "Emerging Frontiers in Plant Sciences" took place attracting over 40 PhD students from 10 different countries. In addition, the Competence Area on Food Security celebrated its kick-off symposium with an interdisciplinary symposium on the global challenge of Food Security.
Our two Humboldt Professors, Wolf B. Frommer and Jijie Chai, started their research groups in May 2017 and are both contributing with their expertise to the conception of the CEPLAS II proposal. A delegation from CEPLAS and both universities attended the award ceremony in Berlin where the renowned awards were presented by the Federal Minister of Education and Research Prof. Johanna Wanka. Shortly after starting his group at HHU, Wolf B. Frommer received a highly renowned grant from the Bill and Melinda Gates Foundation as well as the Tsungming Tu Award, Taiwan's leading award for foreign researchers.
Alga Zuccaro succeeded with her application for a DFG Priority Programme. From 2018, she will lead the new PP DECRyPT – Deconstruction and Reconstruction of the Plant Microbiota – which will complement the research programme of CEPLAS II. Gunther Döhlemann received an ERC Consolidator Grant for the project conVIRgens – De- and reconstructing virulence strategies of fungal plant pathogens – and will be funded for five years by the European Union. Matias Zurbriggen received funding in the frame of the highly renowned Human Frontier Science Programme for an interdisciplinary project with partners from the US, the Netherlands and South Korea.
We continued to evaluate the cluster in the frame of the annual members survey established in 2016. The survey addresses issues such as the members' satisfaction, interactions between the research areas or implementation of the early career programmes. We gladly note that for some areas, such as transparency of decisions, the satisfaction increased compared to last year which we consider a result of modifications of the programme, implemented after the last survey.
Our Bachelor Programme in Quantitative Biology became increasingly attractive, with the number of new enrolments from Cologne and Düsseldorf now rising to 16. Moreover, many of the QB students participated as a joint UoC and HHU team in this year's iGEM competition. The first cohort of Bachelor students finished their Bachelor degrees in 2017.

Goals for 2018

- Submission of the CEPLAS II proposal for a seven-year renewal
- Relocation of CEPLAS groups to the new research building (Center for Synthetic Life Sciences)
- Start of pilot experiments for CEPLAS II

Overview 2017

Meeting with Nicola Beer, Member of the German Bundestag

Visit of René Röspel, Member of the German Bundestag

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Release of new CEPLAS Homepage

Launch of the Competence Area "Food Security"

CEPLAS Workshop at Fascination of Plants Day

Excursion to KWS SAAT SE, Einbeck, Germany

CEPLAS Career Speed Dating

CEPLAS Berufsfeld-praktikum "Research & Eduaction"

Interna-tional CEPLAS Summer School

January | February | March | April | May | June

CEPLAS Friday @ HHU

CEPLAS Friday @ UoC

CEPLAS Friday @ MPIPZ

Start of new CEPLAS / AvH Professors

CEPLAS Friday @ HHU

Networking | Outreach | Early Career Researchers | Research | Equality

CEPLAS at "sitzungswoche agrar"

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CEPLAS at Panel Discussion at Bayer CropScience

IHK Panel Discussion "Rechtsrahmen für die Gen-Schere Crispr-Cas in Deutschland"

Start of Guided Tours in the Botanical Garden

Start of Public Lecture Series "Vom Maisanbau der Maya zu heutigen Superfoods"

Start of Public Lecture Series "Food and Nutrition Security"

Release of CEPLAS Image Movie

Leaflet

First internal CEPLAS Newsletter: "CEPLAS Leaflet"

Excursion to WeGrow GmbH, Tönisvorst, Germany

July | August | September | October | November | December

CEPLAS Friday @ UoC

CEPLAS Friday @ HHU

General Assembly & CEPLAS Friday @ UoC

Early Career Researchers Retreat & CEPLAS Symposium 2017

CEPLAS Women in Plant Sciences Day 2017

Authentic Networking for Women in Academia

Organisation

CEPLAS, the Cluster of Excellence on Plant Sciences, is a joint initiative of Heinrich Heine University Düsseldorf, University of Cologne, Max Planck Institute for Plant Breeding Research Cologne and Forschungszentrum Jülich. Apart from cutting-edge science, CEPLAS focuses on the promotion of early career researchers.

CEPLAS is comprised of four **Research Areas,** each headed by a Research Area Coordinator and Co-coordinator. The Research Area Coordinators are responsible for the scientific development of the respective Research Area and the distribution of the allocated funds within the Research Area.

All Research Area Coordinators are part of the **CEPLAS Steering Committee,** together with the Cluster Speaker and Deputy Speaker, the Equal Opportunity Representative and the Early Career Researchers' Representative. Additionally, one representative of the Forschungszentrum Jülich is invited to the Steering Committee meetings. The committee is responsible for the overall operation and development of the cluster, allocation of resources and preparation of site evaluations.

CEPLAS is consulted by a **Scientific Advisory Board (SAB)** that is composed of scientists from academia and industry. The SAB is in charge of the external evaluation of the cluster (annual evaluation report, mid-term and comprehensive assessment) and provides advice on hiring decisions, scientific and structural planning, development of the training programmes, implementation of equal opportunity measures, and overall development of the cluster. The Board meets on an annual basis and provides an assessment to the **Supervisory Board.**

The **CEPLAS Training Committee** is responsible for the general management of the training programmes and evaluation of training measurements and events. Furthermore, the committee oversees the recruitment procedures of early career researchers and approves admission of externally funded researchers to the CEPLAS training programmes. Additionally, the committee decides about Mobility Fund applications. Members of the Training Committee are the speaker and coordinator of the CEPLAS Graduate School and Postdoc Programme, representatives of the doctoral and postdoctoral researchers and a representative of the MPIPZ. The Training Committee reports to the Steering Committee.

Duties of the **Equal Opportunity Office** include all equality-related issues or concerns such as work-family-integration and support for female scientists. The Equal Opportunity Office cooperates with the respective departments of the partnering institutions. The Equal Opportunity Board is composed of a representative of the junior faculty group, a female professor and a male professor.

The **Central Office** is responsible for the operational business and administrative management of the cluster and supports the Cluster Speaker and Deputy Speaker, the Steering Committee as well as the Scientific Advisory Board.
It organises the meetings of the various committees and boards within CEPLAS, the General Assembly as well as conferences, workshops and symposia. The Central Office cooperates with the finance departments and human resources departments of the partnering institutions in administrative issues.

In addition, it is responsible for all reporting and correspondence (DFG, president's offices), for the design and organisation of the homepage and for public relation work.
At University of Cologne, administrative issues are carried out by a project manager.
Each CEPLAS Early Career Research Programme is managed by a coordinator taking care of all organisational issues related to the programme and curriculum. Furthermore, the coordinator is the first contact point for Early Career Researchers interested in participating in the programmes.

Scientific Advisory Board
Mike Bevan, Justin Borevitz, Natalia Dudareva, George Freyssinet, Karin Herbers, Jane Langdale, Steve Long, Magnus Nordborg, John Rathjen, Kazuki Saito, Johanna Schmitt, Klaas van Wijk, Dabing Zhang

Supervisory Board
Presidents of HHU and UoC, Deans of Math. Nat. Faculty HHU and UoC, Directors MPIPZ and FZJ

Steering Committee

Executive Board
Speaker - Andreas Weber
Deputy Speaker - Stanislav Kopriva

Equal Opportunity Representative - Ute Höcker
Early Career Researchers' Representative - Filipa Tomé
Research Area Coordinators
George Coupland, Peter Westhoff, Alga Zuccaro, Markus Pauly

Training Committee

Ute Höcker, Rüdiger Simon, Maria Albani, Filipa Tomé, Meike Hüdig, Justine Groenewold, Juliane Schmid

Equal Opportunity Board

Ute Höcker, Ilka Axmann, Benjamin Stich

Management and Administration

Central Office, Coordination of Early Career Researchers' Programmes, Equal Opportunity Office

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Research

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

Coordinator
George Coupland

Co-coordinator
Rüdiger Simon

Faculty
Maria Albani
Petra Bauer
Angela Hay
Ute Höcker
Karl Köhrer
Markus Kollmann
Maria von Korff Schmising
Juliette de Meaux
Peter Nürnberg
Richard Reinhardt
Laura Rose
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Klaus Theres
Miltos Tsiantis
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Early Career Researchers
Christos Bazakos
Luise Brand
Panpan Jiang
Nozomi Kawamoto
Gwendolyn Kirschner
Priyanka Mishra
Evelyn Obeng-Hinneh
Udhaya Ponraj
Anna Sergeeva
Vicky Tilmes
Filipa Tomé
Alice Vayssières
Agatha Walla
Jinshun Zhong
Yanhao Zhou

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 flower-

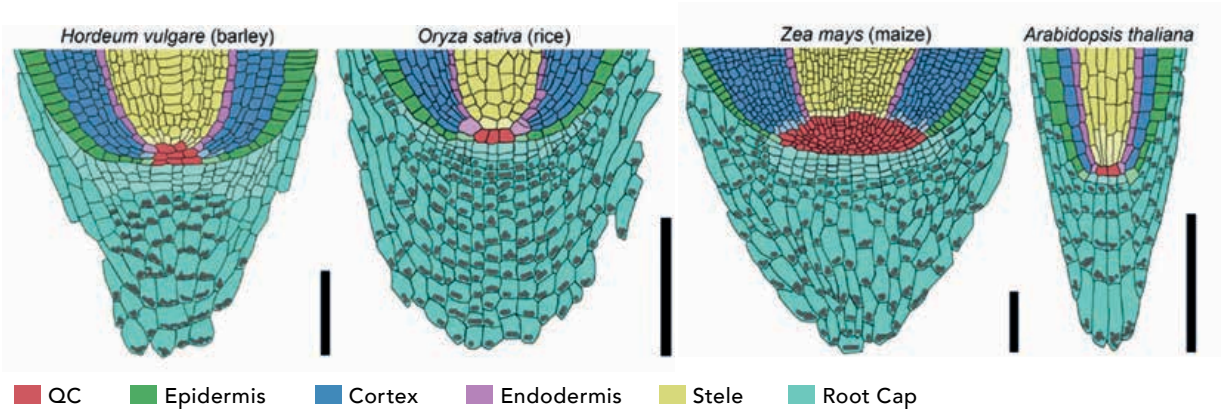
ing traits, vernalisation requirement and perpetual flowering, not found in either parent. We have also mapped short segments of the *Arabis montbretiana* genome that when introgressed into perennial *Arabis alpina* alter flowering time, some of this allelic variation might have arisen during evolution 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*.



Models of barley, rice, maize, and *Arabidopsis* root stem cell niches. Cell types are marked by colour code according to the legend, stem cells that give rise to different tissues are depicted in the respective light colours; grey spheres represent starch granules in the root; the rice stem cell niche was created according to Ni *et al.* (2014) and Wang *et al.* (2014); the maize stem cell niche was created according to Kerk and Feldman (1994), Jiang and Feldman (2005) and Jiang *et al.* (2010); scale bar 100 µm (Kirschner GK, Stahl Y, Von Korff M, Simon R (2017) Unique and Conserved Features of the Barley Root Meristem. Front Plant Sci 8:1240. doi: 10.3389/fpls.2017.01240).

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

A1 Analysis of the roles of PERPETUAL FLOWERING 1 direct target genes in perennial flowering and their divergence in sister annual species

Researcher
Vicky Tilmes

Project leaders
George Coupland
Martin Hülskamp

Project type
Ph.D. project

Project duration
01.11.2013 – 27.06.2017

Cooperation
Julieta Mateos
(Fundación Instituto Leloir, Buenos Aires, Argentinia)
Eva Willing (MPIPZ)
Isabel Lopez-Diaz
(IBMCP, Valencia, Spain)

Aim of the project

Comparison of targets of the orthologous transcription factors (TFs) PEP1 and FLC in perennial *A. alpina* and annual *A. thaliana* to understand the evolution of TF binding sites in plants and the role of targets in the different life histories.

Results

PEP1 and FLC repress flowering in *A. alpina* and *A. thaliana*, respectively. Their differential expression contributes to the different life histories of the species. Here we found that only about 15% of their targets were conserved, but among those were many flowering-related genes. PEP1- and FLC-specific targets included genes involved in responses to GA and cold. In *A. alpina* PEP1 repressed cold induction of gene expression, possibly to allow growth in cold temperatures. GA promoted flowering during vernalisation in *A. alpina* and PEP1 repressed GA signalling, likely to delay flowering in early stages of vernalisation. Thus, PEP1 links environmental and developmental responses within the perennial life cycle of *A. alpina*.

Publication

Mateos J, Tilmes V, Madrigal P *et al.* (2017) *Proc Natl Acad Sci USA* 114(51):E11037-E11046

A2 Nutrient recycling in the perennial plant *Arabis alpina*

Researcher
Anna Sergeeva

Project leaders
Petra Bauer, Stanislav Kopriva

Project type
Ph.D. project

Project start
01.02.2015

Cooperation
Maria Albani, George Coupland
Tabea Mettler-Altmann
(MS Platform, HHU)

Aim of the project
Nutrient recycling capabilities are an important feature of the perennial life style that differ between annuals and perennials and affect metabolism at the whole plant level. We investigate nutrient relocation during perennial life style by applying and combining biochemical experiments, physiological analysis and plant growth as well as molecular analysis and next generation sequencing. The project will rely on the use of model species resources established in the CEPLAS Research Area A. This project addresses goals of Research Areas A, C and D.

Results
Arabis alpina wild type and flowering mutant lines are continuously being grown to harvest and analyse plant tissue samples for stem anatomy, storage compound deposition and metabolic measurements. Using the successfully established methods it was found that nutrient storage capacities are correlated with secondary growth in the perennial zones of the shoot.

A3 Molecular characterization of senescence in annual and perennial plants

Researcher
Luise Brand

Project leaders
George Coupland
Maria Albani

Project type
Postdoc project

Project start
01.08.2015

Cooperation
Petra Bauer
Fabio Fiorani (FZJ)

Aim of the project
This project aims to characterize stem senescence in annual *Arabidopsis thaliana* and perennial *Arabis alpina*.

Results:
The correct timing of stem senescence that is accompanied by resource allocation to sink tissue is an important but not well understood process. In order to characterize stem senescence time courses of sections of inflorescence stem and vegetative stem were harvested from flowering onwards in *A. alpina* and *A. thaliana*. The sampled tissue was anatomically characterized and RNA sent for sequencing. Analysis of the RNAseq data identified known regulators of senescence to be upregulated in both stem sections in *A. thaliana* and in the inflorescence stem of *A. alpina* but not in the vegetative surviving stem of *A. alpina*. Detailed analysis of the RNAseq data is providing a starting point to study the developmental control of stem senescence and its impact on nutrient reallocation in perennial *A. alpina*.

A4 Photoperiodic control of flowering time in *Arabis alpina*

Researcher
Panpan Jiang

Aim of the project:
- To understand the effect of day length on flowering time and inflorescence development and their possible interactions with the vernalisation pathway/perennialism in perpetual and seasonal *A. alpina* accessions.

Project leaders
Ute Höcker
Maria Albani

Project type
Ph.D. project

Project duration
01.10.2013 – 31.05.2017

Cooperation
George Coupland

- To analyse photomorphogenic responses in *A. alpina* (seedling deetiolation, shade avoidance).

Results:
We identified a perpetual *A. alpina* accession that flowers independently of the day length and thus behaves very differently from other perpetual *A. alpina* accessions, which flower earlier in long day than in short day. *AaCO* transcript levels were unaltered in this accession, indicating that constitutive flowering is not related to the circadian clock. *AaFT* transcript levels were only slightly increased in short day, suggesting that early flowering in short day might be caused by a *CO/FT*-unrelated mechanism. Second, we identified *A. alpina* accessions with very divergent shade avoidance responses. These accessions were characterized on the molecular and phenotypic level, establishing links to auxin biosynthesis.

Elucidation of regulatory networks that determine formation and identity of meristems

A5 The control of adventitious root formation in the perennial *Arabis alpina*

Researcher
Priyanka Mishra

Project leaders
Maria Albani
Rüdiger Simon

Project type
Ph.D. project

Project start
01.10.2014

Cooperation
Ulla Neumann (MPIPZ)
Karin Ljung
(UPSC, Umea, Sweden)

Aim of the project
Identify the molecular mechanisms regulating adventitious rooting in the perennial *Arabis alpina*.

Results:
We developed a protocol to induce adventitious roots on soil grown plants by applying synthetic auxin using spraying. We observed that auxin induces adventitious roots in a concentration and genotype dependent manner. Interestingly, flowering plants show spatial pattern of adventitious rooting indicated by the presence of adventitious roots in specific internodes. Adventitious rooting can be correlated with the expression of genes known to participate during adventitious root formation in etiolated *A. thaliana* hypocotyls. Comparison of the transcriptome of rooting and non-rooting internodes after auxin spray suggests a differential regulation of hormonal signalling pathway and responsiveness to auxin promotes adventitious roots in specific internodes. Understanding the molecular mechanisms of adventitious rooting will facilitate efficient clonal propagation practices in horticultural and forest species, and especially for the propagation of long-lived perennial crops that have a long juvenile period.

A6 Developmental basis for asexual reproduction in *Cardamine*

Researcher
Christos Bazakos

Project leaders
Miltos Tsiantis
Angela Hay

Project type
Postdoc project

Project start
01.05.2015

Cooperation
Fabio Fiorani (FZJ)

Aim of the project
The aim of the project is dual: to understand the developmental nature and origin for root sucker formation in *C. resedifolia* and to study the genetic basis for this phenomenon. For the former aim we use microscopy and *in-situ* hybridisation approaches and for the latter we are following two complementary approaches: mutant isolation and QTL analyses.

Results
For the first approach a population of EMS mutagenized M2 seeds is screened for relevant phenotypes. For the second we have constructed a genetic linkage map, using a F2 population derived from a cross between two divergent strains of the species and it was phenotyped for QTL mapping. QTL scans for root sucker number were conducted and significant QTL were identified. Furthermore, additional wild strains were collected in the field for the exploitation of genetics underlying natural variation. In parallel, the *C. resedifolia* genome was sequenced and assembled while comparative transcriptome and methylome studies are ongoing.

A7 Shoot branching in *Arabis alpina* and its role on perennial traits

Researcher
Alice Vayssières

Project leaders
Maria Albani
Wolfgang Werr

Project type
Postdoc project

Project start
01.08.2014

Cooperation
Karin Ljung
(UPSC, Umea, Sweden)

Aim of the project
A. alpina is a perennial plant and survives after flowering in two ways 1) by maintaining vegetative growth from axillary branches and 2) by keeping some buds at dormant state. Dormant buds are clustered in a certain position along the stem creating a dormant bud zone and are always located below the vegetative axillary branches. This project aims to:

- Study the effect of flowering behaviour in branching patterns in a perennial plant such as *A. alpina*
- Understand the factors regulating the maintenance of dormant buds in perennials

Results
We characterized the release of apical dominance in relation to flowering. In *A. alpina* flowering is initiated during vernalisation, which results in the activation of the lower axillary buds forming the vegetative branches. We showed that the maintenance of dormant buds in *A. alpina* correlates with spatiotemporal changes of IAA levels along the stem and expression of auxin response markers. Understanding of plant architecture will contribute optimising plant performance and yield.

A8 The regulation of inflorescence development and outgrowth in *Arabis alpina*

Researcher
Evelyn Obeng-Hinneh

Project leader
Maria Albani

Project type
Ph.D. project, co-funded by SPP1530

Project duration
01.08.2015 – 30.08.2017

Cooperation
Korbinian Schneeberger (MPIPZ)

Aim of the project
To understand the molecular mechanisms regulating inflorescence development and outgrowth in the perennial *A. alpina*.

Results
Inflorescence buds in *A. alpina* develop during the vernalisation period and emerge when plants are transferred to warm temperatures. We characterized the length of vernalisation that is required to ensure complete inflorescence development (with maximal amount of seeds) and outgrowth in *A. alpina*. The quantitative effect of length of vernalisation on flowering and inflorescence fate was associated with the expression patterns of known floral repressors after cold and the accumulation of floral organ identity genes in cold. Overall our results suggest that in perennials besides flower inductions, there is a complex regulation of inflorescence fate that define yield potential.

A9 The regulation of inflorescence development and outgrowth in *Arabis alpina*

Researcher
Yanhao Zhou

Project leader
Maria Albani

Project type
Ph.D. project, co-funded by IMPRS

Project start
01.10.2015

Cooperation
Korbinian Schneeberger (MPIPZ)

Aim of the project
The aim of this project is to identify components that regulate inflorescence development and outgrowth.

Results
Using mapping by sequencing we mapped enhancer mutants showing reduced inflorescence branching in the *pep1-1* background. To identify the genes responsible for *eop055* and *eop077* mutant phenotypes, we are currently fine mapping the genes responsible using more F2 segregating plants. We are also in the process of introgressing the candidate regions into wild type background to assess the role of identified candidates into the perennial growth habit of *A. alpina*. Understanding the molecular mechanisms regulating inflorescence development will facilitate increased yield.

A10 Analysis of axillary meristem initiation in perennial plant *Arabis alpina*

Researcher
Udhaya Ponraj

Project leaders
Klaus Theres
Maria Albani

Aim of the project
To characterize genes that regulate the pattern of axillary meristem formation in *Arabis alpina*.

Results
Microscopic analysis revealed axillary buds that remain vegetative are initiated before the onset of vernalisation and buds initiated after vernalisation were capable of flowering. The role of *LATERAL SUPPRESSOR (LAS)*, known as an important regula-

Project type
Ph.D. project

Project start
01.10.2013

Cooperation
George Coupland

tor of axillary meristem formation, was studied. To compromise *AaLAS* function, an RNAi construct was introduced into *A. alpina* plants. Phenotypic analysis showed, *AaLAS* knockdown plants were compromised in axillary meristem initiation at vegetative stage, which lead to lack of dormant bud zone, important for continuing perennial life cycle. Transcript profiles of young axillary buds initiated at different developmental stages showed high expression of dormancy associated genes in buds initiated at late vegetative stage. This indicates, that *A. alpina* determines the buds that stay dormant soon after their initiation.

A11 Genetic dissection of natural variation in tiller development in cultivated and wild barley

Researcher
Agatha Walla

Project leaders
Maria von Korff Schmising
Rüdiger Simon

Project type
Ph.D. project

Project start
01.02.2014

Cooperation
Laura Rossini
(University of Milan, Italy)
Wilma van Esse
(University of Wageningen, Netherlands)

Aim of the project
Identification and functional characterization of the gene underlying the high tillering *granum-a* mutant locus.

Results
We developed a candidate gene selection approach to identify causative polymorphisms based on RNA-sequencing in induced and natural barley mutants. This technique allowed us to map the causative gene for the high tillering mutant *granum-a (gra-a)* to a 0.4 cM interval on chromosome 7HL. Crosses of the *gra-a* mutant with additional tillering mutants revealed epistatic and additive interactions that allow for further investigations of molecular pathways affecting tillering in barley. Tiller development was also studied in annual and perennial *Hordeum* species. Annual species were characterized by a short period of tiller outgrowth and early whole plant senescence whereas perennial species continuously produced tillers and showed selective senescence of individual tillers. This work contributes to the research goals of CEPLAS decipher the genetic and molecular basis of shoot and inflorescence architecture and different life cycle patterns in grasses.

Publications
van Esse GW, Walla A, Finke A *et al.* (2017) *Plant Physiol* 174(4):2397-2408
Liller CB, Walla A, Boer MP *et al.* (2017) *Theor Appl Genet* 130(2):269-281

A12 Genetic and environmental control of inflorescence development and floret fertility in barley and wheat

Researcher
Filipa Tomé

Aim of the project
Elucidate the mechanisms, which determine floret fertility in response to photoperiod and expression variation of *Flowering Locus (FT)*-like genes in barley and wheat.

Project leaders
Maria von Korff Schmising
Andreas Weber

Project type
Postdoc project

Project start
01.07.2015

Cooperation
Jorge Dubcovsky (University of California, USA)

Results
The specific aims of the project are to elucidate the role of the day length and the effect of major flowering time regulators on spikelet development, flower fertility and seeds set in barley. For this purpose, we generated a mutant population in the background of the winter barley Antonella and selected lines with non-synonymous mutations in the coding regions of major flowering time genes *Ppd-H1*, FLOWERING LOCUS T *(FT1)*, and *FT2*. These lines were grown in the field, and the development and spike fertility were scored. Lines with mutations in all genes showed a delay in development, particularly *ft1* mutant lines, when compared to the wild-type Antonella. More strikingly, we observed that the number of seeds per spike was strongly reduced in the mutant lines. Spike fertility ranged from 30% in *ft1* mutants to 60% in *ppd-H1* mutants. These genotypes will be used to further dissect the genetic and molecular control of flower fertility in barley.

Publication
Gol L, Tomé F, von Korff M (2017) J *Exp Bot* 68(7):1399-1410

A13 Comparative genomics in annual and perennial barley

Researcher
Jinshun Zhong

Project leader
Maria von Korff Schmising

Project type
Postdoc project

Project start
01.08.2017

Aim of the project
Perennial crop plants represent an effective strategy for sustainable and resource efficient agriculture. The objective of the project is to use comparative systems genetics and genomics to dissect the genetic and molecular mechanisms that underlie annual versus perennial growth in the Triticeae.

Results
Phenotypic comparison of annual and perennial *Hordeum* species revealed no repeated cold exposure was needed in perennial *Hordeum* for newly developed tillers to become reproductively competent. Short day repressed flowering in annual and perennial *Hordeum*. However, upon transfer to long days perennial genotypes could resume inflorescence development and flowered while elongated tillers in annual barley plants often aborted and senesced subsequently. In addition, tillering rates and duration were significantly different between annual and perennial *Hordeum*. The results from this work will pave the way for the identification of genes conferring the perenniality in grasses.

A14 Analysis of an evolutionary conserved module regulating root system development in monocot and dicot species

Researcher
Gwendolyn Kirschner

Aim of the project
Understand the genetic modules that determine root meristem growth in barley, using barley homologues of genes known to be important for root development in *Arabidopsis*, maize and rice as a starting point.

Project leaders
Rüdiger Simon
Maria von Korff Schmising

Project type
Ph.D. project

Project duration
01.12.2013 – 11.09.2017

Cooperation
Jafar Imani (University of Giessen)
Raffaele Dello Ioio
(University of Rome, Italy)
Ikram Blilou
(University of Wageningen, Netherlands)

Results
We characterized the barley root meristem growth behaviour and the cell types and division patterns of the stem cell niche by EdU and cell wall staining. We created a set of reporter lines for developmental regulators, including the phytohormones auxin and cytokinin, and confirmed their functionality by RNA *in situ* hybridisations and phytohormone treatments. These reporter lines will serve as valuable tools for the barley community, to analyse developmental processes and mutants. Since our RNAi approaches did not result in mutant phenotypes, we have now initiated CRISPR/Cas-9 knockouts. The results could enable to grow plants with a root system that is more resistant to drought, water stress and could prevent soil erosion.

Publication
Kirschner GK, Stahl Y, von Korff M *et al.* (2017) *Front Plant Sci* 8:1240

A15 Using receptor kinase pathways to modify plant traits

Researcher
Nozomi Kawamoto

Project leaders
Rüdiger Simon
Andreas Weber

Project type
Postdoc project

Project start
01.09.2015

Cooperation
Lucia Colombo
(University of Milan, Italy)
Keiko Torii
(University of Washington, USA)
Naoyuki Uchida
(Nagoya University, Japan)

Aim of the project
Seeds arise from fertilised ovules, which are initiated from a meristematic tissue called placenta. The mechanisms that pattern the placenta, i.e. that specify individual cells as ovule anlagen, is not understood. We aim at revealing these mechanisms and trying to modify seed number as an important agronomic trait via controlling the regulatory pathway(s).

Results
We performed a GWAS and QTL analysis on *Arabidopsis* ecotypes and identified ERECTA, ERECTA-LIKE1 and ERL2 as important regulators of ovule number and ovule density. Furthermore, we also found peptide ligands that interact with these receptor kinases. To analyse mutant phenotypes, we generated CRISPR/Cas9 knockout mutants. Our genetic analyses suggest that one of the peptide functions as a ligand of ERL1 and ERL2 to limit the spaces between ovule primordia, and the other functions with ER to increase the number of cells and thereby interovule spacing. Peptide-receptors interactions were demonstrated by a quantitative biochemical assays. For the remaining project time, we aim to show using transgenic approaches that siliques with higher ovule density and therefore ultimately a higher seed number can be generated by modifying the activities of these peptide dependent receptor signalling pathways.

Summary and Outlook

Model systems for studying divergence of annual and perennial traits have been developed in the *Brassicaceae*, particularly the *Arabideae*, and in *Hordeum* around the crop plant barley. Extensive genomic platforms and genetic tools are now available for studying traits of interest. The carefully chosen biological systems being analysed allow for interesting comparisons in mechanism between different models. For example, does understanding branching patterns in *A. alpina* at the levels of gene expression and auxin transport help to explain differences in the control of tillering between barley and its perennial relatives? Or are there fundamental similarities in the developmental plasticity associated with clonal propagation of shoots from the roots of *C. resedifolia* and of adventitious roots from the shoots of *A. alpina*? In addition, the first examples of exploiting inter-species gene transfer or reverse genetics using CRISPR-Cas9 to test specific hypotheses of how traits diversify during evolution of annuals are now generating results and will test the effectiveness of manipulating such traits by introducing or engineering single genes. These genes were identified as candidates based on our definition of regulatory networks that differ between annuals and perennials, particularly in areas associated with competence to flower and regulation of the duration of flowering. However, the fundamental molecular changes in these genes and networks that confer divergence in life history have not yet been identified. Major next steps will be in understanding the divergence of these traits at higher resolution, at the level of amino acid changes in proteins or nucleotide changes in promoters and how these affect binding of specific transcription factors. The surprisingly high rate at which FLC/PEP1 binding sites differ between annual *A. thaliana* and perennial *A. alpina* provides an instructive example of how extensive changes in transcriptional networks are likely to be among *Brassicaceae* species. Furthermore, the acquisition of high-quality genomes allows us to make direct comparisons among independent occurrences of annualism in the *Arabidopsis*, *Arabis* and *Cardamine* genera. This depth of understanding will create hypotheses to be tested with the transgenic and CRISPR-based tools we have generated as well as in orthogonal systems. These approaches will help reach the long-term goal of engineering the trait in other species with high precision.

Decoding function and development of a C_4 leaf

Coordinator
Peter Westhoff

Co-coordinator
Martin Lercher

Faculty
Oliver Ebenhöh, Georg Groth
Ute Höcker, Martin Hülskamp
Karl Köhrer, Markus Kollmann
Veronica G. Maurino
Juliette de Meaux
Peter Nürnberg, Markus Pauly
Richard Reinhardt, Ulrich Schurr
Kai Stühler, Miltos Tsiantis
Andreas P. M. Weber
Matias Zurbriggen

Early Career Researchers
Kumari Billakurthi, Meike Hüdig
Satish Kumar Eeda
Yuanyuan Li
Otho Mantegazza
Roxanne van Rooijen
Mara Schuler-Bermann
Johannes Schwabroh
Esther Sundermann
Berkley Walker
Silke Weckopp
Eva Willée, Thomas Wrobel

Research Area B aims at understanding the molecular mechanisms underlying the evolutionary trajectory from C_3 to C_4 photosynthesis to a level of detail that enables the construction of C_4 trait modules and their introduction into C_3 model species. The majority of our research was and hence still is targeted at dissecting key characteristics of C_4 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_4 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_3 and C_4 *Flaveria* species to identify putative regulators of C_4 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_4.
4) Studies of selected regulatory modules of C_4 metabolism and investigation of the interaction of C_4 photosynthesis with other metabolic pathways.

Evolutionary transcriptomics of C_3 and C_4 species pairs

The presence of closely related C_3 and C_4 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_4 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_4 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_4 species, but is present in C_3 species and its ontogeny and functional maintenance may therefore be studied in a genetically tractable C_3 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.

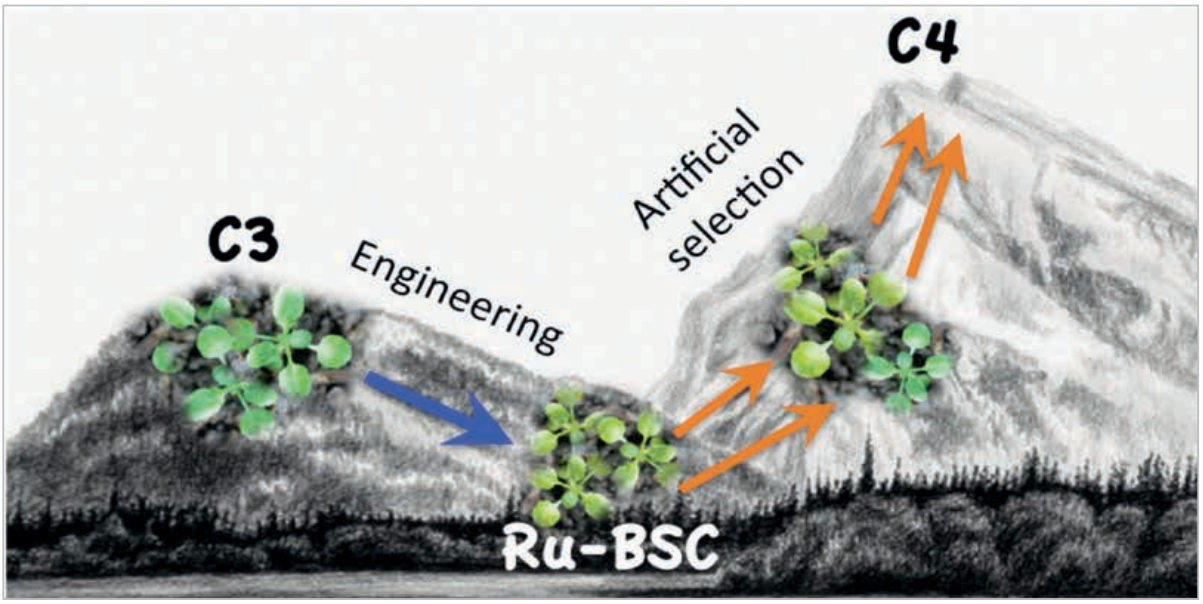
Experimental evolution towards C_4 photosynthesis

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 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.



The strategy for photosynthesis improvement suggested here. If a fitness valley separates the wild type (C_3) from the desired phenotype (C_4), the first mutational steps towards this new phenotype are deleterious and thus not achievable through evolutionary engineering. By genetically engineering the plants into the fitness valley (Ru-BSC plants), evolutionary engineering becomes possible and, because of the now initially very low fitness, efficient (Li Y, Heckmann D, Lercher MJ, Maurino VG (2017) Combining genetic and evolutionary engineering to establish C_4 metabolism in C_3 plants. *J Exp Bot* 68(2):117-125).

Evolutionary transcriptomics of C_3 and C_4 species pairs

B1 Identification of C_4 genetic determinants by a comparative transcriptome analysis of C_3 and C_4 *Flaveria spp.* and by an activation tagging approach with *Arabidopsis thaliana*

Researcher
Kumari Billakurthi

Project leaders
Peter Westhoff
Rüdiger Simon

Project type
Ph.D. project

Aim of the project
To unravel the major genetic regulatory network underlying C_4 specific Kranz leaf anatomy we pursued two approaches. Firstly, we compared transcriptome datasets of *F. robusta* (C_3) and *F. bidentis* (C_4) leaves from different developmental stages to obtain insight into C_4 leaf development. Secondly, we pursued a forward genetics approach with *Arabidopsis thaliana*, using ethyl methanesulfonate (EMS) and activation tagging, to search for mutants affected in Bundle-sheath anatomy and to identify the corresponding genes.

Project start
01.10.2013

Cooperation
Thomas Wrobel
Andreas Weber
Udo Gowik
(University of Oldenburg)
Andrea Bräutigam
(University of Bielefeld)
Gudrun Kadereit
(University of Mainz)
Maximilian Lauterbach
(University of Mainz)
Tammy Sage
(University of Toronto, CA)

Results
Comparative transcriptome datasets were prepared from leaf gradients of both *Flaveria* species. Analysis of the transcriptome datasets revealed no prominent developmental difference between the two species. Nevertheless, we identified a couple of transcription factors that are upregulated during C_4 leaf differentiation.
Arabidopsis thaliana marker lines, whose bundle-sheath cells were labelled with a chloroplast targeted GFP, were mutagenized by EMS treatment or to by random insertion into the genome of an activation tag. Based on the GFP signal we screened for mutants with more or less signal intensity. In the EMS mutagenesis screen five mutant lines were identified with an enlarged vascular tissue and possibly more bundle sheath cells.
Using the Ft-ppcA promoter as an activation tag one stable mutant line with an increased GFP signal intensity was found. The activated gene was isolated by TAIL PCR, and its over-expression recapitulated the mutant phenotype. Analysis of mutant leaves by transmission electron microscopy revealed that the mutant line possessed more plasmodesmata between the chlorenchyma cells than the reference line.

Publications
Lauterbach M, Schmidt H, Billakurthi K *et al*. (2017) *Front Plant Sci* 8:1939
Lauterbach M, Billakurthi K, Kadereit G *et al*. (2017) *J Exp Bot* 68:161-176

B2 Unravelling the mechanisms that control bundle-sheath cell size in leaves of C_4 plants

Researcher
Thomas Wrobel

Project leaders
Andreas Weber
Martin Hülskamp

Project type
Ph.D. project

Project start
01.10.2013

Cooperation
Andrea Bräutigam
(University of Bielefeld)
Kumari Billakurthi
Peter Westhoff
Udo Gowik
(University of Oldenburg)

Aim of the project
The aim of the project is to determine the developmental pattern responsible for the generation of C_4 specific bundles sheath (BSH) traits and its implementation into a C_3 model organism.

Results
We sequenced the transcriptomes of the closely related species *Flaveria robusta* (C_3) and *Flaveria bidentis* (C_4) in leafs at different developmental stages. In order to identify the specific changes during cell division, the cell cycle arrest front and photosynthetic tissue we modelled the abundances of these areas during development using nonnegative factorisation (NMF). The predictions were validated using cleared leaves and cross-sections after araldite embedding.
Major differences in early development are altered patterns of auxin and gibberellin signalling during division and differentiation. Additionally, genes involved in chloroplast biogenesis and its regulation show altered expression levels in the differentiating area.

Publication
Külahoglu C, Denton AK, Sommer M *et al*. (2014) *Plant Cell* 26(8):3243-3260

B3 Establishing a functional link between leaf development and C_4 photosynthesis

Researcher
Otho Mantegazza

Project leaders
Andreas Weber
Matias Zurbriggen

Project type
Postdoc project

Project duration
01.05.2015 – 31.10.2017

Cooperation
Florian Hahn (HHU)
Thomas Wrobel
Mara Schuler-Bermann

Aim of the project
The aim of the project is to learn from leaf developmental transcriptomic series of *Cleome* C_4 and C_3 species the regulatory mechanisms specifying C_4 leaf anatomy and how these mechanisms can be transferred to the C_3 model organism *Arabidopsis thaliana*, with the aim of initiating a C_4-like leaf development in this species.

Results
We have encoded the system that we use to compare the transcriptomes of C_3 and C_4 leaves in an R package. The source code of this package is available and documented on Github, so that it can be used by the scientific community. This tool can be used to identify genetic and transcriptomic differences between C_3 and C_4 leaf development. On side we have tested tools for Cas9 based gene targeting in *Arabidopsis*. These tools can now be used to test specific C_4 modification into the model species *Arabidopsis*.

Publication
Schuler ML, Mantegazza O, Weber AP (2016) *Plant J* 87(1):51-65

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

B4 Unravelling the functional relationship between leaf anatomy and photosynthesis by mutational and synthetic approaches

Researcher
Roxanne van Rooijen

Project leaders
Peter Westhoff
Andreas Weber

Project type
Postdoc project

Project start
01.09.2015

Aim of the project
Identifying endogenous genes in the C_3 plant *Arabidopsis thaliana* that can alter bundle-sheath ontogeny when overexpressed. For this, an *Arabidopsis* reference line was already developed in the laboratory of P. Westhoff before the start of this project for the visualization of GFP-tagged chloroplasts in the bundle-sheath cells of the leaf.

Results
In the *Arabidopsis reference* line, activation tagging was performed with a C_4-derived bundle-sheath specific promoter. Among the mutants that arose, we checked for aberrant bundle-sheath morphology phenotypes and related these phenotypes to the underlying genetics. We found two mutants with increased GFP signal in the chloroplasts of the bundle-sheath cells of *Arabidopsis*, for which we could identify the underlying genetics. This lead to two candidate genes involved in the differentiation of bundle-sheath cells towards C_4-like anatomy. We are now constructing transgenic overexpressor and mutant lines

of these two candidate genes to confirm their role in regulating bundle-sheath ontogeny. After confirmation, we will continue with functional analysis of these two genes.
The project has been suspended since 05/2017 due to parental leave.

B5 Light effects on leaf anatomy in *Arabidopsis*

Researcher
Eva Willée

Project leaders
Ute Höcker, Martin Hülskamp

Project type
Ph.D. project

Project start
01.05.2013

Cooperation
Peter Westhoff
Maarten Koornneef
(formerly MPIPZ)

Aim of the project
1) Identify loci controlling leaf anatomy in *Arabidopsis* using natural genetic variation
2) Help design a leaf with superior anatomical features for efficient photosynthesis

Results
We used the AMPRIL multi-parent mapping population provided by M. Koornneef for QTL mapping of the leaf anatomy traits leaf thickness and vein density at different light intensities. Several significant QTLs were identified that are currently being finemapped using heterogeneous inbred families (HIFs). As a second approach, we generated transcriptome data on developing and fully developed leaves that were exposed to low and high light conditions. These leaves exhibit contrasting leaf anatomy phenotypes and thus will complement the QTL analysis approach.
The project was interrupted in 2016 for 9 months due to maternity leave.

B6 Differentiation of vascular veins and surrounding mesenchyme: the role of leaf meristems within adaptations to C_4 anatomy

Researcher
Satish Kumar Eeda

Project leaders
Wolfgang Werr
Peter Westhoff

Project type
Ph.D. project

Project start
01.10.2014

Aim of the project
The group addresses the activity of marginal or plate meristem with respect to vascularisation during early leaf development.

Results
The initiation of a new primordium starts with mesenchymal identity marked by *WOX3* in marginal and plate meristems before *WOX4* is activated in vascular strands. *WOX4* promoter activity marks a sub-cortical cylindrical domain that connects the fascicular cambiums and extends into vascular system of lateral branches. In leaf primordia *WOX4* is active in the sub-epidermal layer of the petiole and adaxially of the leaf blade, and later in the polyploid palisade parenchyma. *WOX4* expressing cells apparently possess a pluripotent, parenchymal ground state during stem and leaf development. The available data suggest an extensive interplay between the plesiomorphic *WOX3* or *WOX4* stem cell niches during leaf development and lamina expansion, traits that may be relevant with respect to C_3 or C_4 anatomy and vascular density.

B7 Generation of synthetic tools for the engineering of C_4 photosynthesis

Researcher
Mara Schuler-Bermann

Project leader
Peter Westhoff
Andreas Weber

Project type
Postdoc project

Project start
01.08.2015

Cooperation
Berkley Walker
Otho Mantegazza
Jane Langdale
(University of Oxford, UK)
Olga Sedelnikova
(University of Oxford, UK)

Aim of the project
The anatomical shift towards Kranz-like anatomy requires an increase in vein density and a morphological adaptation of bundle sheath cells. Genes playing a major regulatory function early in leaf development are yet to be found and we agree that a cohort of genes must be orchestrated to initiate this specialised anatomy early in development prior to the integration of the two-celled C_4 photosynthetic cycle. This project aims to 1) The detection and functional characterisation of vascular patterning factors inducing higher order veins 2) The identification of *cis*-regulatory modules (CRM) driving tissue specific expression early in vascular development.

Results
1) The 5`flanking region of the *ATHB8* vascular patterning factor will be used for activation tagging in a labelled *Arabidopsis* reference lines to identify new vascular patterning genes. Transgenic seed is already available for screening.
2) Individual transgenic maize, rice and *Arabidopsis* lines carrying different promoter-reporter constructs are currently being analysed. Initial GUS staining experiments of the maize 3 kb 5`flanking region show distinct, but weak vascular-specific expression in rice primordia. Seeds of all transgenic lines are available and need to be analysed in further detail.

The project has been suspended since 10/2017 due to parental leave.

Publications
Schuler ML, Sedelnikova O, Walker B *et al.* (2018) *Plant Physiol* 176(1):757-772
Schuler ML, Mantegazza O, Weber AP (2016) *Plant J* 87(1):51-65

Experimental evolution towards C_4 photosynthesis

B8 *In silico* exploration of paths towards C_4 metabolism

Researcher
Esther Sundermann

Project leaders
Martin Lercher, Andreas Weber

Project type
Ph.D. project

Project start
01.06.2014

Aim of the project
C_4 photosynthesis evolved more than 60 times independently from C_3. To better understand these modes of photosynthesis and their evolutionary emergence, we explore the phenotypic plasticity of resource allocation of C_3 and C_4 plants in terms of nitrogen and energy, and simulate evolutionary trajectories across diverse environments.

Results
We created a mathematical model that considers the effects of leaf nitrogen level, light, temperature, and gas partial pressures on the CO_2 assimilation rate, a proxy for plant fitness. For C_4 plants, the resource allocation is optimally adapted to the

Cooperation
David Heckmann
(University of California,
San Diego, USA)

environment in which these plants have likely evolved, but is not optimised for the conditions under which the plants were grown. We find that a wide range of environments favours C_4 photosynthesis, while others favour C_3. While C_4 is expected to evolve readily in many environments, we find that a reversion from C_4 to C_3 is highly unlikely even when the C_3 state shows higher fitness.

B9 Transforming *Arabidopsis* plants towards C_4 metabolism

Researcher
Yuanyuan Li

Project leaders
Veronica G. Maurino
Martin Lercher

Project type
Postdoc project

Project duration
02.04.2015 – 31.10.2017

Cooperation
David Heckmann
(University of California,
San Diego, USA)

Aim of the project
This project aims to generate C_4 prototype from the C_3 *Arabidopsis* by combining genetic engineering, mutagenesis, and artificial selection. We planned to engineer *Arabidopsis* plants with Rubisco specifically expressed in bundle sheath cells by relocating a regulator of the *rbc*L gene, RLSB (RBCL RNA S1-BINDING DOMAIN protein) to these cells. The plants will later be mutagenized to screen for improved photosynthesis, i.e., with characteristics towards C_4 anatomy and biochemistry.

Results
We have gained the homozygous transgenic *Arabidopsis* plants that express RLSB only in bundle sheath cells. Surprisingly, these transgenic plants still contained certain amount of Rubisco. We generated loss-of-function mutants *rlsb*, which we found able to develop if supplemented with sucrose.
Here, we tried to combine genetic and evolutionary engineering to move towards C_4 photosynthesis in *Arabidopsis*. An even partial success would represent a breakthrough achievement for CEPLAS.

Publication
Li Y, Heckmann D, Lercher MJ *et al.* (2017) *J Exp Bot* 68(2):117-125

Regulation and metabolic interactions of C_4 photosynthesis

B10 Alterations to the regulation of C_4 enzymes during evolution of C_4 photosynthesis

Researcher
Meike Hüdig

Project leaders
Veronica G. Maurino
Peter Westhoff

Project type
Ph.D. project

Aim of the project
The project aims to (1) identify and characterize NAD-malic enzyme (NAD-ME) of *Tarenaya hassleriana* (C_3 plant) and *Gynandropsis gynandra* (C_4) as close relatives to *Arabidopsis thaliana*. The comparison will help to reveal changes that occurred during the evolution of C_4 NAD-ME. Additionally, (2) the role of mitochondrial malate metabolism and its regulation in *A. thaliana* is being studied by using loss-of-function lines of NAD-ME and mitochondrial malate dehydrogenase.

Results
1) In vitro characterization of heterologously expressed NAD-MEs is nearly finished. Comparative NAD-ME transcript anal-

Project start
01.02.2014

Cooperation
Tabea Mettler-Altmann
(MS Platform, HHU)
Gereon Poschmann (HHU)

ysis was performed. A C_4 specific NAD-ME was identified and its composition was described. *In silico* analyses of NAD-ME protein sequences are ongoing.

2) Isolation of 16 knock-out lines (single, double, triple knock-outs) and their molecular and developmental characterization of has been performed. Unexpected phenotypes were found for some double and triple knock-out mutants. Analysis of metabolite levels during the day/night transition is being combined with RNAseq to shed light on the metabolic status and gene regulation.

Publication
Hüdig M, Maier A, Scherrers I *et al.* (2015) *Plant Cell Physiol* 56(9):1820-30

B11 Structural evolution of phosphoenolpyruvate carboxylase kinase (PPCK) in the genus *Flaveria*

Researcher
Johannes Schwabroh

Project leaders
Georg Groth
Peter Westhoff

Project type
Ph.D. project

Project duration
01.10.2014 – 30.09.2017

Cooperation
Veronica G. Maurino

Aim of the project
The project set out to explore the molecular basis of the preferential interaction of C_3- and C_4-PPCK isoforms with their PEPC target.

Results
In the funding period, different PPCK isoforms from the *Flaveria* genus were successfully expressed in soluble form and purified to homogeneity from their bacterial host. Binding studies by MicroScale Thermophoresis confirmed preferential and strong interaction of C_4-PPCKA with C_4-PEPC. The purified protein was successfully crystallized and diffraction data were collected to 3.2 Å resolution. However, structure determination uncovered that carbonic anhydrase (CA) from the bacterial host rather than the anticipated PPCK was found in the crystal structure. This contamination of PPCK by endogenous CA was not obvious on SDS protein gels or SEC used to validate identity and monodispersity of purified recombinant PPCK due to the low difference in MW of both proteins. Subsequently, urea PAGE was used to resolve contamination of recombinant PPCK by bacterial CA and CA was successfully removed by additional washing steps. Crystallization trials on this improved PPCK preparation are in progress.

B12 Nitrogen and sulfur metabolism in C_4 plants

Researcher
Silke Weckopp

Project leaders
Stanislav Kopriva, Peter Westhoff

Aim of the project
The aim of the project is to find out the biological relevance of differential distribution of N and S metabolism in C_4 plants and how this distribution affects use efficiency of these nutrients. Moreover, the general differences in mineral nutrition of C_3 and C_4 plants will be assessed.

Project type
Ph.D. project

Project duration
01.01.2015 – 31.12.2017

Cooperation
Berkley Walker
Tamas Dalmay
(University of East Anglia, UK)

Results
We revealed a gradient in accumulation of reduced S compounds towards increased C_4 characteristics in *Flaveria* species. Similar gradient was seen in sulfate uptake and reduction rate. In C_4 species glutathione is predominantly synthesized in the roots and translocated to the shoots, probably because colocalization with phosphorylated pathway of serine synthesis. A significant change in distribution of sulfate and phosphate between roots and shoots was observed between C_3 and C_4 species. Using interspecies grafting between C_3 and C_4 *Flaveria* we showed that the sulfate distribution is controlled by the root whereas the phosphate is under control of the shoot.

Publications
Weckopp SC, Kopriva S (2015) *Front Plant Sci* 13;5:773
Kopriva S, Calderwood A, Weckopp SC *et al.* (2015) *Plant Sci* 241:1-10

B13 Determining alternative sources of photorespiratory carbon loss

Researcher
Berkley Walker

Project leaders
Andreas Weber
Stanislav Kopriva

Project type
Postdoc project

Project duration
01.06.2016 – 31.12.2017

Cooperation
Dipali Singh (HHU)
Oliver Ebenhöh
Silke Weckopp
Miltos Tsiantis
Francesco Vuolo (MPIPZ)

Aim of the project
Determine inefficiencies in photorespiration by combining measurements of leaf gas exchange with a metabolic model of photorespiration. An additional aim is to provide gas exchange expertise to projects within CEPLAS.

Results
By combining measurements of gas exchange with mutant analysis and a model of photorespiration, we have determined that the efficiency of photorespiration decreases when hydrogen peroxide builds up in the peroxisome to drive wasteful non-enzymatic decarboxylations. We are now using this model to explore if these reactions occur in wild-type plants under elevated temperatures and to design synthetic remediation strategies. Berkley has also shared his gas exchange expertise to other CEPLAS-aligned efforts such as to help resolve the contribution of roots to C_4 photosynthetic function (AG Kopriva) and scale the impact of leaf shape-mediating transcription factors to photosynthetic performance (AG Tsiantis).

Publication
Slattery RA, Walker BJ, Weber APM *et al.* (2017) *Plant Physiol*, doi: 10.1104/pp.17.01234

Summary and Outlook

With the identification of the Golden 2-like (GLK) genes as a central determinant of proto-Kranz leaf anatomy (Wang *et al.*, Curr Biol, 2017) a significant step towards the construction of Kranz anatomy in a C_3 species has been reached. However, it is an open question whether additional regulators beyond the GLK regulon are required. Therefore, forward genetic approaches are still valuable, as an unbiased approach, to search for those additional regulators. Currently, C_3 model species such as *Arabidopsis* or rice are the best systems for forward genetics and synthetic approaches, and their use will most probably continue. Nevertheless, C_3-like C_3-C_4 intermediate species such as *Moricandia arvensis* may represent good models for artificial C_4 evolution under the environmental conditions that led to the appearance of C_4 species in the early Miocene. Given the power of present day whole genome analyses the mutational changes occurring during this process of artificial evolution should be identifiable and give insight into the genetic changes leading to the establishment of C_4 photosynthesis.

The molecular basis of plant-microflora interaction

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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 bioinformatics, 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; Schlaeppi *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 (e.g., project C7 Döhlemann/Kemen).

- **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 (Lahrmann *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).

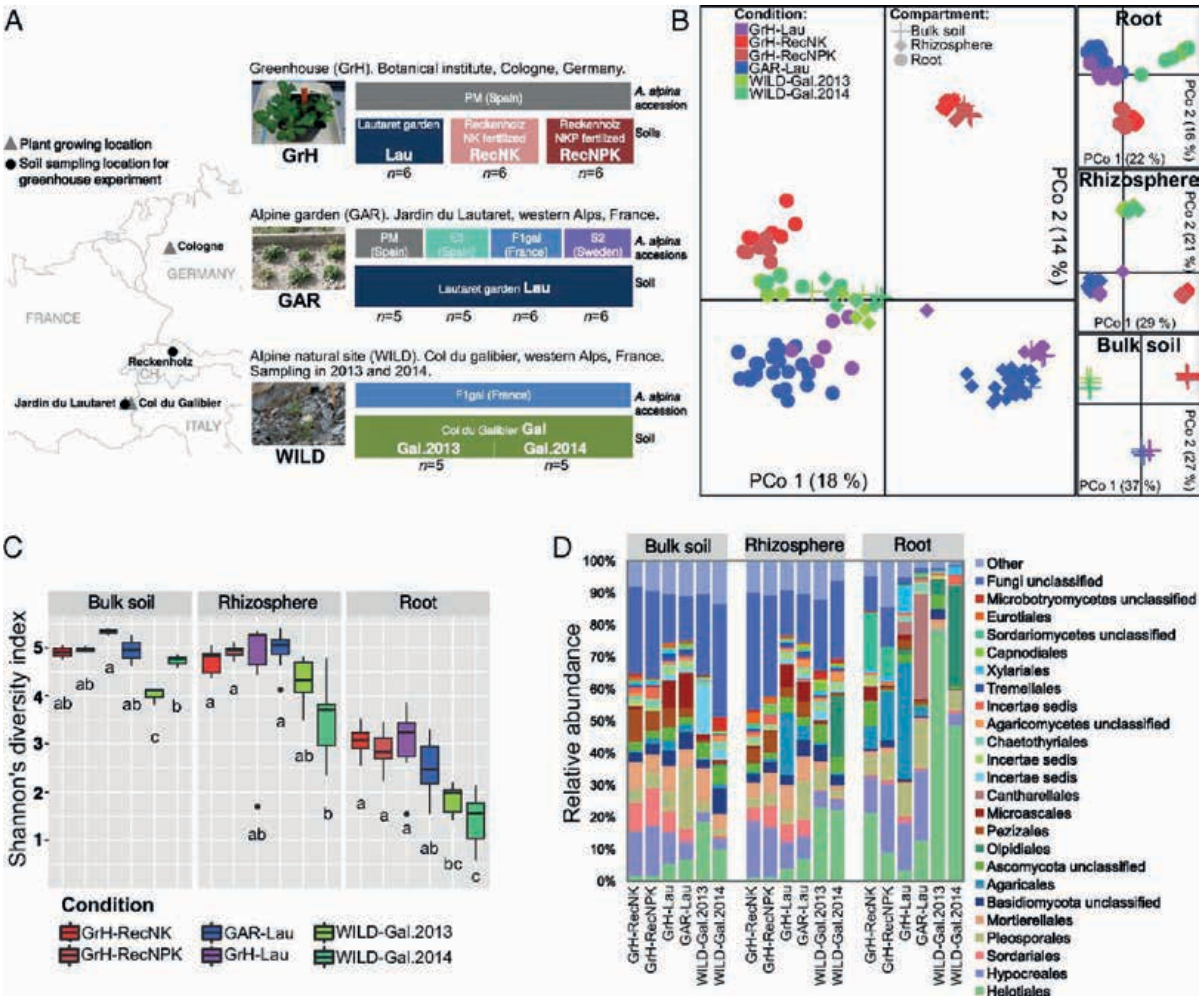
- **Critically assessed methods for assembly, taxonomic profiling and binning** which are key to interpreting metagenome data. We participated in the Critical Assessment of Metagenome Interpretation (CAMI) challenge that engaged the global developer community to benchmark their programmes on highly complex and realistic data sets, generated from ~700 newly sequenced microorganisms and ~ 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 (Sczyrba *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.*, Envirion 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.



Comparison of the fungal communities colonizing *A. alpina* roots and rhizosphere under greenhouse (GrH), common garden (GAR), and natural (WILD) conditions in different soils (RecNK, RecNPK, Lau, and Gal). (A) Experimental setup showing the different plant growing conditions. The geographic origin of the different *A. alpina* accessions is indicated in parentheses. The number of biological replicates per condition (*n*) is indicated. (B) Principal coordinates analysis on fungal community differences (Bray–Curtis dissimilarities) in the different compartments and conditions. (C) Fungal alpha diversity estimated by Shannon's diversity index. Letters a–c indicate significant differences between conditions within each compartment (ANOVA and Tukey's HSD, $P < 0.05$). (D) Mean relative abundance of the major fungal orders in the different conditions and compartments: bulk soil, rhizosphere, and root. As the four *A. alpina* accessions studied exhibited similar fungal communities in the garden experiment, combined results for the four accessions are shown under the "GAR-Lau" condition (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).

Structure, spatio-temporal organisation, function, and ecology of the plant microbiota

C1 Diversity of oomycetes and protists in intimate association with *Arabidopsis*

Researcher
Melanie Sapp

Project leaders
Laura Rose
Michael Bonkowski

Project type
Postdoc project

Project start
07.09.2015

Cooperation
Michael Bonkowski

Aim of the project
Identification of key protists associated with *A. thaliana* and their interaction with the host and other microbes.

Results
We showed that protists are an integral part of the *A. thaliana* microbiome. Key oomycetes belonged to the genus *Globisporangium* (Peronosporales). Key Cercozoa belonged to the *Glissomonadida* and *Cercomonadida*. Niche partitioning was strong for Cercozoa and largely correlated with bacterial diversity. Edaphic factors strongly influenced communities in the rhizosphere, but not in the phyllosphere. Isolates of known pathogens (*G. violae, G. irregulare* and *Pythium brassicum*) caused a drastic reduction in biomass of *A. thaliana* and led to an accelerated life history. We substantially improved methods for oomycete metabarcoding using a specific region of the cytochrome oxidase 2 gene. Our identification of a plant protistbiome and characterization of ecological relationships between plant/oomycetes, as well as Cercozoa/bacteria helped us to reach major aims within CEPLAS.

Publication
Sapp M, Ploch S, Fiore-Donno AM *et al.* (2018) *Environ Microbiol* 20(1):30-43

Insights to how biotic and abiotic factors affect the composition and activities of plant-associated microbial communities and identification of microbial hubs

C2 Shaping of the *Arabis alpina* microbiome by plant host interactions with environmental factors – phosphorous limitation

Researcher
Michael Thielen

Project leaders
Marcel Bucher
Gunther Döhlemann

Project type
Ph.D. project

Aim of the project
The aim of the project is to investigate the genetic underpinnings for plant factors that can shape the root-associated fungal community composition and how the microbes in turn can provide fitness benefits for the host. To approach this goal a synthetic fungal community (SynCom) has been established composed of fourteen species from our fungal isolate collection. We extensively study the effects of various biotic and abiotic factors (nutrient availability, pathogen threat, interaction with bacteria).

Results
After identification of the ideal colonization conditions, we could show that the SynCom reproducibly and consistently colonizes

Project start
01.05.2014

Cooperation
George Coupland

A. alpina roots. Plant growth seems to be unaffected by the SynCom. The fungal community composition is affected by the absence or presence of a bacterial inoculum. The presence of the synthetic community seems to have an inhibitory or delaying effect on the attack of the pathogen *Plasmodiophora brassicae*.

C3 Molecular link between protein stability and sorting (ESCRT) and RNA stability (Pbodies, stress granules) in the context of plant stress responses (ex project: Role of WRK75 in phosphate, pathogen and temperature responses)

Researcher
Heike Wolff

Project leaders
Martin Hülskamp
Jane Parker

Project type
Ph.D. project

Project duration
01.11.2013 – 30.09.2017

Cooperation
Friederike Brüssow
Marcel Bucher
Ute Höcker
Carolin Seyfferth (MPIPZ)
Achim Tresch (UoC)

Aim of the project
The *A. thaliana WRKY75 (AtWRKY75)* gene was published to be involved in plant responses to phosphate (Pi) starvation and the infection by biotrophic and necrotrophic pathogens. This qualified *AtWRKY75* as a good study case to understand the regulatory crosslinks between biotic and abiotic stress pathways. The aim was to study the molecular details of the role of WRKY75 in biotic and abiotic responses and to identify common routes.

Results:
As *AtWRKY75* was reported to be transcriptionally activated upon biotic and abiotic stress and to transcriptionally regulate characteristic downstream genes, we initially focused on these two aspects. We applied various published growth conditions and biotic and abiotic treatments using wild type and two wrky75 mutants. We could neither confirm the transcriptional response of *AtWRKY75* to both biotic and abiotic stress treatments nor could we observe the *AtWRKY75*-dependent regulation of target genes. We therefore abandoned this project and initiated a new project to enable the Ph.D. student Heike Wolff to finish the Ph.D. Towards this end, Heike Wolff analyses a potential link between pathways regulating protein stability and sorting (ESCRT) and RNA stability (P-bodies, stress granules) in the context of stress responses. We established a molecular link and focus on the function of VPS4/SKD1 in the two processes. Proteomics studies revealed a physical association of VPS4/SKD1 with membrane proteins and RNA interacting proteins and we demonstrate their stress-dependent localization to stress granules.

C4 Investigation of the effect of temperature-modulated defense homeostasis on plant-microbe symbioses in *Arabidopsis thaliana*

Researcher
Friederike Brüssow

Aim of the project:
Examine natural genetic variation in the *A. thaliana* defense network in response to temperature at the level of plant growth, stress hormone pathway homeostasis and structuring of leaf-associated microbial communities.

Project leaders
Jane Parker
Paul Schulze-Lefert

Project type
Postdoc project

Project duration
01.09.2013 – 31.07.2017

Cooperation
Eric Kemen

Results:
SA chemotyping of leaves from 105 diverse *A. thaliana* accessions grown in soil at 20o and 16oC uncovered extensive natural variation in response to temperature at the level of SA accumulation. A GWAS analysis for loci underlying temp x SA differences identified 4 statistically well supported peaks. Interrogation of SNPs and genes underlying these peaks revealed one bHLH transcription factor as a strong candidate for temperature modulation of both SA accumulation and bacterial pathogen resistance. SA depletion in selected differential accessions showed that SA differences are causal for the temperature effects on disease resistance. These *A. thaliana* accessions form the basis of further work to i) uncover the bHLH molecular function, ii) determine how certain plant lines effectively buffer growth trade-offs with SA accumulation and disease resistance, iii) measure the effect of differential responses to temperature on leaf microbial population structures, iv) examine the robustness of plant SA, growth and resistance phenotypes under varying environmental conditions.
Results address the central CEPLAS goal of defining complex plant traits and the influence of environment on plant health. The project was interrupted for 12 months due to maternity leave.

C5 Environmental influences on pipecolic acid biosynthesis, defense priming, and systemic acquired resistance: light, nitrogen supply, and the C/N balance

Researcher
Ziba Ajami-Rashidi

Project leaders
Jürgen Zeier
Laura Rose

Project type
Ph.D. project

Project duration
01.10.2013 – 31.01.2017

Cooperation
Shizue Matsubara (FZJ)

Aim of the project
The project investigated how *Arabidopsis* adapts the systemic acquired resistance (SAR) response to different environmental conditions such as light quantity and nitrogen supply. In this context, it re-evaluated the relevance of putative signals previously implicate with SAR.

Results:
The efficiency of SAR establishment and the pathogen-inducible biosynthesis of the SAR regulator pipecolic acid (Pip) are positively affected by appropriate light conditions and nitrogen supply. Genetic analyses with *Arabidopsis* mutants suggest that the previously described SAR signals DEFECTIVE IN INDUCED RESISTANCE1, azelaic acid, and methyl salicylate are dispensable for SAR under different light regimes, and that GLYCEROL INSENSITIVE1-dependent glycerol-3-phosphate production contributes to the strength of SAR. By contrast, Pip accumulation and signalling via FLAVIN-DEPENDENT MONOOXYGENASE1 define a critical and indispensable node for SAR signalling under all kinds of tested conditions.

C6 Mutualistic interactions of pathogenic and non-pathogenic protists in foliar biofilms

Researcher
Alfredo Mari

Project leaders
Eric Kemen
Michael Bonkowski

Project type
Ph.D. project

Project start
01.11.2014

Cooperation
Paul Schulze-Lefert

Aim of the project
Microbial communities have been reported to strongly affect plant physiology and fitness. Protists, due to their ability in re-shaping the bacterial communities by selectively grazing, are likely to hold a top-level role within plant-microbe interactions. In this study, we aim to define a new framework for ecological studies, by reaching the most complete picture on leaf microbiome, and using this as a model for terrestrial ecosystems. We aim to investigate the role of autotrophs vs. heterotrophs, as well as rare taxa impact in microbial systems.

Results
We have developed an 18S Illumina sequencing approach to profile protists and other eukaryotic microbes and produced suited analysis pipelines. First insights obtained by co-occurrence based scale free networks, indicate a major role held by autotrophic organisms in the endophytic compartment. Determinant hubs appear to be low abundant taxa. These findings may shade new light on the role of producers and rare taxa in terrestrial ecosystems.

C7 Role of fungal lifestyle and secreted effectors in multitrophic microbe – microbe and microbe – plant interactions

Researcher
Katharina Lentz

Project leaders
Gunther Döhlemann
Eric Kemen

Project type
Ph.D. project

Project start
01.11.2015

Aim of the project
Microbiome composition both on roots and upper ground plant organs is crucial for plant health and immunity. Previous work demonstrated that both mutualistic and antagonistic microbe-microbe interactions are defining the plant microbiome. A yeast-like basidiomycete, *Pseudozyma*, was identified as an epiphyte of *Arabidopsis* leaves. The project aims to study the role of *Pseudozyma* in the *Arabidopsis* phyllosphere. Because of its close relation to pathogens, its potential role as a plant pathogen, and how its lifestyle influences the leaf microbiome, will be studied.

Results
Genome annotation of *Pseudozyma spec.* has been completed and a transformation system has been established. Competition assays demonstrated strong antibiotic activity of *Pseudozyma spec.* against several epiphytic bacteria and the oomycete pathogen *Albugo laibachii.* RNA sequencing has been performed to identify genes which are involved in the antagonistic interactions of *Pseudozyma* to other microbes.

Identification and characterization of host and microbial genes that influence the outcome of interactions and assessment of methods for assembly, taxonomic profiling and binning

C8 Identification of plant growth promoting genes and pathways of bacterial symbionts in the *A. thaliana* rhizosphere

Researcher
Ruben Garrido-Oter

Project leaders
Alice McHardy
Paul Schulze-Lefert

Project type
Ph.D. project

Project duration
01.10.2013 – 06.06.2017

Cooperation
Stanislav Kopriva
Jeff Dangl
(University of North Carolina, USA)
Rob Knight
(University of San Diego, USA)
Ruth Ley
(Cornell University, USA)
Richard J. O'Connell
(INRA Versailles, France)
Ralph Panstruga
(RWTH Aachen)
Simona Radutoiu
(Aarhus University, Denmark)
Elizabeth Sattely
(Stanford University, USA)

Aim of the project

The aim of this work was to develop and apply computational methods for the analysis of sequence data obtained from environmental samples of plant-associated microbes as well as genomic sequences of cultured isolates in a comparative framework.

Results

Employing culture-independent community profiling we were able to describe and characterize the taxonomic structure and functional potential of the plant microbiota across multiple hosts, including the model *Arabidopsis thaliana* and relatives, the crop barley or the legume *Lotus japonicus*. In addition, we have developed a number of culture-dependent methods to study the plant microbiome, including the characterization of a large collection of cultured and sequenced bacterial microbiota members. Finally, we developed a novel phylogenetic approach for determining clusters of coevolving genes and their network organisation by modeling gene gain and loss as a continuous process along the branches of the species tree.

Publications

Schlaeppi K, Dombrowski N, Oter RG *et al.* (2014) *PNAS* 111(2):585-592
Bulgarelli D, Garrido-Oter R, Muench PC *et al.* (2015) *Cell Host Microbe* 17(3):392-403
Bai Y, Mueller DB, Srinivas G *et al.* (2015) *Nature* 528(7582):364-369
Hacquard S, Garrido-Oter R, Gonzalez A *et al.* (2015) *Cell Host Microbe* 17(5):603-616
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Zgadzaj R, Garrido-Oter R, Jensen DB *et al.* (2016) *PNAS* 113(49):E7996-E8005
Dombrowski N, Schlaeppi K, Agler MT *et al.* (2017) *ISME J* 11(1):43-55
Sczyrba A, Hofmann P, Belmann P *et al.* (2017) *Nat Methods* 14(11):1063-1071
Hacquard S, Spaepen S, Garrido-Oter R *et al.* (2017) *Annu Rev Phytopathol* 55:565-589

C9 Evolution of biotrophy in fungal symbionts: What can the genomes reveal?

Researcher
Ganga Jeena

Project leaders
Alga Zuccaro
Marcel Bucher

Project type
Postdoc project

Project duration
01.05.2015 – 30.11.2017

Cooperation
Gregor Langen (UoC)
Debika Sarkar
Juliana Almario
(University of Tübingen)
Philipp Fesel

Aim of the project
Beneficial plant endophytes have huge potential to shape eco-efficient agriculture. To understand ecological lifestyle adaptations comparative genomics and transcriptomics of ecologically diverse fungal species were performed.

Results
1) De-novo transcriptome and genome assembly of the root associated fungal endophytes: *Sebacina sp., Serendipita herbamans, Chaetospermum artocarpi, Chaetospermum camelliae* were generated for comparative genomics and analysed.
2) 85Mb Genome of the *Arabis alpina* root endophyte (Helotiales) F229 was assembled and annotated. The response of fungal genes *(in planta)* at low Pi was analysed and comparison of CAZymes against 52 fungal species was performed.
3) RNA-Seq data from the pathogen *Bipolaris sorokiniana* (Bs) infected and the endophyte *S. vermifera* (Sv) colonised barley roots were analysed. The data indicate that colonization by the beneficial root symbiont significantly and robustly reduces pathogenic root infection and disease symptoms locally and systemically. The transcriptomic analysis of bipartite and tripartite interactions in soil suggests that different mechanisms are involved in fungal response to other fungi in presence and absence of the plant host.

Publication
Almario J, Jeena G, Wunder J *et al.* (2017) *PNAS* 114(44), E9403-E9412

C10 Functional characterization of candidate effector proteins in the *Sebacinales*

Researcher
Heidi Widmer

Project leaders
Alga Zuccaro
Stanislav Kopriva

Project type
Ph.D. project

Project start
01.01.2015

Aim of the project
Serendipita indica and *S. vermifera* belong to the ecologically widely distributed order Sebacinales. These root endophytes display wide-host spectrum beneficial effects such as growth promotion and increased pathogen resistance. With respect to insights into how sebacinoid fungi establish themselves in metabolically active root cells of different hosts and how the plants are reprogrammed for enhanced performance, we are functionally characterizing fungal effector proteins to find answers to the following questions: Are there functionally conserved effectors between *S. indica* and *S. vermifera*? How do sebacinoid effector proteins mediate symbiosis?

Results
Effector candidates were selected based on mass spectrometry of apoplastic fluid of *S. indica*-colonized barley roots and transcriptomics of *S. indica* or *S. vermifera in planta*. One of these proteins was functionally characterized and proved to be an extracellular endonuclease. Currently, the effect of this pro-

tein on the symbiosis is studied using *S. indica* overexpression and deletion strains as well as *A. thaliana* lines heterologously expressing the endonuclease. Moreover, a *S. indica* protein expression system based on a modular vector with the promoter of FGB1 (see project C12) was established.

Publication
Wawra S, Fesel P, Widmer H *et al.* (2016) *Nat Commun* 7:13188

C11 Elucidation of β-glucan perception in plants

Researcher
Hanna Rövenich

Project leaders
Alga Zuccaro
Laura Rose

Project type
Postdoc project

Project start
07.09.2015

Cooperation
Jane Parker
Jürgen Seibel
(University of Würzburg)

Aim of the project
Chitin and β-glucans are main structural components of fungal cell walls and trigger plant immune responses. While the mechanisms underlying chitin recognition have been well described, little is known about the perception of β-glucans. Here, we aim to identify components of the plant β-glucan perception machinery, and to characterize β-glucan-triggered immunity in both monocots and dicots.

Results
Immune responses to microbial ligands include ROS generation, calcium influx, MAPK activation and changes in gene expression. Confirming earlier results, we find that responses to β-glucans vary greatly between dicots (*A. thaliana* and *N. benthamiana*) and depend on ligand length and structure. Newly established assays to assess β-glucan-triggered immunity in the monocots barley and *B. distachyon* show that both are responsive to linear β-1,3- as well as branched β-1,3/1,6-glucans. Based on these findings, we are now using mutant lines/VIGS to screen β-glucan receptor candidates in the different plant species.

C12 Role of fungal WSC lectin-like proteins in the interaction of endophytic fungi with plant roots

Researcher
Philipp Fesel

Project leaders
Alga Zuccaro
Paul Schulze-Lefert

Project type
Ph.D. project

Project duration
01.09.2014 – 19.12.2017

Aim of the project
Branched β-glucans are crucial components of the fungal cell wall and additionally activate plant defense responses when recognized by so far unknown plant receptors. This project aims to elucidate the role of beta-glucan as a MAMP during the interaction of the beneficial root endophyte *Serendipita indica* and its experimental host plant *Arabidopsis thaliana*.

Results
We could identify the lectin WSC3 which is able to bind branched β-1,3/1,6-glucans leading to reduced exposure and overall increased cell wall stress resistance. Furthermore the β-1,6-glucan specific lectin FGB1 was shown to act as a suppressor of plant defense responses leading to increased root coloniza-

Cooperation
Stanislav Kopriva
Jürgen Seibel
(University of Würzburg)

tion of S. *indica*. The investigation of β-glucan-induced defense responses in *A. thaliana* revealed great natural variation of this trait, which was employed to identify several genetic loci by a genome wide association screen. This all together underlines the importance of β-glucans as cell wall constituents and MAMPs that are guarded in the mutualistic root endophyte S. *indica* by sophisticated mechanisms involving specific fungal proteins.

Publications
Fesel PH, Zuccaro A (2016a) *Fungal Genet Biol* 90:53-60
Fesel PH, Zuccaro A (2016b) *Curr Opin Microbiol* 32:103-112
Wawra S, Fesel P, Widmer H *et al.* (2016) *Nat Commun* 7:13188

C13 Characterization of a leaf-specific *Ustilago maydis* α-L-arabinofuranosidase

Researcher
Elaine Jaeger

Project leaders
Gunther Döhlemann
Eric Kemen

Project type
Ph.D. project

Project start
01.01.2015

Cooperation
Markus Pauly

Aim of the project
Infection of Zea mays by *Ustilago maydis* causes smut disease and provides an important model for biotrophic host-pathogen interactions. *U. maydis* penetrates the maize epidermis and leads to tumor formation in all aerial organs. This project aims for a functional characterization of a leaf-specific *U. maydis* effector, which is predicted to encode an α-L-arabinofuranosidase (Ara1).

Results
Ara1 is required for tumor formation in leaves but not in tassels. Enzyme assays showed that Ara1 is a specific α-L-arabinofuranosidase and by mutational analysis this activity was found to be required for virulence. Moreover, Ara1 contains a carbohydrate-binding domain, whose role for cell-wall binding and virulence is investigated. Infection of different maize lines showed a maize line-specific virulence function of Ara1. However, analysis of cell wall composition in the different maize lines did not reveal significant differences in arabinose contents.

C14 Cysteine proteases and their inhibitors in microbemaize root interactions

Researcher
Jan Schulze Hüynck

Project leaders
Gunther Döhlemann
Marcel Bucher

Project type
Ph.D. project

Aim of the project
Papain-like cysteine proteases (PLCPs) have been identified as pivotal components in plant defense. We propose that the modulation of PLCPs by endophytes and pathogens displays a conserved mechanism during plant-microbe interactions. We aim to identify and characterize small microbial effector molecules secreted by root fungal and bacterial endophytes in maize that modulate, either inhibiting or enhancing, PLCP activity to overcome plant defense responses.

Results
MS analysis identified a new root PLCP in maize, CP1C. Overexpression constructs for transient studies of CP1C have been

Project start
01.05.2016

Cooperation
Stanislav Kopriva

used to characterize its activity. A screen of 96 bacterial endophytes resulted in three candidates showing inhibition of PLCPs and seven showing activation of PLCPs. Putative candidates and a stable bacterial SynCom are tested in root colonization assays using sterile microcosms for maize. Parallel, PLCPs in *Arabidopsis* are tested for modulation by a bacterial Syncom.

C15 Characterization of the molecular mechanisms underpinning fungal beneficial effects in roots

Researcher
Debika Sarkar

Project leaders
Alga Zuccaro
Marcel Bucher

Project type
Ph.D. project

Project start
01.10.2014

Cooperation
Michael Bonkowski
Michelle Watt (FZJ)

Aim of the project
This project aims to understand how host-microbiota interactions in natural soil shape and are shaped by local and systemic responses to beneficial and pathogenic root associated fungi. In order to address this, we have recently established a reductionist approach 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 these interactions.

Results
RNA-seq and qPCR data of genes induced during fungus-fungus interaction suggest that *S. vermifera* hydrolytic enzymes might play a role in antagonism against *B. sorokiniana*. In the tripartite interaction the presence of *S. vermifera* led to a significant decreased colonization of *B. sorokiniana* in natural soil and modified barley transcriptomic response locally as well as systemically to this pathogen. *In planta*, *S. vermifera* induced 33% of its genes encoding CWDEs whereas, *B. sorokiniana* exploited more than 60% of its CWDEs, suggesting an aggressive pathogenic interaction for *B. sorokiniana*. In summary, our data indicates that these two fungi uses different strategies for root colonization and also during fungus-fungus interaction in soil.

C16 Development of a model system for smut fungus – *Brassicaceae* interaction

Researcher
Vera Göhre

Project leaders
Michael Feldbrügge
Laura Rose

Project type
Postdoc project

Aim of the project
We aim at understanding the infection biology of the smut fungus *Thecaphora thlaspeos* infecting *Brassicaceae* to develop a pathosystem with two genetically tractable partners. This will allow engineering of fungal metabolic pathways (RA-D) for the plant benefit, give insight into the immune system of annual vs. perennial plants (RA-A), and provides an additional player that can shape the plant microbiome (E. Kemen).

Results
On the technical side, we have 1. established a transformation protocol for *T. thlaspeos* and generated reporter strains and 2. developed a culture infection protocol, which leads to coloniza-

Project start
01.10.2013

Cooperation
Kaitlyn Courville (HHU)
Kristin Bösch (HHU)
Ronny Kellner
Eric Kemen

tion of the root cortex. In combination, these two methods enable us to genetically engineer the fungus and track is in life-imaging to investigate plant beneficial factors. In parallel, analysis of the natural microbiome revealed that *T. thlaspeos* behaves like an endophyte to the microbial community in that it does not influence its composition. Hence, we can deploy this fungus to bring in novel factors without associated changes in the microbiome.

Publications
Frantzeskakis L, Courville KJ, Pluecker L *et al.* (2017) *Mol Plant Interact* 30(4):271-282
Kellner R, Göhre V (2017) *BIOspektrum* 23(1):498-500

C17 Towards the establishment of synthetic symbiosis studying plant/endophyte interactions

Researcher
Ronny Kellner

Project leaders
Michael Feldbrügge
Eric Kemen

Project type
Postdoc project

Project start
01.01.2016

Cooperation
Vera Göhre
Stanislav Kopriva
Sabine Metzger
(MS Platform, UoC)
Lamprinos Frantzeskakis
(RWTH Aachen)

Aim of the project
We aim to understand how the fungal endophyte *Thecaphora thlaspeos* (Tt) interacts with host microbiota and how this influences host fitness. Using genetic approaches, we want to characterize the molecular basis underlying niche formation by Tt on *Brassicaceae* hosts. Based on its extensive proliferation within the plant we hypothesize massive interference of Tt on host microbiota.

Results
To investigate Tt-microbiota and Tt-host interactions, we first sampled Tt-infected *Arabis hirsuta* in the field in 2 consecutive years and analysed microbial diversity; second, we determined host glucosinolate levels and composition in the presence and absence of Tt. Our microbial profiling revealed no impact of an infection on host microbiota. Further, glucosinolate levels and composition of infected plant are indistinguishable from healthy plants. This suggests Tt to pursue a strategy of stealth biotrophy, which facilitates our long-term goal to establish Tt as a synthetic endophyte to study plant-microbe and microbe-microbe interactions.

Publications
Frantzeskakis L, Courville KJ, Pluecker *et al.* ((2017) *Mol Plant Microbe Interact* 30(4):271-282
Kellner R, Göhre V (2017) *BIOspektrum* 23(1):498-500

C18 Evolutionary Footprints in the genome of *Arabidopsis thaliana*

Researcher
Ovidiu Popa

Aim of the project
The genome of *A. thaliana* ecotypes acquired mutations during the migration process after the last glacial period. Each domiciled ecological niche is described by a combination of abiotic and biotic constraints. The aim of this project is to characterize

Project leader
Oliver Ebenhöh

Project type
Postdoc project

Project start
01.01.2015

Cooperation
Ahmad Mannan
(Imperial College London, UK)

the region of the genome that was affected by the migration process and to define putative links to environmental factors.

Results
In this study we measured the degree of polymorphism between 80 *Arabidopsis thaliana* strains using the Shannon entropy. Our observation revealed a non-random distribution of polymorphisms along particular genomic regions. Here we could identify hotspots of high polymorphisms in genes that are related to several environmental stress response such as interaction with fungi, as well as regions of low variation which points to a general, niche independent response to environmental signals. Our results reveal putative genetic pioneers, which are important for the adaptation of *A. thaliana* to new condition that results from the migration process.

Determination of reciprocal effects of interactions on the production of secondary metabolites in hosts and microbes

C19 Exploring the coordination of indole glucosinolate metabolism and ER body formation in plant fitness

Researcher
Ryohei Thomas Nakano

Project leaders
Paul Schulze-Lefert
Tamara Gigolashvili

Project type
Postdoc project

Project start
01.07.2015

Cooperation
Pawel Bednarek
(Polish Academy of Science, Poland)
Soledad Sacristan
(UPM-INIA, Spain)
Antonio Molina
(UPM-INIA, Spain)
Kei Hiruma
(NAIST, Japan)
Alga Zuccaro
Ruben Garrido-Oter

Aim of the project
The aim of this study is to reveal the impact of indole glucosinolates (IGs) and other secondary metabolism on the plant-associated microbiota.

Results
We have grown *A. thaliana* mutants that are impaired in IG metabolism in three types of natural soils and revealed a slight but significant difference in the taxonomic structure of the root microbiota compared to wild-type plants. In addition, we identified GSTU13 as a key factor in PEN2-mediated IG metabolism for extracellular defense responses against fungal pathogens (Piślewska-Bednarek *et al.* 2017). Together these results point to a fundamental role of IG metabolism in interactions with both pathogenic and commensal microbes.

We also found that root-derived bacterial commensals belonging to the order Rhizobiales, a core lineage of the root microbiota, are able to alter host primary and secondary metabolism via secreted metabolites and promote root growth. Unexpectedly, a subset of these isolates is also capable of suppressing MAMP-triggered immunity, providing strong evidence that the plant immune system exerts direct selection pressure on root commensals of the plant microbiota.

Publications
Hiruma K, Gerlach N, Sacristan S *et al.* (2016) *Cell* 165(2): 464-474

Nakano RT, Piślewska-Bednarek M, Yamada K *et al.* (2017)
Plant J 89(2):204-220
Piślewska-Bednarek M, Nakano RT, Hiruma K *et al.* (2018)
Plant Physiol 176(1):538-551

C20 Influence of the rhizosphere microbiome on shoot metabolism, systemic plant immune responses, and defense priming in *Arabidopsis*

Researcher
Stefan Schuck

Project leader
Jürgen Zeier

Project type
Postdoc project

Project duration
01.04.2016 – 28.02.2017

Aim of the project
This project investigated the impact of rhizobacteria (RB) strains isolated from *Arabidopsis* roots on the plant´s capacity to establish systemic acquired resistance (SAR) against pathogens.

Results
An aeroponic cultivation system to grow *Arabidopsis* plants has been successfully developed in our lab. This cultivation system facilitates the manipulation of RB communities and rhizosphere parameters such as nutrient availability. Similar to soil-grown *Arabidopsis*, plants raised aeroponically were unstressed and naïve, and developed a strong SAR against the bacterial pathogen *Pseudomonas syringae*. Initial experiments were performed to test the effect of 16 RB strains on SAR strength. Given that the root microbiome composition potentially influences plant performance, the effect of the different RB inoculi on plant growth was determined by regular rosette size measurements. Most of the RB strains exhibited plant growth promotion properties and strongly affect SAR capacity.

Construct synthetic symbioses to test hypotheses

C21 Modelling bacterial communities associated to plant roots

Researcher
Antonella Succurro

Project leaders
Stanislav Kopriva, Oliver Ebenhöh

Project type
Postdoc project

Project start
01.09.2016

Cooperation
Paul Schulze Lefert
Richard Jacoby

Aim of the project
The ecosystem composed by a plant and its rhizosphere (the bacterial community associated to the roots) involves a complex network of interactions still not completely understood. With this project we aim at understanding what drives the assembly of microbial communities associated with plant roots under different environmental conditions. Mathematical and computational models can reveal important information on the regulation of metabolism, on signalling pathways and on environmental effects. With strong exchange with experimentalists from RA D, we start by focusing on plant root extracts and exudates and bacteria from the MPI Root collection to identify the relevant metabolic pathways in N utilisation and relate it to root colonization. We implement bioinformatics pipelines to analyse genomic features in relation to metabolic pathways and vice-versa and integrate this added knowledge into stoichiometric models, used to study the metabolic adjustments to different conditions.

The development of algorithms and models that can be easily generalized to different organisms looks forward to the end goal of engineering the microbiome in order to obtain a target plant phenotype.

Results

We are testing genome scale metabolic network models of two MPI Root bacteria strains to reproduce their experimentally measured growth under different media condition reproducing possible root colonization environments. With Richard Jacoby we are planning focused experiments to validate model hypotheses.

Publication

Jacoby R, Peukert M, Succurro A *et al.* (2017) *Front Plant Sci* 8:1617

Summary and Outlook

The proposed research programme in Area C aims at a pragmatic understanding of the plant microbiota by application of systematic reductionist approaches, including the use of tripartite systems (plant/beneficial microbe/pathogenic microbe) and deconstruction and reconstruction of microbial assemblages to test the impact of different microbial microcosms and split root systems on plant fitness parameters such as disease resistance, nutrient acquisition or abiotic stress tolerance under laboratory conditions. Fitness of plants in their natural environment and the yield of crops in agriculture are influenced by plant-associated microbiota and soil type. The composition and activity of plant microbiota are determined by nutritional (mineral nutrients), metabolic (primary and specialized metabolites), and edaphic factors (physical, chemical, and biological properties of soil) interacting with different plant genotypes. The central hypotheses of Area C that evolved from the past research are that (i) metabolic partnership between the plant host and its associated microbes is important for the establishment of inter-organismal nutritional networks; and that (ii) variation in this metabolic connectivity is a major force underlying edaphic adaptation and plant health. The key innovative aspect of this research topic is to integrate analysis of plant nutrition as a combined function of plant roots and leaves, its associated microbial communities, and soil properties.

From our recent discoveries based on working with beneficial and pathogenic microbes of plants, we start to address one of the grand challenges in our field, which is to develop an integrated molecular concept that explains how plants simultaneously manage pathogenic and beneficial interactions to ensure plant survival and maximise plant fitness. The current framework of the innate immune system of plants is conceptually quite mature and can explain the molecular recognition of patho-

genic microorganisms and activation of plant immune responses to limit or terminate pathogen growth. However, it leads to an apparent paradox as it falls short explaining how plants can discriminate pathogenic from beneficial microbes to both eliminate foes and accommodate friends. There is accumulating evidence that at least part of the innate immune system is necessary for the accommodation of beneficial microbes. This calls for a conceptual realignment or even re-definition of evolutionary paths and functions of the innate immune system. In Research Area C, we feel we are on the right track for CEPLAS to make a novel and distinctive contribution to resolve this paradox over the next several years.

Plant metabolism: from biotic challenges to synthetic biology

Coordinator
Markus Pauly

Co-coordinator
Karl-Erich Jaeger

Faculty
Ilka Axmann
Michael Bonkowski
Thomas Drepper
Oliver Ebenhöh
Ulf-Ingo Flügge
Tamara Gigolashvili
Stanislav Kopriva
Lutz Schmitt
Vlada B. Urlacher
Jürgen Zeier

Early Career Researchers
Nikolas Ditz
Henning Frerigmann
Katharina Gräfe
Jennifer Hage-Hülsmann
Richard Jacoby
Sakshi Khosa
Sarah Kranz-Finger
Anita Loeschcke
Manuela Peukert
Kalpana Shanmugarajah
Suraj Sharma

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.

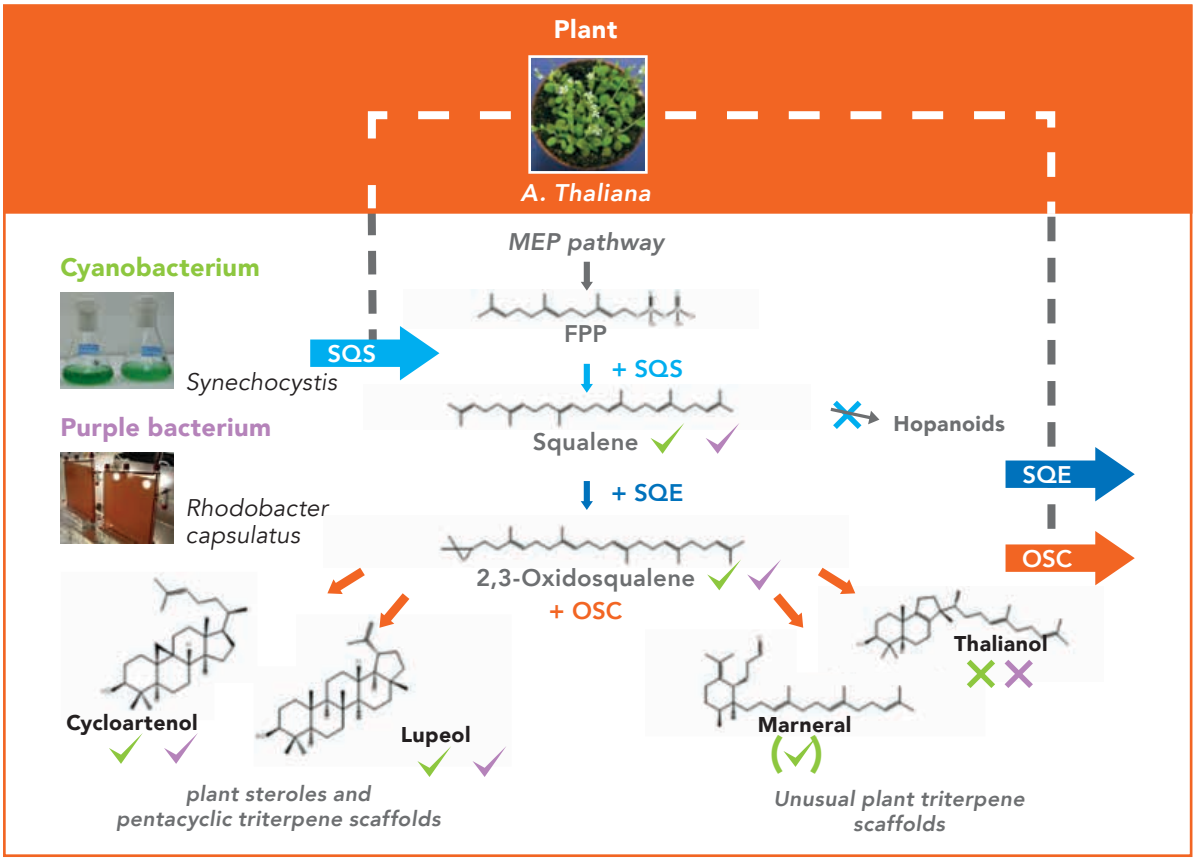
Using *Arabidopsis* accessions and specific microbes plant genes have been identified, which are involved in the ability to shape the microbiome in response to nutrient deficiency. As a result, candidate genes including sulfatases, cytochrome P450s and various transporters are currently further investigated 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.

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 (sequiterpenes, triterpenes and products derived thereof) in orthogonal systems. By introducing plant and myxobacterial genes into the cyanobacteria *Synechocystis* or *Rhodobacter* terpenoids were successfully produced. 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.



Successful production (✓) of triterpenes in cyanobacteria and purple bacteria by introducing plant genes from *A. thaliana*.
In *Synechocystis*, squalene accumulation can be achieved by deletion of the hopanoid biosynthetic pathway. In *Rhodobacter*, this step was newly introduced by expression of squalene synthase SQS. In both hosts, introduction of squalene epoxidase SQE led to the biosynthesis of 2,3-oxidosqualene, which could be further converted to different cyclic triterpenes by additional expression of different enzymes of the oxidosqualene cyclase (OSC) family (Loeschcke A, Dienst D, Wewer W, Hage-Hülsmann J, Dietsch M, Kranz-Finger S, Hüren V, Metzger S, Urlacher VB, Gigolashvili T, Kopriva S, Axmann IM, Drepper T, Jaeger K-E. (2017) The photosynthetic bacteria *Rhodobacter capsulatus* and *Synechocystis* sp. PCC 6803 as new hosts for cyclic plant triterpene biosynthesis. *PLoS ONE*, 12(12): e0189816).

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

D1 Comparative analysis of soil- and root-inhabiting bacterial communities in mutants affected in the biosynthesis and regulation of secondary metabolites: from "metabolic regulons" towards "synthetic plants"

Researcher
Henning Frerigmann

Project leader
Paul Schulze-Lefert

Project type
Postdoc project

Project start
01.08.2016

Cooperation
Stephane Hacquard (MPIPZ)
Pawel Bednarek
(Polish Academy of Sciences, Poland; MPIPZ Cologne)
Tamara Gigolashvili
Stanislav Kopriva
Ute Höcker

Aim of the project
Secondary metabolites are an important feature of plants to communicate with their biotic environment. In *Brassicaceae*, glucosinolates (GLS) define lineage-specific plant secondary compounds and previous work indicated that GLS and their breakdown products might serve as key components in the establishment of stable associations with plant growth-promoting fungi in roots. Therefore, we use GLS and chemically related tryptophan-derived phytoalexins as a model for secondary metabolite networks to probe their potential role in the assembly and function of the root microbiota. This project aims to better understand the role of GLS regulation and their metabolic products in plant-microbe associations. A deeper understanding of the underlying metabolic regulon might serve in future as test case to generate plants with an altered ability to engage in beneficial plant-microbe associations.

Results
We have shown that myrosinase-dependent GLS metabolism products serve also as a signal that impacts transcriptional responses in roots to boost the production of phytoalexins.

Publications
Frerigmann H, Pislewska-Bednarek M, Sanchez-Vallet A (2016) *Molecular Plant* 9(5):682-695.
H. Frerigmann (2016). Advances in Botanical Research: In S. Kopriva (Ed.), Glucosinolates (pp. 57–97).

D2 Mathematical models of glucosinolate metabolism in plants

Researcher
Suraj Sharma

Project leaders
Oliver Ebenhöh
Stanislav Kopriva

Project type
Ph.D. project

Aim of the project
Glucosinolates (GSLs) are sulphur-rich plant's secondary metabolites. Depending on the type of microbes, specific GSLs can act as feeding deterrent or attractants. So, understanding the biosynthesis is key to decipher plant-microbe interaction. We develop mathematical models to explain the factors governing the diversity and biosynthesis of these metabolites.

Results
Our model can realistically simulate different patterns of GSL accumulation and facilitates the identification of regulatory factors that control the biosynthetic flux. Furthermore, by combin-

Project start
01.10.2014

Cooperation
Dan Kliebenstein (UC Davis, USA)

ing our computations with bioinformatics analyses, our model provides a framework to investigate the link between the genotypic and phenotypic characteristics. The knowledge gained from our model serves CEPLAS in i) the targeted design of pathways producing desired GSLs, ii) understanding how genomic differences influence the biosynthetic flux, and iii) how complex traits emerge as a result of evolutionary pressure.

D3 Natural variation in the interaction of plants and bacteria in the rhizosphere

Researcher
Anna Koprivova

Project leader
Stanislav Kopriva

Project type
Postdoc project

Project start
01.11.2013

Cooperation
Stefan Schuck
Oliver Ebenhöh
Karl-Erich Jaeger
Jürgen Zeier
Irene Klinkhammer (UoC)
Achim Schmalenberger (University of Limerick, Ireland)
Lutz Schmitt
Paul Schulze-Lefert

Aim of the project
We used natural variation in *Arabidopsis* accessions and genome-wide association studies to identify genes underlying variation in function of plant microbiome. Thematically, the project is in the centre of RA D. It will identify genes underlying the ability of plants to shape their microflora. The function of such genes, particularly those involved in synthesis of secondary plant metabolites, will be elucidated in cooperation with other projects of RA D.

Results
We analysed 360 *Arabidopsis* accessions and observed at least 5-fold difference in the sulfatase activity in the rhizosphere of individual accessions. The GWAS resulted in 55 candidate genes, which are being analysed in cooperation with other CEPLAS groups. We are currently analysing two cytochrome P-450 isoforms linked to camalexin synthesis and two ABC transporters that affect the response of plants to bacteria.

Publications
Zgadzaj R, Garrido-Oter R, Jensen DB *et al.* (2016) *PNAS* 113(49):E7996-E8005
Jacoby R, Peukert M, Succurro A *et al.* (2017) *Front Plant Sci* 8:1617

D4 Plant secondary metabolites crucial in plant-microbe interactions

Researcher
Manuela Peukert

Project leaders
Stanislav Kopriva
Alga Zuccaro

Project type
Postdoc project

Aim of the project
The interaction between plant roots and soil microbes is regulated by multiple genetic and metabolic factors. Interactions strongly depend on the plant variety and microbial strain and presumably on the exuded metabolic cocktails and the recognition by interaction partners. The aim of the project is to identify (secondary) metabolites that are crucial for the interaction of plants with plant growth promoting microbes. This will be achieved by analysis of root exudates of *Arabidopsis* accessions and mutants under different nutritional conditions and co-cultivation with bacteria. This project is central for RA D and utilises also resources generated within RA C.

Project duration
01.08.2015 – 31.03.2017

Cooperation
Sabine Metzger
(MS Platform, UoC)
Antonella Succurro
Udo Seiffert (IFF Magdeburg)
Michael Kertesz (University of Sydney, Australia)

Results
Experimental set-ups for exudate collection established and tested, showing effects of nutrient on exudate composition. A new pipeline for analysis of the metabolomics data is being tested. Differences in response to co-cultivation with *Pseudomonas* were observed between WT *Arabidopsis* and *tt4* mutant deficient in flavonoids.

Publication
Jacoby R, Peukert M, Succurro A *et al.* (2017) *Front Plant Sci* 8:1617

Signalling molecules and metabolic modules

D5 Dissection of plant-microbe nutrient exchange using metabolomics and proteomics

Researcher
Richard Jacoby

Project leaders
Stanislav Kopriva
Oliver Ebenhöh
Ulf-Ingo Flügge

Project type
Postdoc project

Project start
01.06.2015

Cooperation
Paul Schulze-Lefert

Aim of the project
This project seeks to deepen our understanding of how bacterial metabolism supports plant N & S nutrition from organic molecules. We are conducting proteomic studies of root-associated bacterial strains, to determine which specific metabolic pathways are induced by organic-N nutrition. Also, we are using metabolomics to characterize the plant metabolites that are consumed as growth substrates by root-inhabiting bacterial strains.

Results
We have conducted the first study of MS-exometabolomics applied to rhizospheric microbes using plant root extract as the sole carbon source. This approach defines the metabolic niche of a given strain, which boosts our mechanistic understanding of how diverse microbial strains can coexist in the rhizosphere. Also, we have generated a detailed proteomic dataset of several bacterial strains grown under diverse organic N-sources. This gives new insights into microbial metabolism and its link to plant nutrition.

Publication
Jacoby R, Peukert M, Succurro A *et al.* (2017) *Front Plant Sci* 8:1617

Production of key secondary metabolites in orthogonal systems

D6 Synthetic microbes for the production of plant secondary metabolites

Researcher
Jennifer Hage-Hülsmann

Aim of the project
Synthetic modules are constructed for the production of plant terpenoids in microbes. Also, the interaction of *Burkholderia glumae* with *Arabidopsis thaliana* is studied.

Project leaders
Karl-Erich Jaeger
Thomas Drepper

Project type
Ph.D. project

Project start
01.11.2015

Cooperation
Ilka Axmann
Ulf-Ingo Flügge
Tamara Gigolashvili
Stanislav Kopriva
Sabine Metzger
(MS Platform, UoC)
Lutz Schmitt
Vlada Urlacher
Anita Loeschcke

Results
We have successfully constructed synthetic operons which were transferred and expressed in different bacteria and functionally connected with intrinsic metabolic processes of the microbial hosts. Based on the bacterial carotenoid pathway, the production of 2,3 oxidosqualene was implemented in the photosynthetic bacterium *Rhodobacter capsulatus* and subsequently combined with different cyclisation reactions catalysed by the representative oxidosqualene cyclases CAS1 (cycloartenol synthase), LUP1 (lupeol synthase), THAS1 (thalianol synthase) and MRN1 (marneral synthase) derived from model plant *A. thaliana*. CAS1 catalysed conversion to cycloartenol, expression of LUP1 yielded lupeol and a putative lupeol oxidation product.
B. glumae wild-type significantly reduced the growth of *A. thaliana* which could be correlated with a reduction of the nitrate concentration in the plant shoots.

Publications
Loeschcke A, Dienst D, Wewer V *et al.* (2017) *PlosOne*, 12(12): e0189816
Binder D, Bier C, Grunberger A *et al.* (2016) *ChemBioChem* 17(4):296-299

D7 Functional expression and biochemical characterization of plant P450s

Researchers
Sarah Kranz-Finger

Project leaders
Vlada Urlacher
Karl-Erich Jaeger

Project type
Ph.D. project

Project start
01.04.2013

Cooperation
Ulf-Ingo Flügge
Tamara Gigolashvili
Lutz Schmitt

Aim of the project
Development of synthetic modules for the production of plant triterpenes and their oxygenated derivatives in microbes. Plant cytochrome P450 monooxygenases (CYP) will be produced and characterized. In the context of CEPLAS, the project aims to:
(i) Understand the biosynthesis of triterpenoids.
(ii) Transfer of synthetic modules into plant-associated bacteria (RA C).

Results
1) Molecular tools for the expression of plant CYPs and cyclase genes in *E. coli* were established.
2) CYP71A16 and CYP705A12 from marneral pathway as well as their redox partner ATR 2 from *A. thaliana* were expressed in *E. coli* in functional state.
3) Marnerol was synthesized in *S. cerevisiae*, purified by HPLC and applied for the biochemical characterization of CYP71A16 *in vitro*.
4) Binding studies with CYP71A16 and CYP705A12 were performed for substrate screening.
5) Bacterial CYP102A1 mutants with activity towards α-amyrin, β-amyrin, cycloartenol and lupeol were constrcuted.
6) Oxygenated products of the above mentioned reactions were purified and analysed by NMR.

The project is suspended since 06/2017 due to parental leave.

Publications

Kranz-Finger S, Mahmoud O, Ricklefs E *et al.* (2018) *Biochim Biophys Acta* 1866(1):2-10
Loeschcke A, Dienst D, Wewer V *et al.* (2017) *PlosOne*, 12(12): e0189816

D8 Expression and characterization of P450s and oxidosqualene cyclases involved in plant terpenoid biosynthesis

Researcher
Nikolas Ditz

Project leaders
Vlada Urlacher
Karl-Erich Jaeger

Project type
Ph.D. project

Project start
01.05.2016

Cooperation
Stanislav Kopriva

Aims

Whereas plant mono-, di- and sesquiterpene cyclases have been intensively studied in the recent years, much less information is available about biochemical and biocatalytic properties of plant triterpene cyclases. Thus, one of the aims of the project is cloning, expression and biochemical characterization of selected triterpene cyclases. Furthermore, biochemical studies on plant CYPs involved in triterpene biosynthesis will be continued. In the context of CEPLAS, the project aims to:
(i) Understand the biosynthesis of triterpenoids.
(ii) Creation of triterpenoid producing microbial hosts.

Results

13 *A. thaliana* oxidosqualene cyclases were cloned into *E. coli* (marnerol synthase also in *P. pastoris*);
E. coli expression of CAS1 from *A. thaliana* yielded active protein which produced cycloartenol *in vivo* and *in vitro*;
An *E. coli* host strain capable of the production of 2,3-oxidosqualene starting from glucose was produced and successfully used to produce cycloartenol.

D9 *In vitro* analysis of selected ABC transporters from plants

Researchers
Katharina Gräfe
Kalpana Shanmugarajah

Project leaders
Lutz Schmitt, Andreas Weber
Karl-Erich Jaeger

Project type Ph.D. project

Project start 01.06.2013

Cooperation
Andreas Weber, Jürgen Zeier

Aim of the project

Identification of the substrate spectrum of ABC transporters

Results

We have established heterologous expression and purification protocols for the ABC transporters Pdr2, Pdr8 and ABCG1 (WBC1) from *A. thaliana*. Recently, we focused on ABCG1, because of its basal ATPase activity. Importantly, this activity could be stimulated in the presence of long chain fatty acids and alcohols that present precursors of the cell wall component suberin. This stimulation is specific as long chain fatty acids that are no suberin precursors or substrates of related ABCG1 proteins from different plants do not show this kind of stimulation. Currently, we are generating knock out plants (cooperation with Andreas Weber) for subsequent in vivo analysis (cooperation with Jürgen Zeier).

D10 *In vitro* analysis of selected ABC transporters from plants

Researcher
Sakshi Khosa

Project leaders
Lutz Schmitt
Karl-Erich Jaeger

Project type
Postdoc project

Project duration
01.01.2016 - 31.10.2017

Cooperation
Stanislav Kopriva

Aim of the project
Structural investigation and identification of the substrate spectrum of ABC proteins

Results
We have successfully established the heterologous expression and purification protocol for the ABC protein NAP2 from *A. thaliana*. Initial crystallization trials have been performed, resulting in some initial crystal hits. However, further optimisations of the diffraction quality were not possible. Simultaneously, studies employed to determine the substrate(s) of the ABC protein via mass spectrometry were performed. Here, the tagged wild type protein and the ATPase deficient mutant were used to create an affinity matrix and incubated with the cytosol of the plant roots in the absence or presence of ADP and ATP, respectively. MS analysis identified a β-glucosidase (AAB38783). In parallel, plant studies are performed in the laboratory of Stanislav Kopriva.

Summary and Outlook

The interlinked projects are grouped around the identification and characterisation of plant secondary metabolites involved in plant-microbe interactions. They encompass the characterization of plant secondary metabolites as well as plant signalling molecules, which are decisive for shaping the root microbiome with respect to plant nutrition. The “synthetic” biology approach has been successful in producing triterpenoids in bacteria. This will allow not only to further exploit their bioactive potential but also aid in understanding the interaction on a mechanistic level.

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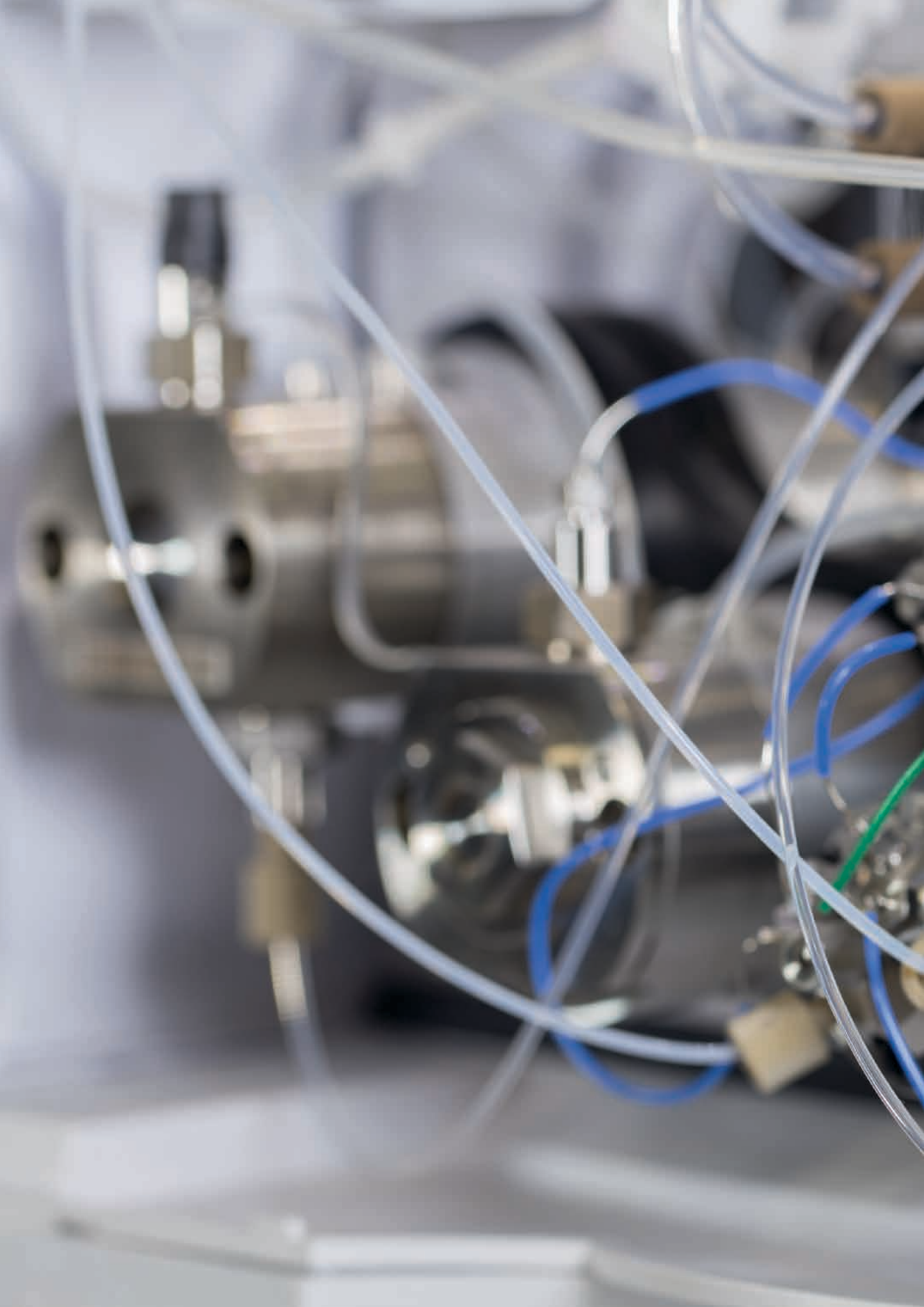

CEPLAS
ANNUAL
REPORT
2017

Plant Metabolism and Metabolomics Platform

The Cluster runs two state-of-the-art analytical laboratories for quantification of metabolites of central and specialized metabolism. Gas and liquid chromatographic separation techniques are coupled to a range of mass spectrometric detections, enabling us to run high-throughput protocols, targeted and untargeted metabolite analyses, perform isotope labelling experiments and identify unknown compounds based on accurate mass determination.

The CEPLAS Plant Metabolism and Metabolomics Laboratories provide expertise and instrumentation for the identification and quantification of metabolites. We measure metabolites for CEPLAS members of all four research areas as well as for external collaborators. The aim of the laboratories is to apply routine methods and establish new methods for the extraction and subsequent analysis of primary and secondary metabolites, mainly via liquid and gas chromatographic separation methods that are hyphenated with mass spectrometry detection.

Metabolites of the central metabolism are measured at the CEPLAS Plant Metabolism and Metabolomics Laboratory at the Heinrich Heine University in Düsseldorf led by Tabea Mettler-Altmann. Metabolites of the specialized metabolism are measured at the CEPLAS Plant Metabolism and Metabolomics Laboratory at the University of Cologne led by Sabine Metzger. Both our laboratories are involved in teaching as well. We contribute to yearly practical courses for undergraduate students within our universities and organise specialized CEPLAS scientific courses for PhD students and post-doctoral fellows. In these courses, the participants not only learn the basics of metabolite extraction, quantification and statistical analysis but get real hands-on experience with our analytical instruments. Also, we contribute to the Bachelor (B.Sc.) Study Programme "Quantitative Biology" in the Module "System Biology". Additionally, we supervise Bachelor, Master and PhD theses and applied successfully for own third party research funding.

The CEPLAS Plant Metabolism and Metabolomics Laboratory at the Heinrich Heine University Düsseldorf

On our GC-qTOF instrument we routinely measure lipids after derivatisation to fatty acid methyl esters (FAMEs) and published an according book chapter that describes the method for absolute quantification of fatty acid residues in *Arabidopsis thaliana* seeds (Hilscher *et al.*, 2017). We also improved this method by combining it with a solid phase separation (SPE) proceeding the derivation step. This allows us to separate three lipid classes (triacylglycerol, glycolipids and phospholipids) and quantifying them separately. On the same instrument, we also routinely measure amino acids, organic acids and sugars in matrices ranging from algal extracts to mammalian tissue. In 2017, this resulted in two publications where sugars in blood plasma from *Canis lupus* was measured to improve the treatment of a haemorrhagic shock (Truse *et al*, 2017; Vollmer *et al.*, 2017). Two more publications applying these two methods studying seed germination in *Arabidopsis thaliana* were submitted already (Chaves *et al.*, submitted, Yazdanpanah *et al.*, submitted).

For absolute quantification of phosphorylated sugars and sugar alcohols, we established an ion-pairing liquid chromatographic separation coupled to a

triple quadrupole mass spectrometric detection in the last years. This year, we extended this method and can now also absolutely quantify nucleotides in green algae with this method. A second LC-based method coupled to diode array detection (DAD) is used for absolute quantification of amino acids and challenging matrices such as cyanobacteria, red algae and *Paulinella chromatophora* (in collaboration with Eva Nowack, Heinrich Heine University Düsseldorf).

Our EA-IRMS instrument is used to quantify total carbon, total nitrogen, the C/N ratio and the carbon isotope ratio ($\delta^{13}C$). In 2017, our long-term collaboration with Andreas Hussner (Heinrich Heine University Düsseldorf) resulted in a publication, which suggests that the composition of dissolved inorganic carbon driven by climate change can interact with environmental stressors such as nitrate pollution and potentially affects growth, biomass allocation and physiology of submerged aquatic plants (Dülger *et al.*, 2017).

Third party funding by the state of North-Rheine Westphalia (BioSC funded project "AlgalFertilizer") enabled a post-doc position in our lab for the last two years to study phosphate metabolism in green algae. In particular, the dynamic of the phosphate storage compound polyphosphate was studied. We showed that two methods, an enzymatic assay and a spectrometric method fit to accurately quantify this polymer in the green algae *Chlorella vulgaris* (Moudřikova *et al.*, 2017). In collaboration with Ladislav Nedbal (Forschungszentrum Jülich), *Chlorella vulgaris* was applied to wheat plants and performed equally successful as a sustainable phosphorus fertilizer compared to fossil phosphorus containing mineral fertilizer widely used in today's agriculture (Schreiber *et al.*, submitted).

The CEPLAS Plant Metabolism and Metabolomics Laboratory at the University to Cologne

The CEPLAS Plant Metabolism and Metabolomics Laboratory at the University of Cologne is focused on measuring secondary metabolites. The platform provides and develops analysis methods for targeted and non-targeted metabolomics. In cooperation with the research groups we establish new methods

for sample collection, extraction, preparation, identification and quantification of secondary metabolites. Methods are optimised and validated together with the researchers based on their biological questions.
Quantification of metabolites is performed with the AB Sciex QTRAP 5500 coupled to an Agilent 1260 HPLC. Using MRM, we routinely quantify more than 20 flavonoid aglycones in parallel in different genera such as *Arabidopsis, Maize, Lotus* and *Petunia*. The high resolution QTOF mass spectrometer Maxis4G (Bruker) is routinely used for metabolic profiling and identification of unknown compounds. Both MS instruments can be equipped with different ion sources to analyse polar (atmospheric pressure ionization, API) and nonpolar (atmospheric pressure chemical ionization, APCI) compounds and to analyse compounds in very low concentration (nano electrospray ionization).
In the last year we extended our spectrum of MS methods. After analysing and quantifying triterpenes in the last years the mono-, di- and sesquiterpenes have come into focus. Here we used the APCI source to detect the nonpolar substances like valencene, rhizathalen, courcumin and caryophyllene.
Additionally we established, based on the high-resolution mass spectrometer, a method to determine the nitrogen incorporation rate into amino acids after labelling with ^{15}N.
In cooperation with AG Zuccaro we developed a method to quantify the phytohormones cis- and trans-zeatin in *Arabidopsis thaliana*. This includes an optimised extraction protocol, followed by an enrichment step with SPE.
The established LC-MS method for glucosinolates was extended also to the desulfo-glucosinolates. Both are measured in the negative mode. The spontaneous fragmentation of desulfo-glucosinolates yields a specific fragment with m/z 195.0327, which can be used to search for unknown glucosinolates.
In cooperation with AG Kemen (University of Cologne) and AG Feldbrügge (Heinrich Heine University Düsseldorf) we used this method for profiling of glucosinolates and desulfo-glucosinolates by LC-MS in *Arabis alpina* and *Arabis hirsuta* shoots and roots. Compared to *Arabidopsis thaliana* the LC-MS analysis of *Arabis alpina* and *Arabis hirsuta* showed very different glucosinolate profiles. We were able to identify two desulfo-glucosinolates which have not been described till now for *Arabis*.

In addition to the development of completely new methods we can currently offer the following established methods to CEPLAS researchers:
- Targeted quantification of metabolites
- Untargeted profiling of plant extracts and exudates
- Labelling studies with non-radioactive isotopes
- Identification of unknown compounds based on accurate mass determination

Routinely analysed metabolites – substance classes:
- Flavonoids, sulfoflavonoids
- Glucosinolates, desulfoglucosinolates
- Terpenes (Tri-, Di-, Sesqui-, Mono-)
- Amino acids

Accepted Publications:

Dülger E, Heidebüchel P, Schumann T, **Mettler-Altmann T,** Hussner A (2017) Interactive effects of nitrate concentrations and carbon dioxide on the stoichiometry, biomass allocation and growth rate of submerged aquatic plants. *Freshwater Biology* 62(6):1094-1104

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; The photosynthetic bacteria Rhodobacter capsulatus and 1 Synechocystis sp. PCC 6803 as new hosts for cyclic plant 2 triterpene biosynthesis. *PlosOne* 12(12): e0189816

Moudřikova S, Sadowsky A, **Metzger S,** Nedbal L, **Mettler-Altmann T,** Mojzeš P (2017) Quantification of Polyphosphate in Microalgae by Raman Microscopy and by a Reference Enzymatic Assay. *Anal Chem* 89(22):12006-12013

Stoffels C, Oumari M, Perrou A, Termath A, Schlundt W, Schmalz HG, Schäfer M, **Wewer V, Metzger S,** Schömig E, Gründemann D (2017) Ergothioneine stands out from hercynine in the reaction with singlet oxygen: resistance to glutathione and TRIS in the generation of specific products indicates high reactivity. *Free Radic Biol Med* 113:385-394

Truse R, Hinterberg J, Schulz J, Herminghaus A, Weber APM, **Mettler-Altmann T,** Bauer I, Picker O, Volmer C (2017) Effect of topical iloprost and nitroglycerin on gastric microcirculation and barrier function during hemorrhagic shock in dogs. *J Vasc Res* 54(2):109-121

Vollmer C, Weber APM, Wallenfang M, Hoffmann T, **Mettler-Altmann T,** Truse R, Bauer I, Picker O, Mathes AM (2017) Melatonin pretreatment improves gastric mucosal blood flow and maintains intestinal barrier function during hemorrhagic shock in dogs. *Microcirculation* 24(4)

Book chapter:

Hielscher B, Charton L, **Mettler-Altmann T,** and Linka N (2017) Analysis of peroxisomal beta-oxidation during storage oil mobilization in *Arabidopsis thaliana* seedlings. *Methods Mol Biol* 1595, 291-304

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Promotion of Early Career Researchers

CEPLAS offers first-class interdisciplinary training programmes for students, doctoral and postdoctoral researchers to educate the next generation of plant scientists and bioinformaticians. Based on the needs of early career researchers and the demand from future employers, we set up adequate comprehensive training activities for different career levels.

Discover plant sciences – research internships for undergraduates

To raise interest in research, especially in plant sciences, at a very early career stage, CEPLAS awards fellowships to undergraduate students in Cologne and Düsseldorf. Each year twelve fellowships are offered to 3rd/4th semester B.Sc. Biology and Biochemistry students. In addition, students with background in mathematics, physics or computer science and interest in plant biology are encouraged as well to join one of the interdisciplinary CEPLAS groups. During their stay in the lab, students work on their own short research projects. After successful completion and a final report, they are awarded a certificate.
Based on our evaluations of the internships, the students highly value to work independently on their own research projects, to get insights into everyday lab work and the work in a research group. The success of the programme is reflected in the fact that many participants continue to perform research in CEPLAS labs, e.g. for their Bachelor thesis.

- Participating student and CEPLAS research groups in 2017
- Farida Bandesha (Weber lab, HHU)
- Malin Hannah Eh (Bucher lab, UoC)
- Sophie Gatzmanga (Pauly lab, HHU)
- Sarah Luise Gerlich (Höcker lab, UoC)
- Maike Hansen (Kopriva lab, UoC)
- Marina Klimke (Rose lab, HHU)
- Anna Tatjana Mänz (Döhlemann lab, UoC)
- Julia Nauen (Zuccaro lab, UoC)
- Celina Michelle Schulze (Urlacher lab, HHU)
- Dominik Steffens (Zurbriggen lab, HHU)
- Larissa Willing (Maurino lab, HHU)
- Lisa Maria Wolpers (Axmann lab, HHU)

Bachelor Programme in Quantitative Biology

The joint study programme of the universities of Cologne and Düsseldorf, that was established in 2015, is attracting more and more students: In fall 2017, sixteen students were admitted to the programme and thus, the third cohort began their studies in the Bachelor Programme Quantitative Biology, with four more students than in 2016. Eleven out of the enrolled students are from Heinrich Heine University Düsseldorf and five are from University of Cologne.
Within the first year of their studies, students are trained in Bioinformatics, Mathematical Modelling, Biostatistics, Physical Biology of the Cell, Systems Biology and Synthetic Biology. To familiarise students with non-biological topics, new methods and approaches, e.g. informatics and mathematical mod-

elling, are taught in the frame of biological research questions. During the second year of the programme, students apply the newly acquired quantitative knowledge to address biological research questions. Thus, the curriculum optimally prepares the students for the current and future needs in life sciences and for the requirements of future employers.
Course evaluations indicate that favourite topics of the students are biological modelling and synthetic biology. Interestingly, the majority of our second cohort of students is part of the current iGEM team Cologne-Düsseldorf called "artico". The iGEM team 2017 aims at creating an artificial compartment toolbox for yeast that revolutionizes synthetic biology and metabolic engineering. Based on our experience, most important requirements for a successful career in Quantitative Biology seem to be good grades especially in mathematics, course motivation and engagement. The first cohort has successfully completed their Bachelor in summer and fall 2017.

"For me, the programme was an essential stepping stone to change into theoretical biology. The multiplicity of acquired methods and the tight proximity to practical research were special and strongly inspired me."
Tim Daniel Rose

"The Bachelor Programme in Quantitative Biology gave me insights into mathematical and computational methods and showed me their necessity for biological research. This has encouraged me to choose a Master in Computational Biology."
Ronja Johnen

Speaker Study Commission Quantitative Biology	Stanislav Kopriva
Coordinator Quantitative Biology	Veiko Krauß
Student representatives in Study Commission	Sandra Heepen, Bastiaan Tjeng
Current number of listed students	29
Proportion of female students	28%

CEPLAS Graduate School

The CEPLAS Graduate School offers a comprehensive and international 3-year structured graduate programme for doctoral students in the field of cutting-edge plant sciences. It is integrated into the highly interdisciplinary and international research network created by the four participating CEPLAS institutions. The graduate programme comprises three elements: (1) the Ph.D. research project, (2) the scientific and (3) the career training programme. The Ph.D. research projects cover a broad spectrum of plant-related topics within all CEPLAS research areas including molecular biology, biochemistry, synthetic biology, genetics, developmental biology, plant-microbe interactions as well as computational and theoretical biology. Regular meetings with the individual thesis advisory committee (TAC) monitor the progress of each Ph.D. research project. Moreover, CEPLAS doctoral researchers are highly encouraged to participate in scientific meetings and conferences to frequently exchange with the scientific community in- and outside of CEPLAS. The comprehensive scientific and career training programmes (see early career researchers' trainings and activities) promote CEPLAS early career researchers to broaden their scientific knowledge and skills as well as to prepare for their future career in academia, industry or other professions.

Development of the programme

The CEPLAS Graduate School has currently 25 members. In addition to the 21 regular doctoral researchers, 4 doctoral researchers are associated members, who participate in the structured graduate programme but are financed by CEPLAS-independent sources. Since start of the Graduate School in 2013, seven CEPLAS Ph.D. students successfully graduated, four in 2017: Ruben Garrido-Oter and Gwendolyn Kirschner graduated at the Heinrich Heine University Düsseldorf, Panpan Jiang as well as Vicky Tilmes at the University of Cologne.

CEPLAS Graduate School Alumni (since 2016)

Dr. Ruben Garrido-Oter

Current position:
Junior Group Leader,
Integrative Bioinformatics, MPIPZ

Dr. Panpan Jiang

Current position:
Postdoctoral research associate,
Dümmen Orange, The Netherlands

Promotion of Early Career Researchers

Dr. Gwendolyn Kirschner

Current position:
Postdoctoral researcher,
Institute of Developmental Genetics, HHU

Dr. Anna Matuszyńska

Current position:
Postdoctoral researcher,
Institute for Quantitative and Theoretical Biology, HHU

Dr. Armin Sadat-Khonsari

Current position:
Postdoc at the University Hospital Cologne,
Department Functional Genomics und Computational Biology

Dr. Elia Stahl

Current Position: Research assistant,
Department of Plant Molecular Biology,
University of Lausanne, Switzerland

Dr. Vicky Tilmes

Current position:
Postdoctoral researcher,
Department of Plant Developmental Biology, MPIPZ

Speaker Graduate School	Ute Höcker
Coordinator Graduate School	Justine Groenewold (since February 2017), Esther Jawurek (until March 2017)
Ph.D. representatives	Meike Hüdig, Michael Thielen (deputy)*
Current number of doctoral researchers	25*
Proportion of female scientists	68%*
Internationality:	32% international students from 3 countries (India, Italy, Switzerland)*
Alumni	7* (since start of programme in 2013)

* Reference date: 31/10/2017

CEPLAS Postdoc Programme

CEPLAS offers a comprehensive scientific and career development programme for postdoctoral researchers. The programme comprises scientific training and interaction, mentoring, coaching and networking with industry. The wide range of advanced training provides support for our postdoctoral fellows to prepare for their next career step in- or outside academia.

Development of the programme

At the end of the funding period in October 2017, the postdoc programme had 21 active programme members: Twelve postdocs funded by CEPLAS and nine associated postdocs funded by other sources. Until October 2017, three postdocs left CEPLAS and one externally funded postdoc joined the Postdoc Programme.

Four of the externally funded postdocs have raised their own funding. Two postdocs that were initially appointed by CEPLAS successfully applied for their own fellowship: Filipa Tomé started her Humboldt Postdoctoral Fellowship in 2017. Richard Jacoby received even two grants at the same time. He was awarded an Alexander von Humboldt and a Marie Skłodowska-Curie Individual Fellowship. Moreover, we are happy that Berkley Walker from the University of Illinois, USA, joined CEPLAS during his Alexander von Humboldt Postdoctoral Fellowship at HHU from 2016 until 2017. Hanna Rövenich, who did her PhD at Wageningen University, Netherlands, joined the CEPLAS Postdoc Programme in 2017 and is currently funded by a UoC Postdoctoral Fellowship.

The bridge funding provided by the DFG until the decision on the CEPLAS renewal, offered the possibility to extend some postdoc projects beyond the official funding end in October 2017. Based on an internal call and a transparent assessment by the CEPLAS Steering Committee, eight postdoc positions could be extended until December 2018.

Currently, the CEPLAS Postdoc Programme has 17 alumni*. The majority of the former CEPLAS members found positions in academia or at non-university research institutions, three of them took up a position in industry (analytical scientific instrumentation; medical communication software; molecular diagnostics).

Speaker Postdoc Programme	Rüdiger Simon
Coordinator Postdoc Programme	Juliane Schmid
Postdoc representatives	Filipa Tomé, Luise Brand (deputy)*
Current number of postdoctoral researchers	21*
Proportion of female scientists	62%
Internationals	62% international postdocs from 10 countries (Australia, China, France, Greece, India, Italy, Japan, Netherlands, Portugal, USA)*
Alumni	17* (since start of the programme in 2013)

*Reference date: 31/10/2017

Early career researchers' trainings and activities

Training programme

CEPLAS aims to offer CEPLAS early career scientists a comprehensive scientific and career training programme in the field of plant sciences to extend and deepen their scientific knowledge and to prepare their future career path. Trainings provided by all four CEPLAS partner institutions are open to CEPLAS doctoral and postdoctoral researchers independent of their institutional allocation. Additionally, CEPLAS organises own workshops and courses tailored to the needs and interests of CEPLAS early career researchers. Consequently, CEPLAS early career scientists have the opportunity to compose their individual training programme depending on their personal needs and interests at a certain time of their career. In 2017 CEPLAS has organised in total 7 internal workshops. In addition, CEPLAS doctoral and postdoctoral researchers participated in multiple courses offered by the partnering institutions (see table below). Furthermore, CEPLAS organised an international Summer School entitled "Emerging Frontiers in Plant Sciences". 47 students from 10 countries participated, among them were 3 CEPLAS doctoral researchers (please see "Outreach activities" for more information). The course programme for 2018 is currently in preparation. The selection of courses that will be organised next year is based on the results of the annual evaluation and polling among the early career scientists.

Workshops attended by early career researchers in 2017

Scientific training:	Career training:
Applied Plant Genetics & Career Development at NPZ Innovation GmbH	
Data Analysis	Be fit for the Future
Gentechnische Arbeiten in gentechnischen Anlagen	Conflict Management
Mass Spectrometry in Protein Analysis	**Design Thinking** (in cooperation with STARTPLATZ Düsseldorf)
Natural Plant Variation	Discussing (in) Science - How to Succeed in (Interdisciplinary) Discussions, Debates and Meetings
Scientific Image Processing and Analysis	Get into Teaching
Stats Literacy	Introduction into Patent Issues
Basic Bioinformatics Training for Biologists	Introduction to Project Management and Working in Teams
Creating Graphs with R (in cooperation with iGRAD-Plant)	Leadership Skills
RNA-Seq Data Analysis	Optimising Strategies for Publishing Research in English
Linux and Perl	Presenting (in) Science - How to Own Stage on (International) Conferences
	Projekte erfolgreich managen
	Proposal Writing
	Success in Companies
	Time and Selfmanagement
	Time Management - Get More Done with Less Effort
	Science - How to succeed in (interdisciplinary) discussions, debates and meetings

In bold: CEPLAS-organised workshops

Networking with industry

CEPLAS Speed Dating

Due to last year's success, we organised a second "Career Speed Dating" in April 2017 at the "Haus der Universität" in Düsseldorf. This year, 12 CEPLAS early career researchers and 13 representatives from different professional fields (e.g. plant breeding and biotechnology industry, life science, funding agencies or patent law) were invited. At the beginning of the meeting all external guests gave short presentations about their individual career path describing the decisions, hurdles and curiosities they encountered during their careers. In the second part of the meeting, CEPLAS early career researchers had the opportunity for short one-to-one-meetings with all guests. During the short, 10-minutes meetings, early career researchers received feedback on their CVs and future career plans. Apart from the valuable feedback, the doctoral and postdoctoral researchers received by the experts, the event was a good opportunity for them to extend their professional network. For the invited guests the event was a good chance to present their companies or institutions as attractive potential future employers.

"It is very useful to spend time to share experiences with early or experienced professionals. There are similar challenges and questions along the road of our career. Plus meeting persons live helps to print out the message and create the connections."

Dr. Lucie Cardon, programme manager breeding at Dümmen Orange, The Netherlands

"Well organised, highly interactive, very open atmosphere: A great way to quickly get to know both early career scientists and peers."

Dr. Remco van Poecke, researcher at Keygene N.V., The Netherlands

Promotion of Early Career Researchers

External guests:

- **Dr. Katrin Beckmann**
  Manager of Pre-Breeding, Phytopathology and Molecular Markers lab at NPZ Innovation GmbH, Germany
- **Dr. Lucie Cardon**
  Program Manager Breeding at Dümmen Orange, The Netherlands
- **Dr. Jon Falk**
  Managing Director at Saaten Union Biotech GmbH, Germany
- **Dr. Suzan Gabriels**
  Scientist Postharvest Technology at Wageningen Food & Biobased Research, The Netherlands
- **Dr. Frederike Horn**
  Spinach Breeder at Nunhems Netherlands BV, The Netherlands
- **Dr. Marieke Louwers**
  Research Manager Systems Biology of Yield at VIB - UGent Center for Plant Systems Biology, Belgium
- **Dr. Andreas Mahn**
  Bioökonomie/FB Ressourcenökonomie at Projektträger Jülich, Germany
- **Dr. Andreas Menze**
  Head of Bioinformatics at KWS SAAT SE, Germany
- **Dr. Aurelie Nowack**
  Business Development Manager at VIB Gent BE, Belgium
- **Dr. Remco van Poecke**
  Researcher at Keygene N.V., The Netherlands
- **Dr. Gerald Schock**
  Associate Director, Global Product Management at QIAGEN GmbH, Germany
- **Dr. Thomas Stratmann**
  Advisor ERC, Marie Skłodowska-Curie Actions (Horizon 2020) at KoWi (European Liaison Office of the German Research Organisation), Germany
- **Dr. David Wilcke**
  Head of Laboratory at Bayer CropScience, Germany

Excursions to industry

In January 2017, CEPLAS early career researchers visited the headquarter of the fourth largest producer of crop seeds worldwide: **KWS SAAT SE in Einbeck, Germany**. The group of 18 early career researchers gained impressive insights into the KWS production facilities and brand new greenhouses and became an idea of the challenges and technical solutions of producing high quality crop seeds. The early career researchers were introduced to different positions within the company and discussed differences between university and company employment conditions. Fruitful discussions took place at the subsequent poster session where CEPLAS early career researchers had the opportunity to present their projects to KWS SAAT scientists. In the evening, the group took the opportunity of a guided city tour to get an insight into the history of Einbeck.

As a second excursion, 18 CEPLAS early career researchers visited the headquarter of the company **WeGrow GmbH in Tönisvorst, Germany** in October 2017**.** WeGrow develops and implements Kiri tree cultivation projects for sustainable timber production. The technical manager and founder of WeGrow welcomed the group and enthusiastically introduced them to the founding history of WeGrow, which is originally a spin-off company from the University of Bonn. In an active discussion, the participants received first-hand information on Kiri tree cultivation, the application of Kiri timber as well as the experience and challenges when founding a company. On a tour through the company, the CEPLAS scientists visited WeGrow laboratories, green houses and Kiri plantations. At the end of the visit, all CEPLAS early career researchers received a small gift: a Kiri plant in a test tube and an instruction for home cultivation!

Early Career Researchers Retreat

In 2017, the CEPLAS Early Career Researchers Retreat was integrated in the annual CEPLAS Symposium, which took place on October 19 and 20 at the Mediapark Cologne. More than 100 CEPLAS scientists as well as members of the Scientific Advisory Board participated in the two-day event.
On the first day, selected early career researchers presented their own as well as other thematically related projects in short elevator pitches to advertise the posters. Within the following poster session, all conference guests were allowed to vote for their three favourite posters. Poster Prizes were awarded to three CEPLAS doctoral researchers: Agatha Walla (No. 1.), Katharina Gräfe (No. 2) and Silke Weckopp (No. 3).
At the joint dinner, doctoral and postdoctoral researchers had the opportunity to network with their colleagues, alumni of the CEPLAS Graduate School and Postdoc Programme, the CEPLAS faculty and the invited speakers.

Support for individual research stays abroad – the CEPLAS mobility fund

CEPLAS provides extra funding for early career researchers who want to gain new insights and extend their competences and scientific network by spending several weeks or months in other labs abroad. In 2017 five motivated early career researchers were supported by the mobility fund and went to different labs in the US and in Japan (see list below).
Furthermore, CEPLAS co-funded the research stay of an incoming international master student from the US, who spent three months at one of the CEPLAS institutions. Moreover, the CEPLAS mobility fund supported the participation of a CEPLAS postdoc at an intensive workshop on RNA-Sequencing in Berlin.

> This allowed me to improve my project by fruitful discussions (…) I learned more about various in silico methods, which were utilized in a wide range of applications.
> **Esther Sundermann, doctoral researcher at HHU**

> We also took the chance to work on one of our current collaborations and discovered novel common interest in phosphate metabolism.
> **Tabea Mettler-Altmann, Head of Plant Metabolism and Metabolomics Laboratory, HHU**

> It was inspiring to integrate in another group and experience slight differences in e.g. handling of lab organisation, way of working and discussing. I extended my scientific network by meeting local and international scientists, strongly fostering my interest in working abroad and engaging with different perspectives.
> **Anika Wiegard, postdoc at HHU**

> I am very thankful for the opportunity to travel to Germany that was provided by the DAAD-Rise programme and the CEPLAS mobility fund. I have been able to expand my scientific network, learn interesting new methods, and meet some amazing new friends.
> **Justin DuRant, master student at the University of South Carolina, USA**

In 2017, the following exchanges were supported:

- **Esther Sundermann,** HHU
  Visit to University of California, San Diego, USA
- **Tabea Mettler-Altmann,** HHU
  Visit to University of California, Los Angeles; Solazyme, San Francisco and California Center for Algae Biotechnology, San Diego, USA
- **Justin DuRant,** University of South Carolina, USA
  Visit to CEPLAS (Albani lab)
- **Anika Wiegard,** HHU
  Visit to Ritsumeikan University, Kustatsu-Shiga, Japan
- **Suraj Sharma,** HHU
  Visit to UC Davis, USA
- **Florian Schwanke,** UoC
  Visit to Nara Institute of Science and Technology (NAIST), Japan

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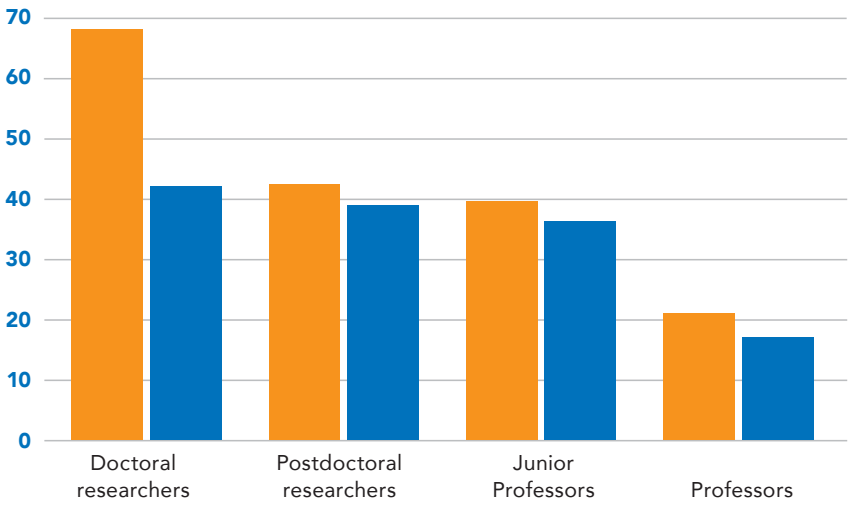
CEPLAS Equal Opportunity Programme

CEPLAS strives to create equal opportunities for all members. Therefore, we have established the CEPLAS Equal Opportunity Programme covering various offers to ensure greater equality and diversity within our research community.

Female scientists in CEPLAS

In CEPLAS the proportion of female (47%) and male (53%) scientists is almost balanced. However, female scientists in advanced positions including the group leader and (junior) professor levels are still underrepresented. Nevertheless, the proportion of CEPLAS female scientists is higher at almost all career stages when compared to average data from other Clusters of Excellence (see figure below).

Female researchers within CEPLAS at different career levels compared to other Clusters of Excellence



*Source: DFG Chancengleichheits-Monitoring 2017

CEPLAS "Women in Plant Sciences" Day

The CEPLAS "Women in Plant Sciences" Day evolved from the initial CEPLAS Women's Career Day into a full-day workshop and information event specially designed for women scientists. After its great success last year, it was provided in a similar format again in 2017, offering several modules taught by professional coaches. The goal of this year´s event was to provide a compact, interactive and women-oriented workshop programme covering the topics self-marketing, negotiations and spontaneous speech. In total 21 female CEPLAS researchers participated in the CEPLAS "Women in Plant Sciences" Day 2017 which took place at the University of Cologne in July. Experienced coaches from different professional disciplines were invited to share their knowledge and experiences including practical exercises about the following topics:

- "Branding for Female CHAMPS"
- "Negotiation for Women – Between Empathy and Assertiveness"
- "Quick on Your Feet! – Spontaneous Speaking Practice for Women Scientists"

The CEPLAS "Women in Plant Sciences" Day also serves as a platform for networking, where participants can discuss with coaches and colleagues. Besides the interesting workshops the informal and relaxed atmosphere during the entire event contributed to the event's success.

Career development workshops

In collaboration with the Equal Opportunity Offices at HHU and UoC, CEPLAS members have access to a plethora of career development workshops specially tailored to female scientists. Additionally, depending on the needs and interests of female CEPLAS researchers, CEPLAS also organises and offers own workshops. In October 2017, the one-day workshop "Authentic Networking for Women in Academia" was offered to female CEPLAS members. The experienced coach and University lecturer for diversity/gender management provided information and advice on authentic networking strategies to the 14 participating female scientists.

Individual coaching

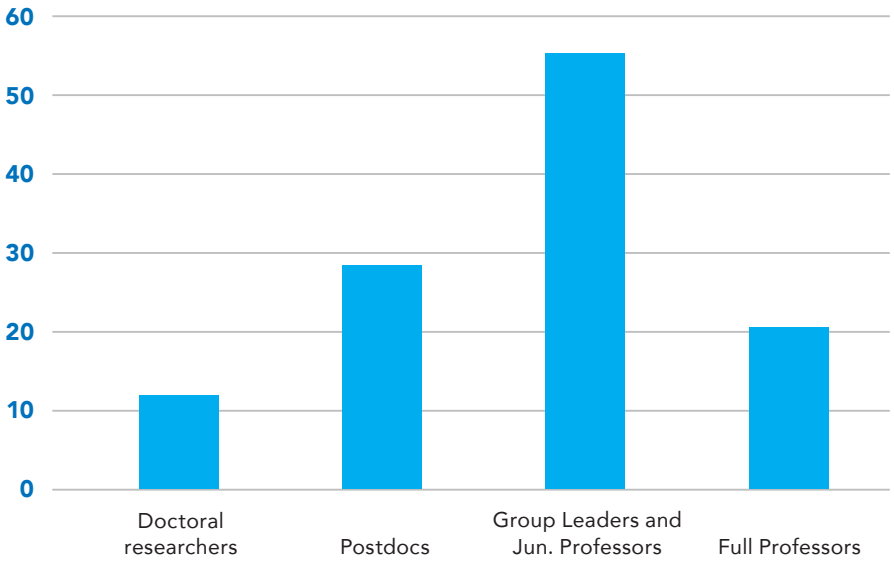
Especially female scientists, striving for an academic career path, are facing many hurdles and challenges, e.g. the acquisition of own funding and the setup of an own research group but also issues related to work/life balance and family planning. Therefore, CEPLAS undertakes great efforts to support female scientists during this challenging phase. We therefore offer individual coachings as a person-targeted career development measure. During one-to-one consultations with an experienced coach CEPLAS female researchers are guided to achieve their professional goals in a self-reflective manner. In 2017, CEPLAS supported individual career coaching for four female CEPLAS members.

Equal Opportunity measures

Helping hands programme

Pursuing a successful research career and managing private life with young children or relatives in need of care comes along with challenges. To help balance family obligations and a career in research CEPLAS successfully runs the "helping hands" programme offered to all CEPLAS members. Within this programme, CEPLAS provides funding for student assistants who support CEPLAS members, performing routine tasks relevant for the research work. Support is granted during pregnancy, for parents with children (up to an age of 10 years) and for scientists with other care responsibilities. In 2017, eight CEPLAS members including doctoral and postdoctoral researchers as well as junior group leaders benefited from the "helping hands" programme.

Percentage of scientists within CEPLAS with children under 10 years



Child-care during CEPLAS events and parents-child rooms

Child-care is provided during all CEPLAS events. It is mostly organised in cooperation with the Family Support Services of the Universities Cologne and Düsseldorf but if needed CEPLAS also hires external child-care services. In 2017, CEPLAS parents requested child-care during CEPLAS Friday seminars and the CEPLAS Symposium/Early Career Researchers Retreat. At the Universities Cologne and Düsseldorf parents also have access to parents-child rooms equipped with child care needs and work places.

CEPLAS Equal Opportunity Programme


SKATE

Outreach Activities

Our outreach activities seek to raise awareness among the general public of the importance of plant science and its contributions to solving major global challenges. In addition, we aim to transport our fascination for plant sciences to general audience.

CEPLAS Homepage and social media activities

User-friendly online access and exchange of information is nowadays essential, as internet resources are becoming the central hub for information. Therefore, we redesigned and relaunched the entire CEPLAS homepage (www.ceplas.eu) to make it a central, more user-friendly source of information, providing the latest news but as well general information for target groups. The main page provides quick information about latest news and upcoming events, and furthermore offers several customer-suited entry opportunities into the different sections of the homepage. We put special effort into a new "Discover" section, providing insights into the CEPLAS research programme in an easily understandable way and promoting several public events on plant science, e.g. free guided tours through the CEPLAS section of the Botanical Gardens.

CEPLAS Homepage

CEPLAS has expanded its social media presence to widen the network within the plant sciences community and to increase the general visibility. Via our Twitter channel (@ceplas_1) we share current activities, news and backgrounds and interact with cluster members and other scientists and research networks. The CEPLAS Facebook page (https://www.facebook.com/plantsoftomorrow), which has close to 1,000 followers, was revised and now keeps followers updated with cluster news, upcoming and past events or new publications. In a more career-related approach, we have created a LinkedIn group to connect our early career researchers within the plant scientists' community and CEPLAS cooperation partners from academia and industry. For our followers on YouTube we provide selected media features on our YouTube channel either produced by the cluster, with contribution from CEPLAS scientists or of direct relevance to CEPLAS science. In addition, we established an online newsletter in late 2017 to keep CEPLAS members, alumni, the Scientific Advisory Board and others updated with current activities.

As before, our early career researchers publish a monthly "Planter's Punch" edition where one special aspect of the CEPLAS research programme is explained in an easy understandable way. The "Planter's Punch" is distributed via all our online channels and, as a new feature, we implemented a subscription tool for the Planter's Punch on our website. Through authoring the "Planter's Punch", our early career researchers build a track-record in science communication and gain experience with communicating science to a general interest audience.

CEPLAS Image Movie

A particular highlight of the CEPLAS outreach activities in 2017 was the production of a short CEPLAS movie. For the recordings, we teamed up with a professional production company, specialized on science communication. The intention behind the movie was to raise the public interest for plant sciences and to demonstrate the importance of plant sciences for coping with today's global challenges, e.g. food security. Furthermore, the movie addresses also other scientists interested in the ongoing CEPLAS research.
The movie introduces all four CEPLAS research areas as well as the CEPLAS early career researcher programmes and the general concept of the cluster. Recordings for the movie took place at all four CEPLAS sites. The movie is available on our homepage, the websites of partner institutions and was distributed via several social media channels.

Lecture series „NutzPflanzen – Pflanzen nutzen" and guided tours in the Botanical Garden

As in previous years, we organised a public lecture series during the summer term in 2017. The newly designed lecture series "Vom Maisanbau der Mayas zu heutigen Superfoods" covered current topics such as the advantages and disadvantages of monocultures compared to mixed crops, the evolution of high performance wheat from its wild ancestors and current trends in human nutrition, such as "super foods". After the talks, the audience was invited for informal discussion where they also had the possibility to sample some of the foods mentioned in the lectures. The lecture series was combined with several guided tours in the Botanical Garden, where Peter Westhoff demonstrated the topics of the lecture series such as the mixed crop system Milpa and the superfoods Chia and Quinoa. In addition, Andreas Weber offered several tours on "Crop plants for the future – energy and food for next generations" during which he introduced different energy plants, e.g. Miscanthus and Silphie within the CEPLAS section in the Botanical Garden.



CEPLAS Symposium 2017

On October 19/20, the CEPLAS Symposium took place as a combined event with the Early Career Researchers Retreat at the Mediapark, Cologne. Same as in the last years, members from the Scientific Advisory Board participated for the annual evaluation of the cluster. On day one, each research area coordinator gave an overview about recent research activities, followed by several elevator pitches from early career researchers to advertise their posters. During the poster session, posters were highly frequented and the best ones were honoured with a poster award. On day two, one specific research project from each research area was highlighted. In addition to the cluster-internal speakers, three key note speakers were invited: Prof. Dr. Stefan Jansson (Umeå University, Sweden), Dr. Isabel Vercauteren (CEO from the start-up Aphea.Bio) and Prof. Dr. Bernd Müller-Röber (University of Potsdam).
Overall, the combined event covered many interesting talks and provided space for scientific exchange and networking. Several CEPLAS alumni joined the CEPLAS Symposium and thereby caught up with former lab mates and friends.

International CEPLAS Summer School 2017 „Emerging Frontiers in Plant Sciences“

During the first CEPLAS Summer School at the Sportschule Hennef, 47 participants from 10 countries, mainly PhD students, had the opportunity to learn about the latest developments in plants science.
Talks were given by CEPLAS investigators and three international guests: Ben Blackman (UC Berkeley, USA), Ian Graham (CNAP in York, UK) and Rob Last (Michigan State University, USA). During the second half of the week, students attended workshops which focused on communicating, networking & career

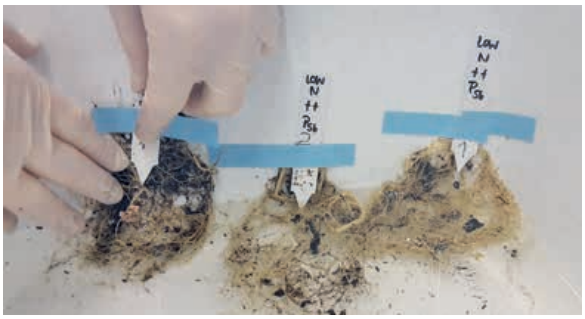
building (led by Mary Williams), research misconduct (Leonid Schneider) or science journalism (Claudia Ruby).
The aim of the CEPLAS Summer School was to provide a platform for PhD students to learn about CEPLAS topics and promote an information exchange between peers and PIs.

Fascination of Plants Day

150 pupils from 3rd and 4th grade from different elementary schools in Düsseldorf visited Heinrich Heine University Düsseldorf on May 18th in frame of this year's international Fascination of Plants Day. The interactive workshop day on the topic "Plants have superpowers" included a short lecture, hands-on experiments and a short tour in the Botanical Garden, all specially designed for kids to stimulate the interest and enthusiasm for plant sciences. At the end of the day the pupils proudly received a certificate about their participation. Furthermore, instructions and seeds for experiments with plants at home were handed out. Due to the very positive feedback, CEPLAS will again participate at the next Fasciation of Plants Day.

Research and Education

The CEPLAS Research and Education programme aims to increase the awareness of pupils and teachers on plant science topics and therefore brings them together with CEPLAS scientists and university students who are in pre-service training to become teachers of biology. Initially, in a three-week laboratory phase, CEPLAS scientists familiarise student teachers with current plant molecular biology research and basic lab work. After the lab phase, the student teachers develop teaching concepts and experimental settings, which are applicable in biology lessons in secondary school. These concepts are then tested in a six-day internship with highly motivated pupils from partnering schools. In 2017, 21 pupils from five partner schools participated in the programme guided by five student teachers. The programme offered a great benefit for the students as they could gather first teaching experience and at the same time train themselves in current research. This year's topics were "Bodenmikroflora - Vielfältigkeit und Einfluss von Protisten auf die pflanzliche Entwicklung (AG Bonkowski)" and "Erforschung von Symbiosen im Wurzelraum bei Erbsenpflanzen mit Schwerpunkt auf a) Mykorrhiza (Pilzsymbiosen) und b) Knöllchensymbiosen (AG Bucher)". Furthermore, in May 2017, four pupils participated in the "Our common future youth congress" in Bremerhaven, organised and hosted by the Bosch foundation. At the congress, they shared their first experience in the frame of the Research and Education programme.



Competence Area „Food Security"

A new Competence Area (CA) on Food Security was established at the end of 2016 as one part of the Institutional Strategy of the University of Cologne. The mission of the CA is to build an international network of university scientists, partners from industry and non-governmental organisations to address all aspects of food security such as plant science, economics, law, politics in an interdisciplinary approach. Furthermore, the Competence Area promotes efficient knowledge transfer between relevant disciplines as well as to the public.

At the kick-off symposium in May, the international speakers Prof. Dr. Daniel Chamovitz from the Manna Center Program for Food Safety & Security at Tel Aviv University and Dr. John Ingram from the ECI Food Programme from the University of Oxford, as well as national speakers from different areas such as plant sciences (Prof. Dr. Andreas Weber, Prof. Dr. Alga Zuccaro) and law Prof. Dr. Stephan Hobe) discussed about the global challenge of food security, which can only be tackled in interdisciplinary, global approaches. During the winter semester, the Competence Area offered a lecture series on "Food and Nutrition Security" in the frame of the "Studium integrale" at University of Cologne with speakers from the Universities Cologne and

Düsseldorf or invited speakers from industry and non-governmental organisations. The lectures covered biological, legal, economical as ethical aspects and addressed students and the interested public.
The activities of the Competence Area are organised by a coordinator as well as several members from the CEPLAS Postdoc Programme.

Political Outreach and selected participation at expert panels

CEPLAS aims to draw the attention of policy makers to the role of plant sciences in Germany, both as a research location and as part of Germany's national strategy in response to the global challenges of food security and sustainability.
To inform politicians about ongoing challenges with regard to plant sciences, especially in Germany, CEPLAS researchers compiled a short political letter in summer 2017 providing information on currently relevant scientific topics but as well appealed to the politicians to focus more on the importance of plant sciences.
In 2017, CEPLAS representatives met several members of the German Parliament in Berlin or were visited by members of the German Parliament.
One example is CEPLAS' participation in the third "sitzungswoche agrar" meeting in summer 2017 in Berlin where representatives from politics, science and industry were discussing how digitalization can lead to a resource efficient and sustainable agriculture and which innovations will form the agriculture of the future.
Invited guests were Andreas Weber, speaker of CEPLAS, the chair of the committee for nutrition and agriculture Alois Gerig (CDU), committee member Harald Ebner (Bündnis90/Die Grünen), Norbert Lemken from Bayer AG and Georg Larscheid from John Deere. In their keynote speeches Andreas Weber, Harald Ebner and Alois Gerig addressed the chances as well as the challenges in the course of digitalization. Andreas Weber focused in his talk on the importance of plant sciences for the agriculture of the future

and showed, on the example of the vegan "Impossible Burger" a perspective, how new innovations could in the future contribute to the protection of resources and climate. Together with representatives from industry the discussion on how digitalization can support resource protection and which framework conditions are required for the implementation, was continued within a panel debate.

Photos: sitzungswoche / Henrik Andree

In October, Andreas Weber, in his capacity as the speaker of CEPLAS, was invited to a panel discussion at Bayer CropScience. At the beginning of the event, the movie "Food Evolution" by Academy Award®-nominated director Scott Hamilton Kennedy was shown which deals with the debate on GMO from various perspectives. At the following panel discussion several experts from Bayer and external experts including the movie director Scott Hamilton Kennedy (via videoconference) discussed with about 150 participants.

Photo: Bayer Crop Science

CEPLAS in the media

- Press release of the Heinrich Heine University Düsseldorf 14.12.2017: Pflanzenforschung verständlich erklärt.
- WILA Arbeitsmarkt 04.12.2017: Wenn das Herz für die Pflanzenforschung schlägt.
- Press release of the University of Cologne 02.11.2017: Neu entdeckter Pilz füttert Pflanze mit Phosphor.
- Focus online 26.10.2017: Humboldt-Forschungsstipendiatin in den Pflanzenwissenschaften Düsseldorf.
- RP online 02.10.2017: Auf dem Uni-Dach keimt Zukunft.
- Press release of the Heinrich Heine University Düsseldorf 29.09.2017: Exzellenzstrategie: HHU mit CEPLAS II im Finale.
- Press release of the University of Cologne 29.09.2017: Uni Köln in starker Position für Exzellenzstrategie.
- DBG news 29.09.2017: DFG wählt zwei pflanzenwissenschaftliche Antragsskizzen für Exzellenz-Endrunde.
- Press release of the Heinrich Heine University Düsseldorf 03.07.2017: Superfoods – was sind und was können Chia, Goji und Co?
- Press release of the Heinrich Heine University Düsseldorf 28.06.2017: Vom Einkorn zum Hochleistungsweizen – die Entstehung und Bedeutung des Weizens für die Menschheit.
- Genius science & dialogue, Newsroom 26.06.2017: Sitzungswoche Agrar – Nachhaltige Landwirtschaft 4.0 – Ideen für Ressourceneffizienz.
- Press release of the Heinrich Heine University 07.06.2017: Vom Maisanbau der Mayas zu heutigen Superfoods.
- Press release of the Heinrich Heine University Düsseldorf 17.05.2017: HHU holt mit Unterstützung der Humboldt-Stiftung renommierten Biologen nach Düsseldorf.
- Deutsche Welle (DW) 16.05.2017: Forscherstars kommen für ihre Karriere nach Deutschland.
- Land NRW 10.05.2017: Alexander von Humboldt-Professuren: Erneut drei Auszeichnungen für Nordrhein-Westfalen.
- Press release of the Heinrich Heine University Düsseldorf 04.05.2017: Struktur einer altertümlichen biologischen Uhr enthüllt.
- Video feature Spektrum.de 27.04.2017: Humboldt-Professor Frommer: SWEET-Proteine lassen Pflanzen besser wachsen.
- Rheinische Post 21.04.2017: Prominente Verstärkung für die Pflanzenforschung an der Heinrich-Heine-Uni und das dortige Exzellenzcluster CEPLAS.
- Press release of the Heinrich Heine University Düsseldorf 03.04.2017: Grundschüler entdecken Pflanzen mit Superkräften an der HHU.
- DBG news 01.04.2017: Alexander von Humboldt-Professor Wolf B. Frommer nimmt Dienst auf.
- Press release of the Heinrich Heine University Düsseldorf 01.04.2017: Alexander von Humboldt-Professor Wolf B. Frommer nimmt Dienst auf.
- Press release of the Forschungszentrum Jülich 01.04.2017: Fünf Millionen Euro für gemeinsamen Antrag von Düsseldorf, Jülich und Köln.
- Video feature University of Cologne 25.01.2017: Universitätspreis 2017 // Forschung // Prof. Dr. Ulf-Ingo Flügge.
- CEPLAS Image Movie, Youtube

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Technology Transfer and Cooperation Management

The aim of our technology transfer activities is to expand our contacts with industry and to establish long-term strategic partnerships to ensure the transfer of CEPLAS research results. In addition, we seek to establish an entrepreneurial mind-set within the CEPLAS community and sensitize for the opportunity of founding a start-up as an alternative career path.

Expanding contacts with industry

In preparation for the renewal of CEPLAS, discussions with companies have been re-directed and intensified; focusing on the establishment of more comprehensive, long-term "Strategic Partnerships". In addition to the classical licensing of IPR-protected CEPLAS research results, our goal is to include in such "Strategic Partnerships" elements of the following cooperation opportunities:

- Establishing and sponsoring of additional Graduate Schools in areas of common interest.
- Funding of Postdoctoral Fellows within the CEPLAS programme in research areas of common interest.
- Sponsoring of Junior Professorships in areas of mutual interest.
- Mentoring of Postdoctoral Fellows working in research areas of interest for partner companies.
- Mutual exchange programmes between CEPLAS scientists and scientists working at companies.
- Invitation of partner company scientists to scientific presentations and seminars of CEPLAS.
- Support/participation of partner companies in the foundation of Start-up businesses, on the basis of CEPLAS research results.

For each of these elements, CEPLAS is open for negotiations on the associated rights of cooperation partners, respecting the regulations of the individual CEPLAS institutions.
Discussions with various potential partners from the private sector on such a "Strategic Partnership" are ongoing. Also contacts with GFPi (R&D section of the German Plant Breeders' Association, BDP), representing nearly all plant breeding companies with R&D activities in Germany have been intensified, in this context.

Compendium on application-relevant aspects within CEPLAS projects

In 2017, the compendium "Excellent science for tomorrow's plants – Opportunities for Public-Private Partnerships", originally published in the beginning of 2016, has been updated, after evaluating the status of each individual CEPLAS project. The compendium presents the progress of research projects in the CEPLAS programme, indicates the potential for translation of results into novel products and processes with economic value and, thereby, should stimulate the interest of companies in discussions about partnerships with CEPLAS. The updated version of the compendium supports discussions with companies on opportunities for cooperation between CEPLAS projects and partners from the private sector.

Technology Transfer and Cooperation Management

Re-organization of "Technology Transfer and Cooperation Management"

Based on our experiences in previous years, we plan to optimise the processes and structures of "Technology Transfer and Cooperation Management" within the next putative CEPLAS funding period. The goal is to especially improve effectiveness and efficiency in scouting for application-relevant aspects in CEPLAS research projects and results, in securing IPR, in mediating and supporting the transition from the research phase of projects to the development of new products and processes (reaching "proof of concept" under conditions close to economic exploitation) and in foundation of "Start-up" companies. For this purpose, a visit at the "Technology Transfer and Cooperation Management" unit of the VIB, Ghent, provided very helpful insights, as a basis for future planning.

Establishing an entrepreneurial mind-set within CEPLAS

The CEPLAS leadership aims at establishing an entrepreneurial mind-set and entrepreneurial skills within the CEPLAS community. In addition, CEPLAS wants to sensitise especially early career researchers to consider the foundation of a start-up company as a realistic career alternative. For this purpose, a first one-day workshop on "start-up culture" was organised together with STARTPLATZ Düsseldorf. Following presentations by successful start-up founders, the focus of the workshop was put on the method of "Design Thinking", a very effective, fast way of collecting ideas and developing first prototypes. Based on the positive feedback, the workshop will likely be organised again in 2018.
Discussions and plannings are ongoing, internally at CEPLAS and with potential partner institutions, to establish structures and elements within the CEPLAS programme that further strengthen and support the thinking and capabilities of CEPLAS scientists in this direction.

Support in career planning of early career researchers

Due to the success of last year's "Career Speed Dating" for young researchers, the event was organised again in spring 2017. Thirteen representatives from companies and other non-academic professional areas participated. The guests presented their own individual career paths and their most decisive decisions during their career. Then, the CEPLAS early career scientists had one-to-one meetings with all guests. During these 10-minute-meetings, they could discuss their CVs, get an impression on professional career opportunities outside academia, and could also obtain first-hand answers on their questions in this context.

In addition, a two-days course on "Applied Plant Genetics and Career Opportunities" was held for young CEPLAS scientists by G. Strittmatter, in May 2017.

Technology Transfer and Cooperation Management

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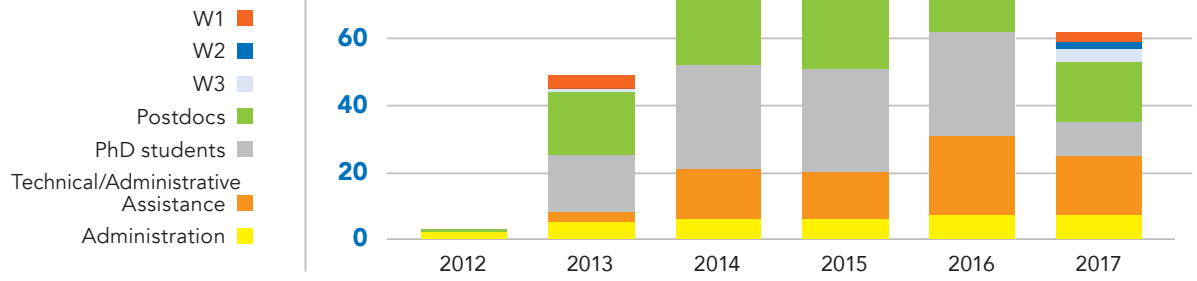
Key Figures

Staff

Internal staffing

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.

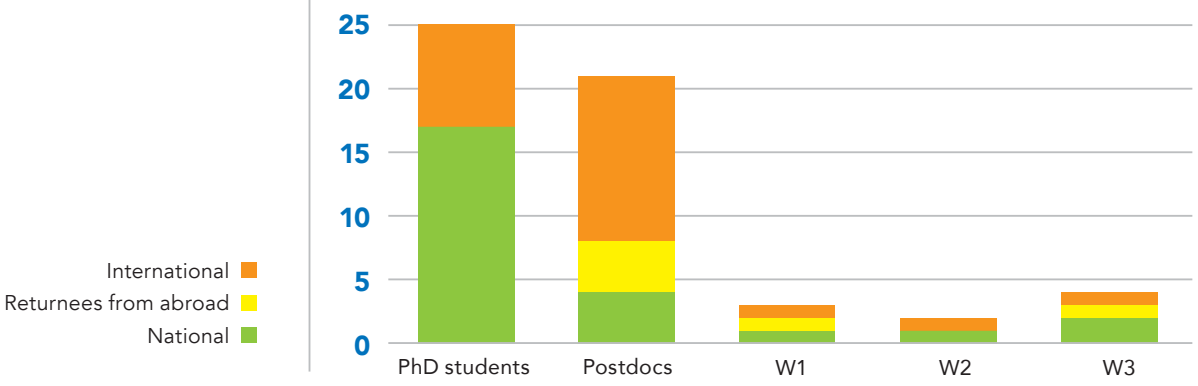
Employment figures from 2012 – 2017 (record date November 1st)



International researchers@CEPLAS

With a highly diverse and international research environment, CEPLAS succeeded to appoint several international researchers and returnees from abroad to the CEPLAS faculty positions. The percentage of international researchers within the early career researchers' programmes remained stable: 32% in the CEPLAS Graduate School and 62% in the CEPLAS Postdoc Programme.

Numbers of international researchers and returnees within the Graduate School and Postdoc Programme and for faculty positions.



New Faculty 2017

Photo: Tsinghua University, Beijing

Prof. Dr. Jijie Chai

Alexander von Humboldt Professorship
Independent Research Group Structural Biology
University of Cologne/Max Planck Institute for Plant Breeding Research
Starting Date: 04/2017

Research focus

The main interests of our lab focus on structural and functional studies of immune-related proteins in animal and plant innate immune systems and plant receptor-like kinases. The innate immune system is the first line of defense against invading micro-organisms. NOD-like receptors (NLRs) are a large family of receptors involved in monitoring the cytoplasmic environment inside cells and initiating immune response to eliminate pathogens. Plant receptor kinases (RKs) are membrane-localized receptors that play vital roles in various biological processes including growth, development and defense. We use X-ray crystallography, cryo-EM, and biochemical techniques to study the autoinhibition, ligand recognition and activation mechanisms of NLRs in immune defense; to elucidate ligand recognition and activation mechanisms of RKs; to discover new ligand-receptor pairs.

Photo: Humboldt-Foundation/Elbmotion

Prof. Dr. Wolf B. Frommer

Alexander von Humboldt Professorship
Institute of Molecular Physiology, Heinrich Heine University Düsseldorf
Starting Date: 04/2017

Research focus

Carbon allocation is critical for crop yield. Our group studies nutrient transport processes in plants, both regarding yield potential and yield when infected by pathogens. We use a wide range of tools to identify the key transporters, for example for carbohydrates in order to study everything from structure to function and regulation. A main tool set includes fluorescent biosensors for a wide range of small molecules, from sugars to hormones. We develop tools for monitoring the activity of proteins in vivo (in particular fluorescent transport activity sensors). We surgically engineer permissive sites in transporters that act as susceptibility factors to create robust broad-spectrum pathogen resistance in crop plants.

Prof. Dr. Gunnar W. Klau

Group Algorithmic Bioinformatics
Heinrich Heine University Düsseldorf
Starting Date: 02/2017

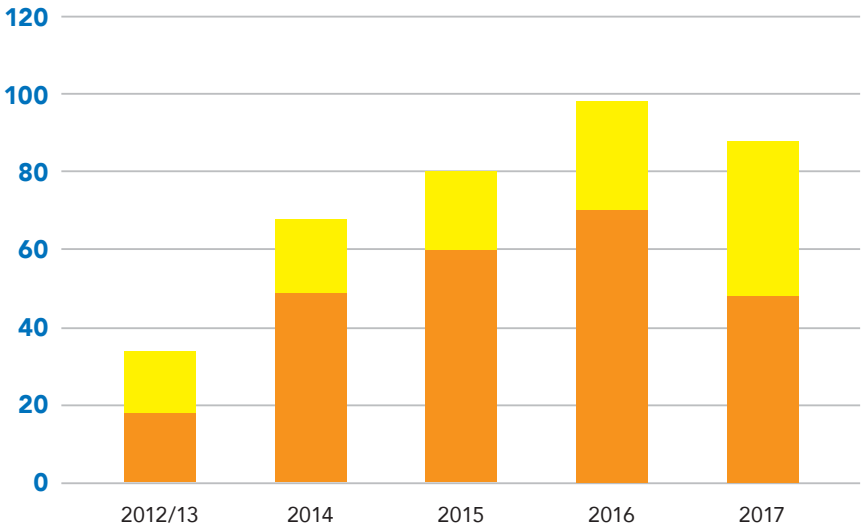
Research focus

We develop data-driven models and algorithmic methods to better understand biological processes. A particular focus lies on network biology, where major steps are to construct high-quality networks, to compare them, to derive network modules from high-throughput data, and to study their properties and their enrichment. Interesting challenges lie in the reconstruction of metagenomic network modules and the integrative analysis of host-microbe interaction networks. Furthermore, we work on new methods for genotype/phenotype studies, for rational growth media design with flux balance analysis models and for reconstructing haplotypes of polyploid organisms.

Publications

Since the beginning of the cluster in 2012, CEPLAS members have published more than 350 publications. The percentage of publications that were achieved with contributions of at least one CEPLAS early career scientist has increased to 45%.

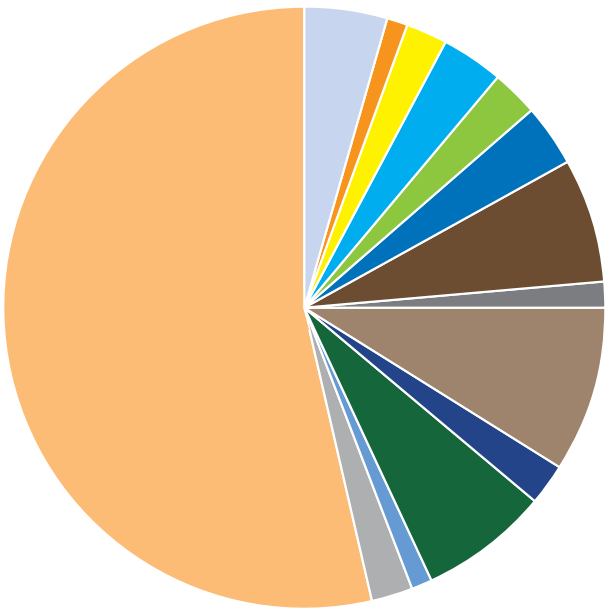
Number of publications and early career researchers' contributions (in yellow) since 2012.



Publication analysis for 2017

In 2017, almost 50% of CEPLAS members' publications were in high-rank journals in plant sciences.

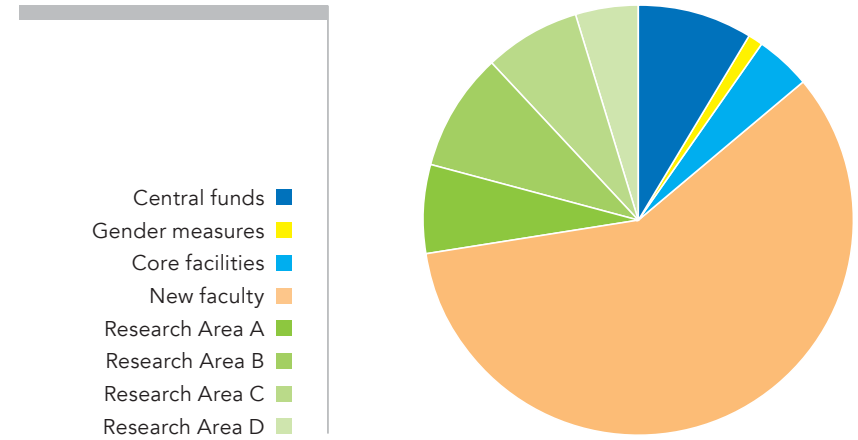
Publication numbers 2017 for the most important journals in plant sciences (total n=88)



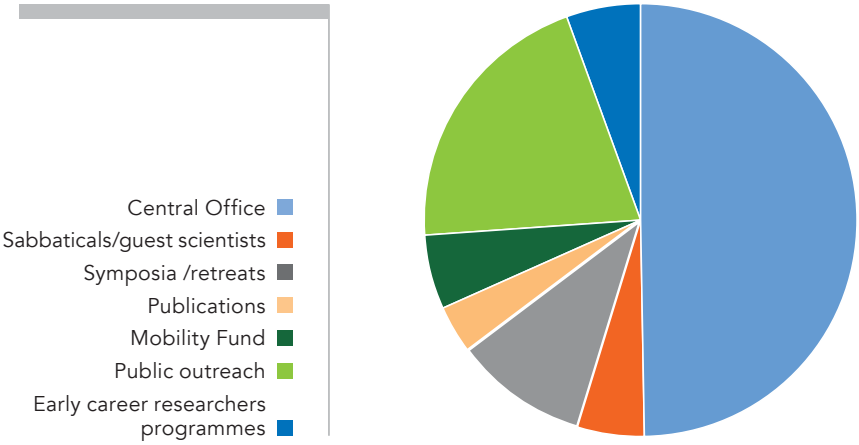
*Nature, Nature Communications, Nature Genetics, Nature Plants, Nature Reviews

Finances

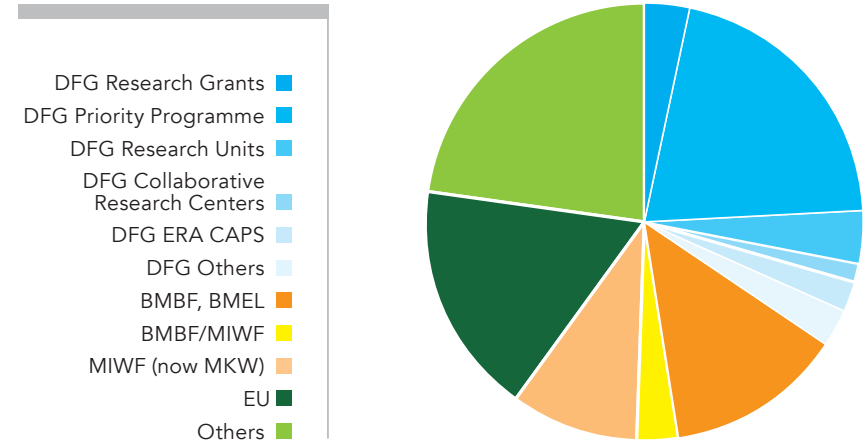
Granted funds and total spending



Overview total spending in 2017 (% of total budget k€ 5,331)



Overview central funds in 2017 (% of central funds budget k€ 444)



Fundraising from additional third-parties (total 29.7 Mio €)

CEPLAS Faculty

Principal Investigators

1	Jun.-Prof. Dr. Maria Albani	Cologne Biocenter, UoC at MPIPZ
2	Jun.-Prof. Dr. Ilka Axmann	Institute of Synthetic Microbiology, HHU
3	Prof. Dr. Marcel Bucher	Cologne Biocenter, UoC
4	Prof. Dr. George Coupland	MPIPZ
5	Prof. Dr. Gunther Döhlemann	Cologne Biocenter, UoC
6	Jun.-Prof. Dr. Oliver Ebenhöh	Institute of Quantitative and Theoretical Biology, HHU
7	Prof. Dr. Michael Feldbrügge	Institute of Microbiology, HHU
8	Prof. Dr. Ulf-Ingo Flügge	Cologne Biocenter, UoC
9	Prof. Dr. Ute Höcker	Cologne Biocenter, UoC
10	Prof. Dr. Martin Hülskamp	Cologne Biocenter, UoC
11	Prof. Dr. Karl-Erich Jaeger	Institute of Molecular Enzyme Technology, HHU at FZJ
12	Prof. Dr. Markus Kollmann	Institute of Mathematical Modelling of Biological Systems, HHU
13	Prof. Dr. Stanislav Kopriva	Cologne Biocenter, UoC
14	Prof. Dr. Martin Lercher	Institute of Informatics, HHU
15	Prof. Dr. Jane Parker	MPIPZ
16	Prof. Dr. Markus Pauly	Institute of Plant Cell Biology and Biotechnology
17	Prof. Dr. Laura Rose	Institute of Population Genetics, HHU
18	Prof. Dr. Lutz Schmitt	Institute of Biochemistry I, HHU
19	Prof. Dr. Paul Schulze-Lefert	MPIPZ
20	Prof. Dr. Ulrich Schurr	Institute of Bio- and Geosciences (IBG-2), FZJ
21	Prof. Dr. Rüdiger Simon	Institute of Developmental Genetics, HHU
22	Prof. Dr. Benjamin Stich	Institute of Quantitative Genetics and Genomics of Plants, HHU at MPIPZ
23	Prof. Dr. Miltos Tsiantis	MPIPZ
24	Prof. Dr. M. von Korff Schmising	Institute for Plant Genetics, HHU at MPIPZ
25	Prof. Dr. Andreas P. M. Weber	Institute of Plant Biochemistry, HHU
26	Prof. Dr. Peter Westhoff	Institute of Molecular and Developmental Biology of Plants, HHU
27	Prof. Dr. Jürgen Zeier	Institute of Molecular Ecophysiology of Plants, HHU
28	Prof. Dr. Alga Zuccaro	Cologne Biocenter, UoC

Associated Investigators

1	Prof. Dr. Petra Bauer	Institute of Botany, HHU
2	Prof. Dr. Michael Bonkowski	Cologne Biocenter, UoC
3	Prof. Dr. Jijie Chai	Cologne Biocenter, UoC / MPIPZ
4	Prof. Dr. Juliette de Meaux	Cologne Biocenter, UoC
5	Dr. Thomas Drepper	Institute of Molecular Enzyme Technology, HHU at FZJ
6	Prof. Dr. Wolf B. Frommer	Institute of Molecular Physiology, HHU / MPIPZ
7	Dr. Tamara Gigolashvili	Cologne Biocenter, UoC
8	Prof. Dr. Georg Groth	Institute of Biochemical Plant Physiology, HHU
9	Dr. Angela Hay	MPIPZ
10	Dr. Eric Kemen	MPIPZ
11	Prof. Dr. Gunnar W. Klau	Institute of Informatics, HHU
12	Prof. Dr. Karl Köhrer	Centre for Biological and Medical Research (BMFZ), HHU
13	PD Dr. Veronica G. Maurino	Institute of Plant Molecular and Developmental Biology, HHU
14	Prof. Dr. Peter Nürnberg	Cologne Center for Genomics (CCG), UoC
15	Prof. Dr. Uwe Rascher	Institute of Bio- and Geosciences (IBG-2), FZJ
16	Dr. Richard Reinhardt	Max Planck Genome Centre, MPIPZ
17	Prof. Dr. Kai Stühler	Centre for Biological and Medical Research (BMFZ), HHU
18	Prof. Dr. Klaus Theres	MPIPZ
19	Prof. Dr. Vlada B. Urlacher	Institute of Biochemistry II, HHU
20	Prof. Dr. Wolfgang Werr	Cologne Biocenter, UoC
21	Prof. Dr. Matias Zurbriggen	Institute of Synthetic Biology, HHU

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Photos
p.8: HHU / Jochen Müller
p.9 and 108 (below): Bayer Crop Science
p.108 (top): sitzungswoche / Henrik Andree
p.76/77/79/80 and 105 (below): Stefan Köhler
p.119: (top) Tsinghua University, Beijing
p.119: (centre) Humboldt-Foundation/Elbmotion
All other pictures: CEPLAS

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