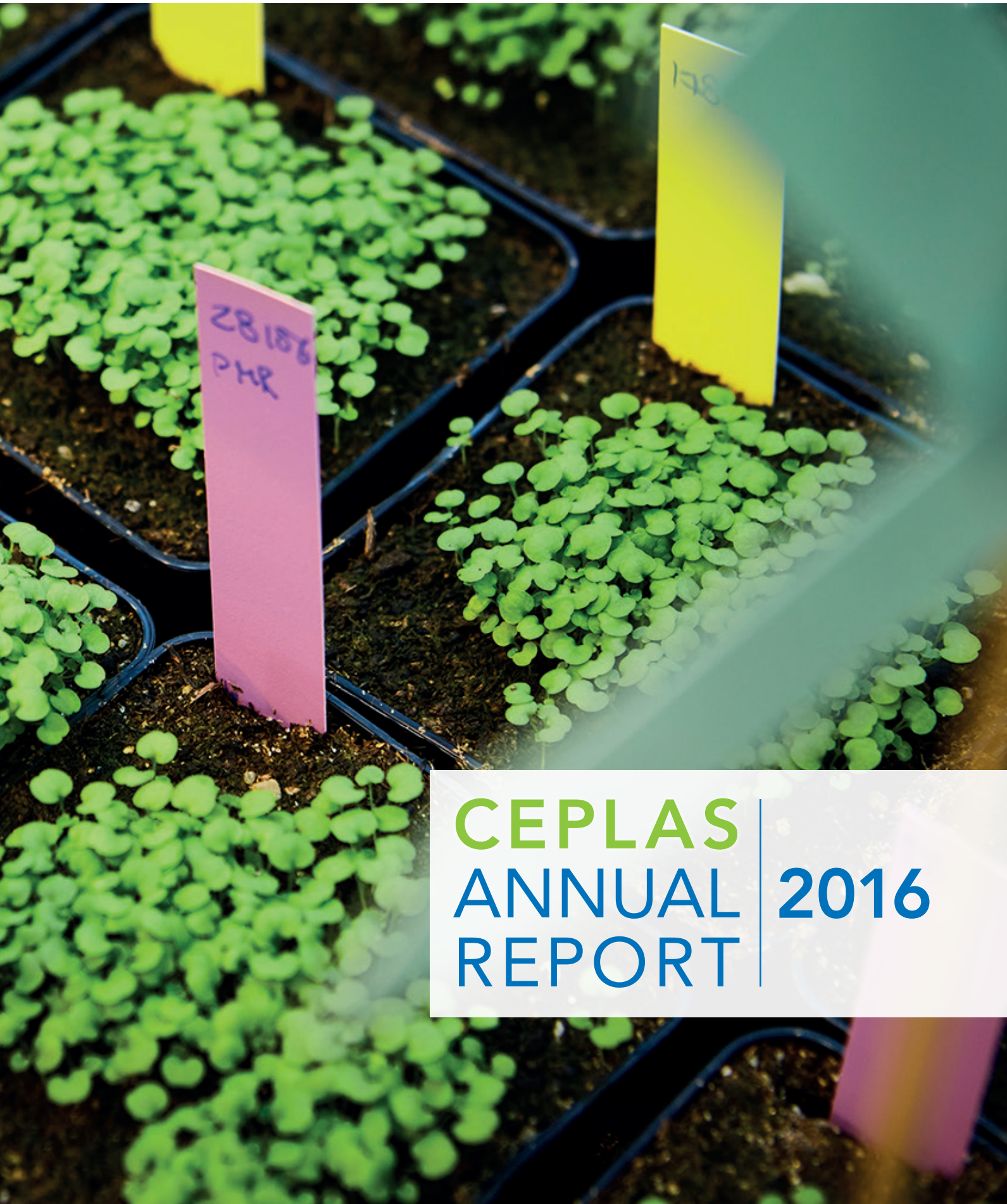
CEPLAS
Cluster of Excellence on Plant Sciences



CEPLAS ANNUAL REPORT 2016

Imprint

Published by
CEPLAS – Cluster of Excellence on Plant Sciences
Heinrich Heine University Düsseldorf
40225 Düsseldorf

Photos
p.76/77/79/85 Steffen Köhler
All other pictures: CEPLAS

Layout
Annegret Koerdt

CEPLAS
Cluster of Excellence on Plant Sciences

CEPLAS ANNUAL REPORT 2016

CEPLAS
ANNUAL
REPORT
2016

Content

CEPLAS
ANNUAL
REPORT
2016

General Presentation

CEPLAS at a glance

The global demand for plant products is increasing with unprecedented pace and it has been estimated that agricultural yields will have to double by the year 2050. However, global change, in particular altered precipitation and temperature patterns, is challenging the sustained production of crops and thus the agronomic base of human civilisation. Simultaneously, arable land is becoming scarce due to increased erosion and population pressure. Meeting the continuously increasing demand will require innovative strategies for crop improvement that aim at enhancing yield, without compromising increases in the use of water, nutrients, and soil, or diminished resistance to 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 completion 11/2017), 34 Mio. € financial volume

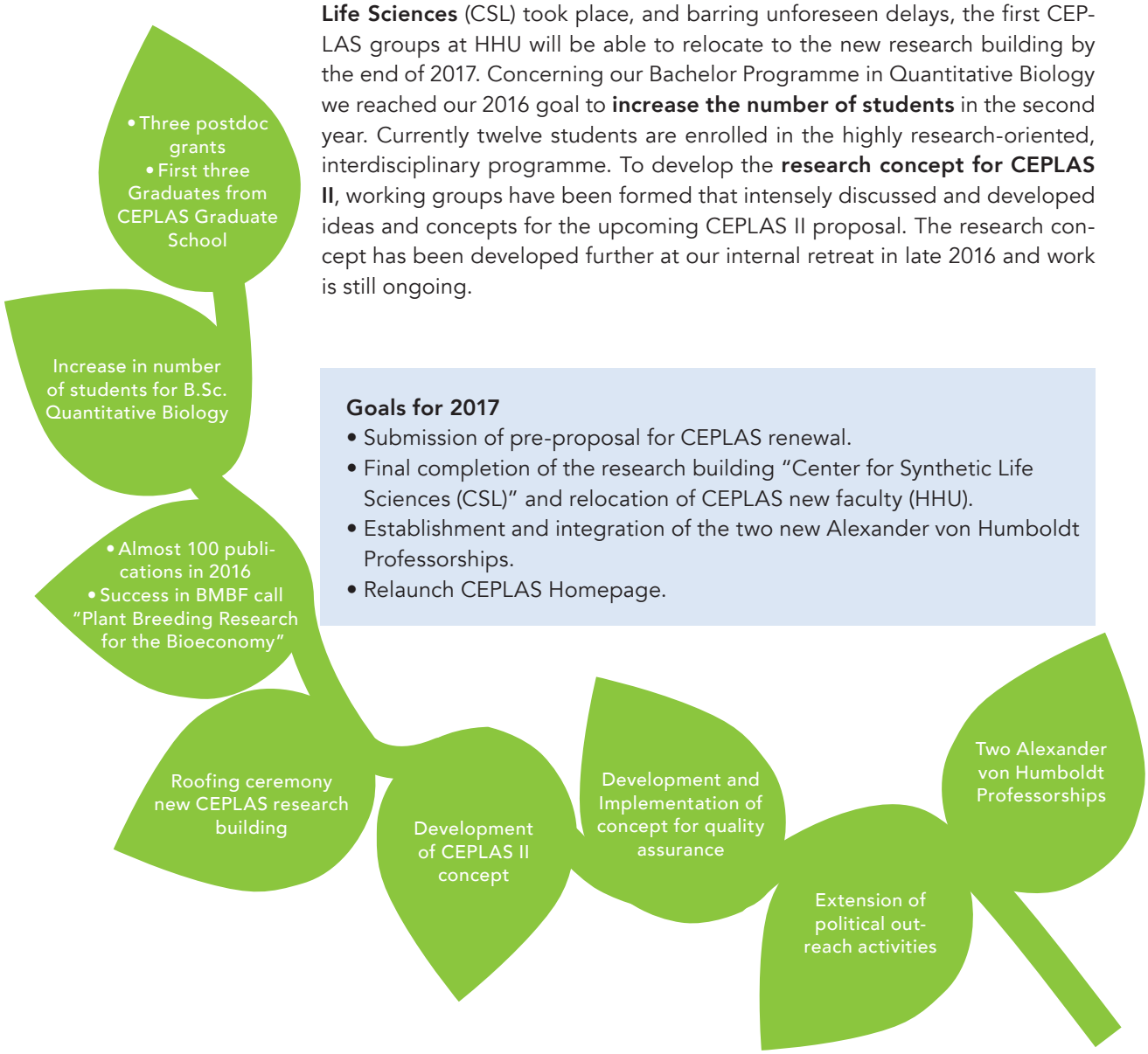
Training & Career Development
- CEPLAS@School
- Research internships for undergraduates
- Bachelor in Quantitative Biology
- CEPLAS Graduate School
- CEPLAS Postdoc Programme

Achievements

In a joint effort, HHU, UoC, MPIPZ and FZJ succeeded in securing **two Alexander von Humboldt Professorships** and appointing two highly renowned experts in the field of plant sciences and structural biology: Wolf B. Frommer at HHU and Jijie Chai at UoC. Both professors will start establishing their groups beginning of 2017. We are excited to report that three of our early career researchers succeeded in the competing for highly **renowned research grants**: one VENI grant from The Netherlands Organisation for Scientific Research (NWO), one Marie Skłodowska-Curie Individual Fellowship and one **Alexander von Humboldt Research Fellowship.**

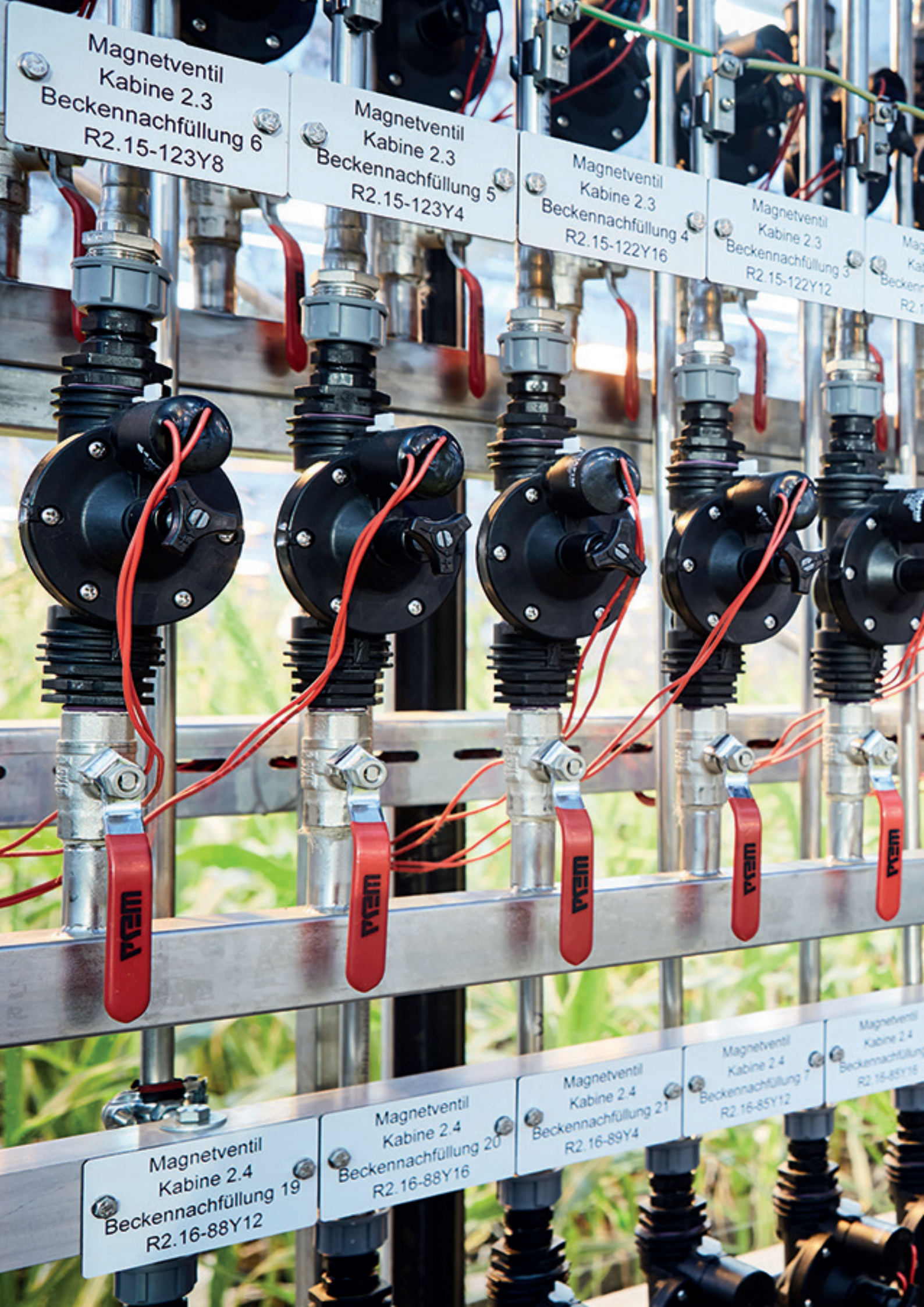
In addition, this year the first three doctoral researchers graduated in the CEPLAS Graduate School. A major achievement in 2016 and evidence of scientific quality was the success of CEPLAS scientists in the highly competitive **BMBF call "Plant Breeding Research for the Bioeconomy"**. Out of a total number of 13 funded projects, 6 are coordinated by or with participation from CEPLAS investigators. In addition, almost 100 peer-reviewed articles have been published in 2016 by the CEPLAS groups, numerous in high rank journals such as Cell, Nature Plants or PNAS. Around 25 % of these publications are with participation of our early career researchers.

In the first half of 2016 we developed a new **concept for evaluation and quality assurance** for the Graduate School and Postdoc Programme and the Cluster as a whole. As a result, we conducted a large survey, which will now be implemented as an annual evaluation. Based on the survey's results, we could implement modifications that serve to improve communication between the Research Areas and identification with the Cluster. Moreover, we extended again our **political outreach activities** and were happy to see that our efforts in this issue are bearing fruits. CEPLAS researchers have been invited for expert discussions and a member of the German parliament will visit CEPLAS in early 2017. In October, the roofing ceremony for the **Center for Synthetic Life Sciences** (CSL) took place, and barring unforeseen delays, the first CEPLAS groups at HHU will be able to relocate to the new research building by the end of 2017. Concerning our Bachelor Programme in Quantitative Biology we reached our 2016 goal to **increase the number of students** in the second year. Currently twelve students are enrolled in the highly research-oriented, interdisciplinary programme. To develop the **research concept for CEPLAS II**, working groups have been formed that intensely discussed and developed ideas and concepts for the upcoming CEPLAS II proposal. The research concept has been developed further at our internal retreat in late 2016 and work is still ongoing.



Goals for 2017

- Submission of pre-proposal for CEPLAS renewal.
- Final completion of the research building "Center for Synthetic Life Sciences (CSL)" and relocation of CEPLAS new faculty (HHU).
- Establishment and integration of the two new Alexander von Humboldt Professorships.
- Relaunch CEPLAS Homepage.


Magnetventil
Kabine 2.3
Beckennachfüllung 6
R2.15-123Y8
Magnetventil
Kabine 2.3
Beckennachfüllung 5
R2.15-123Y4
Magnetventil
Kabine 2.3
Beckennachfüllung 4
R2.15-122Y16
Magnetventil
Kabine 2.3
Beckennachfüllung 3
R2.15-122Y12
Magnetventil
Kabine 2.4
Beckennachfüllung 19
R2.16-88Y12
Magnetventil
Kabine 2.4
Beckennachfüllung 20
R2.16-88Y16
Magnetventil
Kabine 2.4
Beckennachfüllung 21
R2.16-89Y4
Magnetventil
Kabine 2.4
Beckennachfüllung 7
R2.16-85Y12

Organisation

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 one representative of the early career researchers. 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, which meet on an annual basis. The SAB receives and reviews the annual report of the cluster and provides an assessment to the **Supervisory Board**. Moreover, 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 **CEPLAS Training Committee** is responsible for the association of externally funded early career researchers to the CEPLAS programmes, quality control, evaluation of courses, overseeing the recruitment procedures of early career researchers and the general management of the training programmes. Members of the Training Committee are the speakers and coordinators 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 the helping hands programme, career support for female scientists or family support. The Equal Opportunity Board is composed of a representative of the junior faculty group, a female professor and a male professor as well as the coordinator of the Equal Opportunity Office

Organisation

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 coordinated 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

Equal Opportunity Board

Management and Administration

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

CEPLAS
ANNUAL
REPORT
2016

Research

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

Coordinator:
George Coupland

Co-coordinator:
Rüdiger Simon

Members:
Faculty:
Maria Albani
Petra Bauer
Angela Hay
Ute Höcker
Markus Kollmann
Maria von Korff Schmising
Karl Köhrer
Juliette de Meaux
Peter Nürnberg
Richard Reinhardt
Laura Rose
Ulrich Schurr
Rüdiger Simon
Benjamin Stich
Klaus Theres
Miltos Tsiantis
Wolfgang Werr

Early career researchers:
Christos Bazakos
Luise Brand
Wilma van Esse
Panpan Jiang

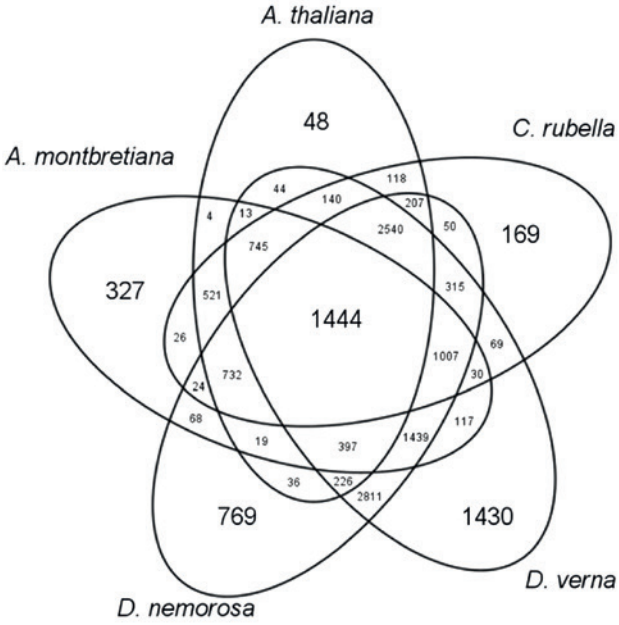
Plant life history varies widely, even among closely related species. In this Research Area we compare annual and perennial species and aim to explain how key traits diversify during the divergence of these life histories. Annuals evolve from perennial progenitors in response to environmental selective 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 this year are mentioned. Genomes of two further annual species (*Arabis iberica and Arabis auriculata*) were obtained using Pacific Biosystems sequencing methodologies, and the resulting high quality assemblies allow us to compare two independent occurrences of annual life history closely related to our perennial model species *Arabis alpina*. By exploiting the population generated by crossing annual *A. montbretiana* and perennial *A. alpina*, we were able to show that introgression of a single chromosomal region of *A. montbretiana* into *A. alpina* was sufficient to generate plants exhibiting a combination of flowering traits, vernalisation requirement and perpetual flowering, not found in either parent. Similarly, 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

Nozomi Kawamoto
Armin Sadat Khonsari
Gwendolyn Kirschner
Priyanka Mishra
Evelyn Obeng-Hinneh
Udhaya Ponraj
Anna Sergeeva
Vicky Tilmes
Filipa Tomé
Alice Vayssières
Agatha Walla
Yanhao Zhou

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.



Venn diagram of gene families in which at least one or more annual species is missing. Each oval corresponds to an annual species and states the number of gene families that do not contain a gene from that species, e.g. there are 48 families that are missing *A. thaliana* genes only and 118 families that are missing *A. thaliana* and *C. rubella* genes (Heidel AJ, Kiefer C, Coupland G, Rose LE (2016) Pinpointing genes underlying annual/perennial transitions with comparative genomics. *BMC Genomics* 17(1):921).

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.

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 – 31.10.2016

Cooperation:
Julieta Mateos
(Fundación Instituto Leloir, Buenos Aires, Argentinia)
Eva Willing (MPIPZ)

Aim of the project:
To use ChIPseq and RNAseq to identify the target genes of the PEP1 transcription factor in perennial *Arabis alpina* and to compare these with the targets of the orthologous protein FLC in annual *Arabidopsis thaliana.*

Results:
PEP1 and FLC are orthologous floral repressors in *A. alpina* and *A. thaliana*, respectively. Their differential expression contributes to the different life histories of the species. Here we compared their target genes. Relatively low proportions, about 15%, were conserved, but this group contained most genetically important flowering targets, including SOC1, SPL15 and SEP3. Among the non-conserved targets, genes involved in GA metabolism and cold responses were present in both species, but were different genes in each case. We demonstrated that in *A. alpina* PEP1 has a role to repress cold-induced genes within the first few hours of cold exposure, and this seems to be important for growth when the plant is exposed to longer durations of cold.

Publication:
Mateos, J, Tilmes, V. *et al.* (2017) Divergence of regulatory networks governed by the orthologous transcription factors FLC and PEP1 in Brassicaceae species (*in revision*).

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

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

(Plant Metabolism and Metabolomics Laboratory, HHU)
Klaus Theres

for metabolomic activities and storage compound production. Methods for anatomical and metabolic measurements have been established.

A3 Molecular characterisation 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

Aim of the project:
This project aims to study similarities and differences in the control of senescence between annual and perennial *Brassicaceae* species.

Results:
A time course of stem sections was harvested from flowering until senescence. This was performed at one internode in the inflorescence that will undergo senescence in *A. alpina*, and at another in the basal shoot that will survive when the inflorescence senesces. Similar samples were harvested from annual *A. thaliana*, where both internodes undergo senescence. RNAseq was performed in triplicate for these two internodes in both species at several time points. Analysis of these data identified *A. alpina* orthologous of known regulators of senescence in *A. thaliana* that are induced specifically in the inflorescence internode that undergoes senescence. Furthermore, sets of genes characteristic of senescence were differently regulated in the basal internode of *A. thaliana* compared to *A. alpina*. These data provide a starting point to study the developmental control of senescence in perennial *A. alpina*.

A4 Photoperiodic control of flowering time in *Arabis alpina*

Researcher:
Panpan Jiang

Project leaders:
Ute Höcker, Maria Albani

Project type:
Ph.D. project

Project start:
01.10.2013

Cooperation:
George Coupland

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.
- 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.

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*.

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

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 identified that

a) Auxin spray induces adventitious roots in a concentration and genotype dependent manner
b) Adventitious root primordia are formed from phloem/phellogen cells
c) Flowering plants show spatial patterns of adventitious rooting

The presence of adventitious roots in certain internodes correlated with the expression of genes known to be involved in the formation of adventitious roots in A. thaliana etiolated hypocotyls. We are currently comparing the transcriptome of internodes that preferentially develop adventitious roots after auxin spray and internodes that do not. Understanding the molecular mechanisms of adventitious rooting will facilitate efficient clonal propagation practices in horticultural and forest species and give insights how to deal with side effects of clonal growth by developing perennial crops.

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 exploit the developmental nature and origin for root sucker formation in *C. resedifolia* and to study the genetic basis for this phenomenon.

Results:
Among several species examined, *C. resedifolia* was chosen for further investigation because it is a diploid self-compatible plant that can be cultivated readily. Phenotypic analyses indicated that C. *resedifolia* propagates vegetatively by forming shoots from roots. To understand the developmental nature and origin of root suckers we use microscopy and *in-situ* hybridisation approaches. To study the genetic basis for this phenomenon we are following two complementary approaches: mutant isolation and QTL analyses. For the first approach a population of EMS mutagenized M2 seeds is screened for relevant phenotypes. For the second we have crossed two divergent strains of the species and F2 generation was phenotyped and genotyped for QTL mapping. In parallel the genome sequencing of *C. resedifolia* is almost completed using Illumina Hi-Seq and PacBio sequencing technologies and will now be assembled. Transcriptome and methylome sequencing is ongoing.

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

Researcher:
Alice Vayssières

Project leaders:
Maria Albani, Wolfgang Werr

Project type:
Associated Postdoc project

Project start:
01.08.2015

Cooperation:
Karin Ljung (UPSC, 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 establishment of dormant buds in perennials

Results:
We characterised 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. Contrary to A. thaliana, we showed that the outgrowth of initiated axillary buds in A. alpina is inhibited until plants experience warm temperatures. This growth habit resulted in spatiotemporal changes of IAA levels along the stem. IAA levels in the dormant bud zone transiently increase after flowering suggesting their growth might be inhibited by other parts of the plant. We are currently developing the molecular tools (e.g. DR5:GUS and DII:VENUS) to

describe at the cellular level the dynamics of plant architecture and vascular connectivity in A. alpina. Understanding of plant architecture will contribute optimizing 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:
CEPLAS funded via AG Albani**
Project also associated and co-funded by SPP1530

Project start:
01.08.2015

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 characterised the length of vernalisation required to ensure complete inflorescence development (with maximal amount of seeds) and outgrowth in *A. alpina*. We also performed an enhancer screen of the *perpetual flowering 1 (pep1)* mutant, which does not require vernalisation to flower. This screen resulted in the identification of several mutants showing early flowering and reduced number of inflorescence branches. In this study we focused in the characterisation of three mutants. Using mapping by sequencing we mapped the mutations responsible for the mutant phenotypes into the same chromosomal region. The candidate region was introgressed into the wild type *A. alpina* background with an active *PEP1 allele*. Introgression lines required shorter vernalisation for complete flowering response suggesting that the identified region contains the candidate gene(s) for the mutant phenotypes. We are currently characterising the identified candidates. Understanding the molecular mechanisms regulating inflorescence development will facilitate increased yield.

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

Researcher:
Yanhao Zhou

Project leader:
Maria Albani

Project type:
CEPLAS funded via AG Albani**
Project also associated and co-funded by IMPRS

Project start:
01.10.2015

Aim of the project:
Similar to project A9 - the aim of this project is to identify components that regulate inflorescence development and outgrowth

Results:
Using mapping by sequencing we mapped two mutants showing reduced inflorescence branching in the *pep1-1* background. Mutations were mapped in different regions than in project A8 suggesting that these mutants carry lesions in different genes. To identify the genes responsible, we are currently fine mapping and backcrossing the chromosomal regions into the wild type *A. alpina* background with an active *PEP1* allele.We are also characterising three mutant alleles of *CLAVATA 1* in *A. alpina*. *Clavata 1* mutants were identified in the *pep1-1* background and showed

Cooperation:
Korbinian Schneeberger
(MPIPZ)

late flowering and enlarged inflorescence and floral meristem compared to pep1-1. This phenotype is consistent to the phenotype of *clavata 1* mutants in *A. thaliana* where it was shown that *CLAVATA 1* plays a role maintaining floral meristem identity and meristem size. We introgressed the *clavata 1* alleles into the wild type *A. alpina* background with and active *PEP1* and we are currently characterising the effect of the mutation in *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

Project type:
Ph.D. project

Project start:
01.10.2013

Aim of the project:
To identify and analyse genes that regulate the pattern of axillary meristem formation during shoot development of *Arabis alpina*

Results: Microscopic analysis revealed that axillary buds in A. alpina that remain vegetative are produced before vernalisation, whereas buds that undergo flowering are produced after the onset of vernalisation. The role of *LATERAL SUPPRESSOR (LAS)*, known as an important regulator of axillary meristem formation, is being studied in *A. alpina*. To compromise *AaLAS* function, an RNAi construct was introduced into *A. alpina* plants. T1 transgenic plants lack axillary buds in vegetative leaf axils, including the dormant bud zone, which is important for perennial life style. RNA-seq analysis of very young axillary buds is being used to study the transcriptome of emerging axillary buds at different stages of the *A. alpina* life cycle. Preliminary results show that dormancy markers, like DRM1, DRM1-like and BRC1, are upregulated in the 8 weeks old buds in comparison to buds harvested at other stages of development.

A11 Genotypic and phenotypic analysis of tiller development in cultivated *(Hordeum vulgare)* and wild barley species *(H. v. spp. spontaneum, H. bulbosum)*

Researcher:
Wilma van Esse

Project leaders:
Maria von Korff
Maarten Koornneef

Project type:
Postdoc project

Project duration:
01.07.2013 – 31.12.2016

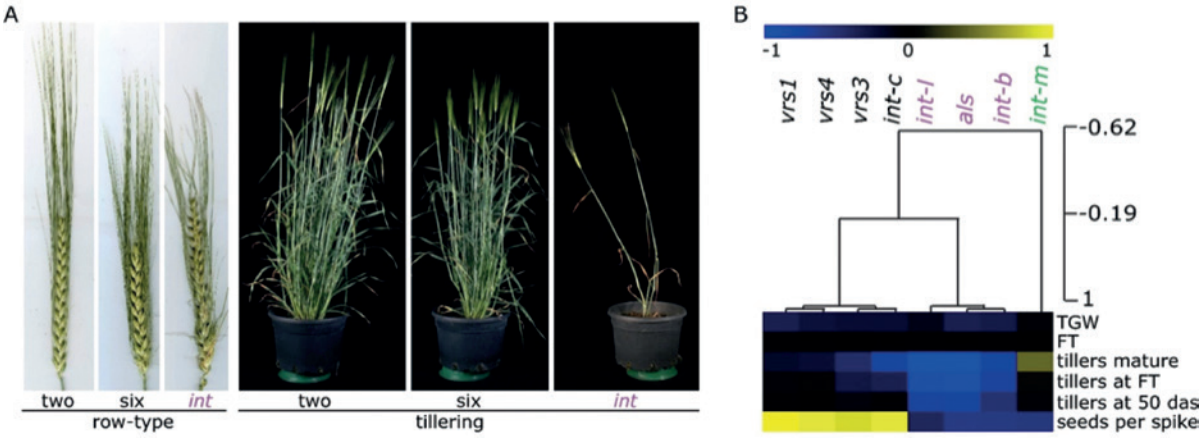
Aim of the project:
Genetic dissection of (axillary) meristem initiation and outgrowth.

Results:
We demonstrated that mutations in barley row-type genes have pleiotropic effects on shoot branching. There were three main groups of row-type mutants identified: 1) increased number of seeds per spike and a tillering phenotype at early development or 2) only at maturity or 3) mutants with a reduction in seeds per spike and tiller number. These results demonstrated that the same genes or regulatory modules affect inflorescence and shoot branching. In addition, RNA sequencing of allelic row-type mutants resulted in the identification of the gene underlying the row-type mutation *vrs3*, which is affected in both seed and tiller

Cooperation:
Laura Rossini
(University of Milan, Italy)
Rüdiger Simon

number, as a histone demethylase. Understanding the genetic and molecular correlation between shoot and inflorescence branching is essential to improve crop yield through the modification of these traits.

Publications:
Liller CB, Neuhaus R, von Korff M et al. (2015) *PloS one* 10(10):e0140246.
Liller CB, Walla A, Boer MP, et al. (2016) *Theoretical and Applied Genetics*:1-13.



Row-type mutants have pleiotropic effects on tillering. A) The architecture of two, six and int spikes and shoots. B) Hierarchical cluster analysis (HCL) using the relative performance of row-type mutants compared to the wild type. Traits are thousand grain weight (TGW), flowering time (FT), tiller number at 50 days after sowing (DAS), tillers at FT and maturity.

A12 Genetic dissection of natural variation in tiller development in cultivated and wild barley

Researcher:
Agatha Walla

Project leaders:
Maria von Korff
Rüdiger Simon

Project type:
Ph.D. project

Project start:
01.02.2014

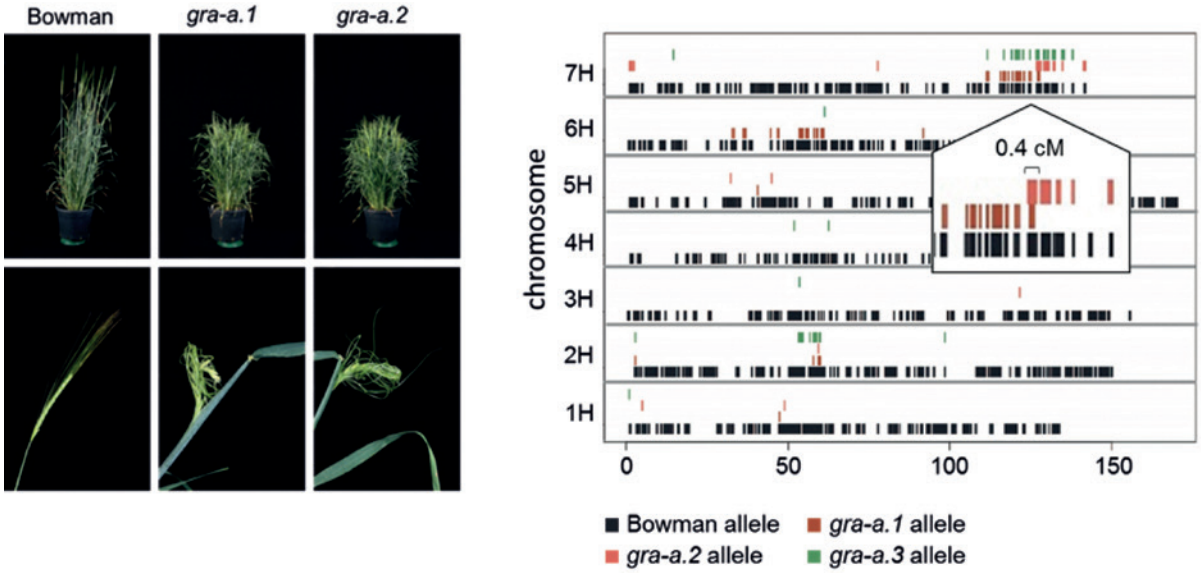
Aim of the project:
Identification and functional characterisation of the high-tillering granum-a mutant

Results:
Detailed phenotypic analysis of the high tillering *granum-a (gra-a)* mutants showed that high tillering was caused by an altered plastochron. RNA-sequencing of three allelic variants (*gra-a.1, gra-a.2 and gra-a.3*) introgressed into a common genetic background allowed to map the causative gene to an interval of 0.4 cM on chromosome 7HL. In addition, we developed a candidate gene selection approach to identify causative polymorphisms based on RNA-sequencing in heterogeneous inbred families derived from wild barley (Liller *et al.* 2016).Tiller development was also studied in a collection of annual and perennial

Cooperation:
Laura Rossini
(University of Milan, Italy)
Rüdiger Simon

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. The collection of annual and perennial *Hordeum* species was subjected to exome sequencing to study natural variation of candidate genes for tiller development.

Publication:
Liller CB, Walla A, Boer MP, *et al.* (2016) *Theoretical and Applied Genetics*:1-13.



Characterisation of the high tillering mutant *gra-a*. Left panel: Phenotype of *gra-a* compared to the wild type Bowman. Right panel: Mapping of *gra*-a to chromosome 7HL using RNA sequencing. Genetic and environmental control of inflorescence development and floret fertility in barley and wheat.

A13 Genetic and environmental control of inflorescence development and floret fertility in barley and wheat

Researcher:
Filipa Tomé

Project leaders:
Maria von Korff, Andreas Weber

Project type:
Postdoc project

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.

Results:
Natural variation at the photoperiod response gene *Ppd-H1* and expression of its downstream target *HvFT1* (FLOWERING LOCUS T) was shown to correlate with an acceleration of flowering time and increased floret fertility under long photoperiods. In order to identify Ppd-H1 targets, which may be causative for the

Project start:
01.07.2015

Cooperation:
Jorge Dubcovsky
(University of California, USA)

different fertility phenotypes, we subjected developing inflorescence meristems of isogenic lines varying at *Ppd-H1* to RNA-sequencing. A preliminary analysis indicated that inflorescences of isogenic lines differed in the expression of genes involved in jasmonate signalling and amino acid metabolism.

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

Researcher:
Gwendolyn Kirschner

Project leaders:
Rüdiger Simon, Maria von Korff

Project type:
Ph.D. project

Project start:
01.12.2013

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.

Results:
We have by now achieved a characterisation of the barley root meristem growth behaviour. The stem cell niche and its cell division patterns were studied using EDU stainings. We found that the barley homologues of CLE peptides also regulate meristem differentiation, and monitored the expression patterns of a set of developmental regulators using reporter lines. Because 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 draught, water stress and could prevent soil erosion.

A15 Using receptor kinase pathways to modify plant traits

Researcher:
Nozomi Kawamoto

Project leader:
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 stem 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 unravelling these mechanisms which would allow to modify seed number as an important agronomic trait.

Results:
We performed a GWAS and QTL analysis on *Arabidopsis* ecotypes and mapped chromosomal regions important for ovule number and ovule density. We found that ERECTA ERECTA-LIKE1 and ERL2 are main regulators of ovule density, and identified peptide ligands that interact with these receptor kinases. Misexpression of ER from a placenta specific promoter reduced the spacing between ovules, indicating that the ER pathway lays a central role in ovule spacing. We identified a ligand for the ERf receptors that controls ovule spacing and is expressed between ovule primordia. Binding of the peptide to the receptors was shown biochemically.

Modelling regulatory modules that differ between annual and perennial plants

In this work package we aim to develop methods that can be used to model regulatory modules from the genetic, RNAseq, ChIPseq data obtained in the work described above. Modelling of such networks would assist in predicting which interventions would be most effective in engineering perennial traits into annuals. At present, the modelling algorithms are tested using the extensive data sets available in yeast.

A16 Transcriptome Data Analysis and Optimal Experimental Design

Researcher:
Armin Sadat Khonsari

Project leader:
Markus Kollmann, Martin Lercher

Project type:
Ph.D. project

Project start:
01.04.2013 – 31.03.2016

Publication:
Khonsari AS, Kollmann M (2015) PloS one 10(5):e0126244.

Aim of the project:
To infer direct interactions between gene activities from transcriptome data

Results:
Generating a comprehensive map of molecular interactions in living cells is difficult and great efforts are undertaken to infer molecular interactions from large-scale perturbation experiments. Here, we develop the analytical and numerical tools to quantify the fundamental limits for inferring transcriptional networks from gene knockout screens and introduce a network inference method that is unbiased and scalable to large network sizes. We show that it is possible to infer gene regulatory interactions with high statistical significance, even if prior knowledge about potential regulators is absent.

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-spe-

cies 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

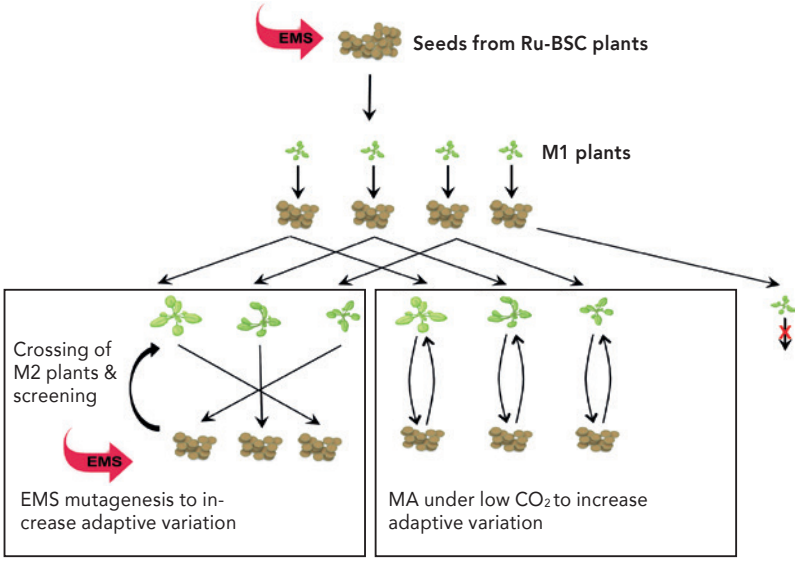
Members:
Faculty:
Oliver Ebenhöh
Georg Groth
Ute Höcker
Martin Hülskamp
Karl Köhrer
Markus Kollmann
Veronica G. Maurino
Juliette de Meaux
Peter Nürnberg
Markus Pauly
Richard Reinhardt
Ulrich Schurr
Kai Stühler
Miltos Tsiantis
Andreas P. M. Weber

Early career researchers:
Kumari Billakurthi
Meike Hüdig
Satish Kumar Eeda
Yuanyuan Li
Otho Mantegazza
Anna Matuszyńska
Roxanne van Rooijen
Mara Schuler-Bermann
Johannes Schwabroh
Esther Sundermann
Alice Vayssières
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 EU 3to4 project, the DFG-funded International Research Training Group 1525, the DFG Research Group Promics, the DFG Priority Programme Adaptomics and HHU Düsseldorf.

Work conducted in this area focussed on four different research topics:

1) Transcriptome comparisons of C_3 and C_4 species of the genera *Cleome* and *Flaveria* were performed to identify putative regulators of C_4 leaf differentiation.
2) Genetic analyses were pursued with *Arabidopsis thaliana* in order to detect genes that are involved in bundle-sheath differentiation, and the natural variation of this species was exploited to identify genes affecting general leaf morphology and anatomy.
3) Approaches of experimental evolution were initiated with *Arabidopsis* to select directly for traits that should appear on the trajectory towards C_4.
4) Projects were designed that targeted regulatory modules of C_4 metabolism and investigated the interaction of C_4 photosynthesis with other metabolic pathways.



Schematic representation of evolutionary engineering.
Seeds from Ru-BSC lines (plants expressing Rubisco only in bundle sheath cells) were subjected to EMS mutagenesis, followed by ar-

tificial selection (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

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. Projects combined within this research section pursue this approach for identifying putative regulators of C_4 leaf differentiation and of the C_4 cycle. The transcriptome analyses included the comparisons of leaf transcriptomes at various developmental stages from the primordia up to fully matured leaves. In addition, the leaf transcriptomes of C_3 (F. robusta) and C_4 (F. bidentis) Flaveria species, which were grown under circadian clock entrained and free-running (constant light) conditions, were compared to infer regulators of C_4 physiology. The developmental transcriptomics studies were accompanied by detail analyses of leaf differentiation at the histological level. The two data sets were used to construct a linear model aiming of correlating changes in gene expression with leaf differentiation state. Two major results emanated from these studies. Firstly, in both species leaf development of the C_4 species is lagging behind that of the C_3 species. Secondly, a set of genes could be identified that is correlated with the differences in leaf differentiation between C_3 and C_4 leaves and will provide candidate genes for future engineering efforts. Furthermore, the model enables the deconvolution of cell-specific transcriptomes from whole leaf transcriptome data sets.

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 behind the C_4 specific Kranz leaf anatomy by two approaches. The first approach is to compare the transcriptome data sets of *F. robusta* (C_3) and *F. bidentis* (C_4) leaves at different developmental stages, to provide insights in understanding C_4 leaf development. In second approach we took the advantage of forward genetics (Activation tagging and EMS mutagenesis approaches) to screen for Kranz anatomy mutants and identify responsible candidates using *Arabidopsis thaliana*.

Project start:
01.10.2013

Cooperation:
Andrea Bräutigam
Udo Gowik (HHU)
Thomas Wrobel

Results:
Comparative transcriptome data sets were prepared from leaf gradients of both *Flaveria* species, *F. bidentis* and *F. robusta*. Analysis of the transcriptome data sets has revealed that leaf differentiation in C_4 (*F. bidentis*) is delayed in comparison to a C_3 (*F. robusta*) leaf development, which has been also reported in the genus Cleome (Külahoglu *et al.*, 2014). *Arabidopsis thaliana* marker lines, whose bundle-sheath cells are labelled with chloroplast targeted GFP have been subjected to Activation tagging and EMS mutagenesis approaches, to identify mutants with altered bundle sheath anatomy. Based on the GFP signal intensity in the bundles of *A. thaliana* leaves we identified mutants with more or less signal intensity. We have analysed 25 mutant lines from EMS screen with more and less signal intensity respectively) with respect to BS and vasculature development, found out that our mutant lines are mostly affected in three different parameters: (1) the size of BS cells, (2) the number of BS cells, and (3) the overall size of the vasculature. Combinations of these three characteristics allowed us to put 25 mutant lines into three different categories. Category 1: increased number of BS cells, smaller BS cells, and no change in the size of the vasculature. Category 2: increased number of BS cells, smaller BS cells, and increased size of the vasculature. Category 3: no effects at the BS combined with an enlarged vasculature.
We have subjected *Arabidopsis* marker lines to Activation tagging approach using Ft-ppcA promoter to randomly activate genes in the entire leaf tissue and have identified one stable mutant line with increased GFP signal intensity. T-DNA flanking sequence of this mutant has been identified by inverse PCR and over-expression of the identified gene (BSOM4) with P-Ft-ppcA, recapitulated the mutant phenotype and quite interestingly over-expression of BSOM4 under the control of Ft-GLDT and 35S promoters has resulted in the same phenotype with respect to GFP signal intensity. Qualitative analysis with Transmission Electron Microscopy has led to the observation that mutant line has high number of plasmodesmata in the entire leaf tissue, in comparison to the reference line. We are further analysing the effect of this gene under the control of the Ft-GLDT and 35S promoters in *Arabidopsis thaliana* and with the Zm-PEPC, Zj-PCK and PV-Ub promoters in rice.

Publication:
Lauterbach M, Billakurthi K, Kadereit G *et al.* (2017) *Journal of Experimental Botany* 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
Kumari Billakurthi
Udo Gowik (HHU)
Sarah Richards (HHU)

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. Leaf development was analysed using cleared leaves and cross-sections after araldite embedding. BSH specific traits emerge during the development of the minor vasculature in the C_4 species. In order to determine the underlying genetic program the leaf was divided into three sections according to its anatomic features. A Linear model was fitted to the expression of genes, using the changes of relative area of the three sections in both species. Analysis of genes correlating with the presence of the differentiating section in the C_4 but not the C_3 species led to the hypothesis that auxin acyl-conjugating enzymes belonging to the Gretchen Hagen 3 (*GH3*) gene family trap auxin in inactive form during early leaf development, followed by a burst of active auxin release through IAA aminohydrolases during later stages of leaf development.

Publication:
Külahoglu C, Denton AK, Sommer M *et al.* (2014) *The 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 start:
01.05.2015

Cooperation:
Mara Schuler-Bermann
Wolfgang Werr
Thomas Wrobel

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:
Quantitative comparison of the transcriptomes from different Cleome species with linear models has been used for identifying candidate genes. A CRISPR-Cas9 based strategy for the directed evolution of the transcriptional and post-transcriptional regulation of candidate genes in *Arabidopsis thaliana* was developed, in which increased mutation rates of cis-regulatory elements are hypermutated by an RNA-guided, modified CRIPRS-Cas9 system.

Publication:
Schuler ML, Mantegazza O, Weber AP (2016) *The Plant Journal* 87: 1-65.

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 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.

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.

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

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

Results:
The research project on *Arabidopsis* has provided three major results within the first two years:
1) Based on WOX3 activity in marginal and plate meristems the initiation of a new primordium starts with mesenchymal identity. There is a delay of at least 12 hours before the cambial marker AtHB8 is detectable.
2) By adding upstream and downstream enhancer elements the WOX4 promoter has been improved and is expressed in the cambium, although later than ATHB8, and possibly during xylem development. This new finding is presently analysed in more detail.
3) Bundle-sheath-like identity in cells surrounding vascular strands is determined significantly later (> 36 hours) than the cambial fate.The project elaborated successive critical steps in leaf and vascular development.

B7 Generation of synthetic tools for the engineering of C_4 photosynthesis

Researcher:
Mara Schuler-Bermann

Project leaders:
Peter Westhoff, Andreas Weber

Project type:
Postdoc project

Project start:
01.08.2015

Aim of the project:
The anatomical adaptation of C_4 plants called `Kranz anatomy' is a major evolutionary prerequisite to evolve the C_4 carbon concentrating cycle and represents a major engineering challenge due to complexity of the trait. It is not yet fully understood which developmental factors determine Kranz anatomy but we agree that a cohort of genes is needed to initiate this specialised anatomy early in development prior to the integration of the two-celled C_4 photosynthetic cycle. Therefore, the conversion of leaf anatomy and the integration of altered photosynthetic components require a toolbox of *cis*-regulatory modules (CRM) to drive cell-type specific expression in specific developmental stages.
Research focuses on biological and methodological aspects. The aims are: 1. The functional characterisation of a putative developmental patterning factor in leaves and 2. The identification of *cis*-regulatory modules driving expression early in vascular development in leaf primordia by analysing the 5`flanking region of a putative procambium initiation factor.

Results:
1) Transgenic lines ectopically expressing our candidate gene in the rice bundle sheath were characterised in detail. Ectopic expression of this candidate has led to a stomata patterning defect in rice.xperiments are completed and manuscript is in preparation for submission.
2) Individual transgenic rice and *Arabidopsis* lines carrying different promoter-reporter constructs were generated. Reporter constructs were assembled by fusing 5' flanking regions of the rice and *Arabidopsis* orthologous of our maize candidate gene with the GUS reporter gene. Strengths and localisation of the reporter gene in young shoots will help to identify CRMs, which confer specific expression in primordial and provascular tissues respectively. Initial GUS staining experiments of the maize 3 kb 5`flanking region show a strong vascular-specific expression in *Arabidopsis* seedlings. Seeds for all transgenic lines are available and will be analysed in detail over the next months.

Publication:
Schuler ML, Mantegazza O, Weber AP (2016) The Plant Journal 87: 51-65.

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*.

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

Cooperation:
David Heckmann (HHU)

Aim of the project:
C_4 photosynthesis evolved more than 60 times independently from C_3. Our goal is to better understand this evolutionary process with a view towards artificial selection. We assess the influence of environmental factors on the relative fitness of C_3 and C_4 plants, and simulate evolutionary trajectories between C_3 and C_4 across different environments.

Results:
We created a mathematical model that considers the effects of light, temperature, and gas partial pressures on CO_2 assimilation rate, which we use as a proxy for plant fitness. The availability of proteins is limited by a total nitrogen budget. We find that a wide range of environments favours C_4 photosynthesis; while other environments favour C_3, a reversion from C_4 to C_3 is only very rarely accessible. The nitrogen distribution across photosynthetic proteins is optimal with respect to the type of environment in which these plants have likely evolved, but is not optimized for the conditions under which the plants were grown.

B9 Transforming *Arabidopsis* plants towards C_4 metabolism

Researcher:
Yuanyuan Li

Project leaders:
Veronica G. Maurino
Martin Lercher

Project type:
Postdoc project

Project start:
01.04.2015

Cooperation:
David Heckmann (HHU)

Aim of the project:
This project aims to generate C_4-like prototype from the C_3 plant *Arabidopsis* by combining genetic engineering, mutagenesis, and artificial selection to implement C_4 traits in the C_3 plant *Arabidopsis*. We will engineer *Arabidopsis* plants with Rubisco specifically expressed in bundle sheath cells. The plants will later be mutagenized to screen for improved, i.e. C_3-C_4-like photosynthesis, and are expected to respond to this artificial selection pressure by evolving further towards C_4 anatomy and biochemistry.

Results:
We have gained the homozygous transgenic *Arabidopsis* plants that express Rubisco only in bundle sheath cells. We are characterizing the transgenic lines physiologically and biochemically under different growth conditions. Now the second phase of the project is beginning.

Publication:
Li Y, Heckmann D, Lercher MJ, Maurino VG (2017) *Journal of Experimental Botany* 68: 117-125.

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.

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

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 NAD-ME evolution in C_4. Additionally, (2) the role of mitochondrial malate metabolism and its regulation in *A. thaliana* will be studied by using loss-of-function lines of NAD-ME and mitochondrial malate dehydrogenases.

Project type:
Ph.D. project

Project start:
01.02.2014

Results:
1) *In vitro* characterization of heterologously expressed NAD-MEs is advanced and nearly finished. Comparative NAD-ME transcript analysis has been performed. Physiological characterization in leaf material is ongoing. Enzyme complex subunits are being identified in a tissue specific manner.
2) Developmental characterization of 14 *knock-out* lines (single, double, triple gene knock-outs) has been performed. Metabolite status of mutants during the day/night transition is being combined with RNAseq to shed light on metabolic compensating mechanisms.

Publication:
Hüdig M, Maier A, Scherrers I, *et al.* (2015) *Plant & Cell Physiology* 56: 1820-1830.

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 start:
01.10.2014

Aim of the project:
The project aims to resolve the molecular basis of the preferential interaction of different PEP-carboxylase kinase (PPCK) isoforms with their C_3- and C_4-PEPC target. Detailed knowledge of the molecular basis controlling PPCK specificity will provide essential information on the regulation of the C_4-module.

Results:
PPCK isoforms were successfully expressed and purified to homogeneity from a bacterial host. Size-exclusion chromatography confirmed that recombinant PPCKs were obtained in monomeric form. Interaction of recombinant PPCK with their PEPC target was studied by microscale thermophoresis (MST). A dissociation constant in the sub-µM range indicating strong interaction was obtained for C_4-PPCKA and C_4-PEPC from *F. trinervia*. Protein crystals obtained for the recombinant PPCKA diffracted to 3.2 Å resolution. Analysis of the collected data set and phasing is in progress.

B12 Nitrogen and sulphur metabolism in C_4 plants

Researcher:
Silke Weckopp

Project leaders:
Stanislav Kopriva
Peter Westhoff

Project type:
Ph.D. project

Project start:
01.01.2015

Cooperation:
Tamas Dalmay
(University of East Anglia)

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.

Results:
We revealed a gradient in accumulation of reduced S compounds towards increased C_4 characteristics in *Flaveria* species. Similar gradient was seen in sulphate uptake and reduction rate. A significant change in distribution of sulphate and phosphate in roots and shoots was observed between C_3 and C_4 species. We also found significant differences in accumulation of several elements in the different *Flaveria* species and significant variation in total P and Cu between C_3 and C_4 grasses. Currently nutrient responsive miRNAs in C_3 and C_4 *Flaveria* are being analysed.

Publications:
Weckopp SC, Kopriva S (2014) *Frontiers in Plant Science* 5:773Kopriva S, Calderwood A, Weckopp SC *et al.* (2015) *Plant Science* 241:1-10.

B13 Mathematical modelling of acclimation processes of the photosynthetic electron transport chain in green algae and plants

Researcher:
Anna Matuszyńska

Project leaders:
Oliver Ebenhöh, Peter Westhoff

Project type:
Associated Ph.D. project

Project start:
01.05.2014 –30.09.2016

Cooperation:
Federica Cariti
(University of Geneva, Switzerland)
Gilles Curien
(CEA, Grenoble, France)
Giovanni Finazzi
(CEA, Grenoble, France)

Aim of the project: Using theoretical methods to understand how various photosynthetic model organisms acclimate to changing light conditions.

Results:
In collaboration with several experimental groups dynamic mathematical models are developed for various scenarios and for different species:

1) A minimal mathematical model for qE to study short-term light memory: with a minimal set of equations we can capture the dynamics of a complex system and can test hypotheses on the role of zeaxanthin in the photoprotective memory (*Arabidopsis*);
2) Include light wavelength as external parameter impact on organisms performance, as so far there is no dynamic model of light photosynthetic acclimation that includes wavelengths to study how light quality affects photosynthetic activity (*Chlamydomonas*);
3) Implement Calvin cycle demand to study supply-demand regulation and make predictions regarding biomass production;
4) Dynamics of state transitions (*Chlamydomonas*).

Activities will support CEPLAS to optimise photosynthetic efficiency.

Serena Flori
(CEA, Grenoble, France)
Michel Goldschmidt-Clermont
(University of Geneva,
Switzerland)
Somayeh Heyidari (Ferdowsi
University of Mashhad, Iran)
Jun Minagawa
(National Institute for
Basic Biology, Okazaki, Japan)
Ryutaro Tokutsu
(National Institute for Basic
Biology, Okazaki, Japan)

Publications:

Matuszyńska A, Heidari S, Jahns P, Ebenhöh O (2016) *Biochimica et Biophysica Acta* 1857: 1860-1869.
Matuszyńska A, Ebenhöh O (2015) *Biochemical Society Transactions* 43: 1133-1139

Summary and Outlook

Despite intense developmental transcriptome and leaf anatomical studies, the evolutionary enablers for Kranz anatomy have still not been identified. Candidate factors that may be used for creating an activated bundle-sheath rich have been deduced from transcriptome analyses or forward genetic approaches, but their validity and usefulness for achieving Kranz anatomy in C_3 species has not been proven yet. Unfortunately, there are no C_3-C_4 intermediate species available that could be easily used for gene identification by genetic approaches. Consequently, C_3 model species such as *Arabidopsis* or rice are currently the best systems for forward genetics and synthetic approaches, and their use will therefore 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

Coordinator:
Alga Zuccaro

Co-coordinator:
Jane Parker

Members:
Faculty:
Michael Bonkowski
Marcel Bucher
Gunther Döhlemann
Michael Feldbrügge
Martin Hülskamp
Eric Kemen
Karl Köhrer
Peter Nürnberg
Richard Reinhardt
Laura Rose
Paul Schulze-Lefert
Jürgen Zeier

Early career researchers:
Ziba Ajami-Rashidi
Juliana Almario
Friederike Brüssow
Philipp Fesel
Vera Göhre
Elaine Jaeger
Ganga Jeena
Ronny Kellner
Katharina Lentz
Alfredo Mari
Ryohei Thomas Nakano
Ovidiu Popa
Melanie Sapp
Debika Sarkar
Stefan Schuck
Jan Schulze Hüynck
Antonella Succurro
Michael Thielen
Heidi Widmer
Heike Wolff

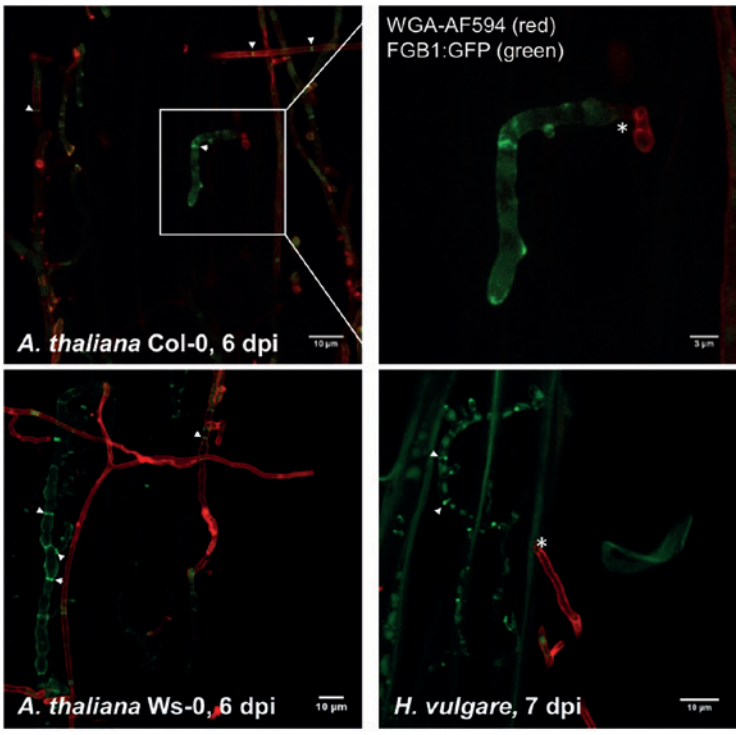
Roots and leaves of healthy plants host a wide range of microbes which enhance resistance to pathogens and/or facilitate plant mineral nutrient uptake in exchange for carbohydrates and other organic metabolites. These associations play a key role in shaping terrestrial ecosystems and are widely believed to have promoted the evolution of land plants. To establish compatibility with their host, plant-associated microbes have evolved diverse colonisation strategies with distinct morphological, functional and genomic specialisations as well as different degrees of interdependence. These associations can be host-specific or display a broad host range and undergo long-term interactions with a large variety of plants. To establish and maintain a compatible interaction, hosts as well as microbes must respond and adapt to different signals. Alternative lifestyle and colonisation strategies may thus be a consequence of this adaptation to highly variable environments. Recent findings indicate that both abiotic factors and host genotype interact to influence plant colonisation by microbes and that a small number of microbial taxa, named microbial "hubs", which are strongly interconnected help shape community structure. The increasing number of available plant and microbe genomes, together with rapid technical advances in computational biology, metagenomics, culture-independent microbe detection and manipulation of microbe-plant interactions, offers unprecedented opportunities for new discoveries to this rapidly evolving field. This is the foundation for exploring how distinct microbial and host symbiosis determinants modulate interactions with different plants. Results from this research will help to develop sound experimental strategies in more complex environments (e.g., in mesocosms) at different temporal and spatial scales. We now know that the composition and activity of the 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. This can be seen as a contribution to the fundamental understanding of the plant-microbiota as a functional entity called the "holobiont" and thereby to the development of novel strategies for plant protection and biological growth promotion.

In Research Area C we aim to:

1) Characterise the structure, function, and ecology of the plant microbiota within and between species of the Brassicaceae.
2) Understand how biotic and abiotic factors affect the composition and activities of plant associated microbial communities. Identify and characterise microbial hubs.
3) Identify genes in both host and microbe that determine the outcome of these interactions.

4) Determine the reciprocal effects these interactions have on the production of metabolites in hosts and microbes (collaboration Research Area D).
5) Construct synthetic symbioses to shape the endophytic microbiota towards increased plant performance.

Complementary expertise in Research Area C groups on evolutionary ecology (e.g., Bonkowski, Rose), effector biology and microbiology (e.g., Zuccaro, Döhlemann, Feldbrügge), plant immunity (e.g., Parker, Zeier), plant physiology (e.g. Hülskamp), and microbiota reconstitution biology (e.g., Schulze-Lefert, Bucher, Kemen) combined with that of our well-established national and international long-term research collaborators and the availability of different central facilities located at the four partner centres within CEPLAS help us dissect plant-microbe ecology and design synthetic microbiota. As a result, groups working in this emerging field within Research Area C are able to fill a new and highly promising research niche that is relevant from both a pure and applied scientific perspective.
Our recent research into the biology and genomics of plant-microbe associations has revealed fascinating insights to the phenotypic and trophic plasticity of these interactions and underlying genomic traits associated with intracellular accommodation of microbes in roots and leaves.



The small secreted *Piriformospora* indica protein FGB1 binds to fungal cell walls and harbours a fungal-specific lectin domain. FGB1:GFP localizes to the fungal septa (arrowheads) and cell wall in colonized *A. thaliana* Col-0 and Ws-0 roots, as well

as barley roots. (Wawra S, Fesel P, Widmer H, Timm M, Seibel J, Leson L, Kesseler L, Nostadt R, Hilbert M, Langen G, Zuccaro A (2016) The fungal-specific ß-glucan-binding lectin FGB1 alters cell-wall composition and suppresses glucan-triggered immunity in plants. *Nature communications* 7:13188)

Structure, function and ecology of the plant microbiome

With respect to this goal, we determined which microbial species (fungi, bacteria, oomycetes and protozoans) prosper endophytically within plant tissues of *Arabidopsis thaliana* and *Arabis alpina* and how these endophytic communities differentiate from the microbes in the surrounding soil. We demonstrate that root microbiota dynamics are dependent on soil type and soil residence time but independent of flowering time (Dombrowski *et al.*, 2017; Sapp *et al.*, unpublished). Additionally, we produced data on how the microbiome differs across a larger taxonomic breadth (Dombrowski *et al.*, 2016; Agler *et al.*, 2016; Ploch *et al.*, 2016; Almario *et al.*, submitted).

C1 Characterization of the mycobiome of *A. alpina*

Researcher:
Juliana Almario

Project leaders:
Marcel Bucher, George Coupland

Project type:
Postdoc project

Project duration:
01.03.2013 - 31.07.2016

Cooperation
Ganga Jeena
Gregor Langen
Alga Zuccaro
Jörg Wunder (MPIPZ)

Aim of the project:
Study the root-associated fungal microbiome of Arabis alpina, a non-mycorrhizal plant growing in alpine environments including low phosphorus (P) soils.

Results:
Using fungal ITS2 sequencing we described the fungal community colonizing the roots of *A. alpina* growing under controlled and natural conditions. From 830 fungal OTUs detected in *A. alpina* roots, 6 were detected in every sample. This core fungal root microbiome included plant-associated fungal genera like *Mortierella, Ilyonectria* and *Fusarium*. Using correlation analysis we identified a *Rhynchosporium* OTU whose relative abundance in plant roots significantly correlated with higher P accumulation in the plant shoot, suggesting this fungus could improve plant P uptake. Indeed, *Rhynchosporium* inoculation promoted plant root growth under low P conditions, and increased plant shoot growth and P content under sterilized soil conditions. Moreover, the fungus facilitated 32P-phosphate acquisition of *A. alpina* plants under experimental conditions. We hypothesize that the studied *A. alpina* population developed a partnership with this fungus in order to adapt to local low P in soil.

C2 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

Aim of the project:
Next Generation Sequencing will be used to characterize the diversity of oomycetes and protists associated with *Arabidopsis thaliana*. We aim to find beneficial protist and oomycete taxa and to manipulate microeukaryote populations for the benefit of the plant.

Results:
The taxonomic coverage of Solexa compatible metabarcoding methods for oomycetes and cercozoa have been tested *in silico* and complemented by a revision of a cytochrome c oxidase subunit 2 assay specific for oomycete communities. Now that the sequencing has been completed, our analyses indicate that communities of oomycetes and protists are strongly shaped both by soil and plant organ. The *A. thaliana* core microbiome - independent of soil origin - consisted of four oomycete taxa (specifically *Globisporangium spp.*) and 13 cercozoan taxa, the majority belonging to the *Glissomonadida* and *Cercomonadida*. In contrast to the oomycetes, cercozoa were consistently found in the phyllosphere.

Understand how biotic and abiotic factors affect the composition and activities of plant associated microbial communities

While genetic differences within host and endophytes are expected to play an important role in structuring microbial communities, we anticipate that the microbiota will also be strongly influenced by abiotic factors. Recent evidence indicates that environmental (abiotic and biotic) factors and host genotype interact to affect plant colonisation by microbes and that particular microbes, termed “hub microbes” due to their central position in a microbial network, are disproportionally important in shaping microbial communities on plant hosts (Agler *et al.*, 2016) and that green algae play an important role in microbiota composition and activities (Mari *et al.*, unpublished). Research on these microbial hubs represents a novel direction in Research Area C (e.g., project C8 Döhlemann / Kemen). A transformation system for one of these hubs, the fungus *Pseudozyma* spec., has been established. Competition assays demonstrated strong antibiotic activity of *Pseudozyma* spec. against several epiphytic bacteria (Lentz *et al.*, unpublished).

C_3 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

Project start:
01.05.2014

Cooperation:
George Coupland

Aim of the project:
The aim of the project is to study the genetic underpinnings for plant factors that can shape the root associated fungal community composition. To approach this goal a synthetic fungal community (SynCom) has been established composed of fourteen fungal species and is currently used on *A. alpina x A. montbretiana* introgression lines to subsequently pinpoint genetic loci responsible for variation in the fungal microbiome.

Results:
A time and cost efficient, semi-quantitative screening method for fungal community comparison has been adapted and established. After identification of the ideal colonization conditions, the reproducibility of the colonization of *A. alpina and A. montbretiana* roots by the SynCom has been tested extensively. The SynCom is now being utilized to investigate the genetic underpinnings linked to microbial community structuring and to study how environmental factors contribute to root-associated fungal microbiome composition. Moreover, functional attributes of the SynCom affecting plant growth are currently being investigated.

C_4 Molecular link between protein stability and sorting (ESCRT) and RNA stability (P-bodies, 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 start:
01.11.2013

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.

C5 Investigation of the effect of temperature-modulated defense homeostasis on plant-microbe symbioses in *Arabidopsis thaliana*

Researcher:
Friederike Brüssow

Project leaders:
Jane Parker
Paul Schulze-Lefert

Project type:
Postdoc project

Project start:
01.09.2013

Cooperation:
Eric Kemen

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.

Results:
SA chemotyping of leaves from 105 diverse *A. thaliana* accessions grown in soil at 20o C and 16o C uncovered natural variation in plant responses to temperature at the level of SA accumulation. A GWAS analysis for loci underlying temp x SA differences identified 4 statistically well supported peaks. SNPs and genes underlying these peaks are being interrogated further. Selected 'extreme' SA x temp differential accessions are undergoing detailed phenotyping for altered stress hormone network and pathogen resistance properties. Differential SA accumulation in response to temperature correlated poorly with growth (biomass), suggesting a high degree of plasticity regulating the stress hormone network and growth trade-offs. SA accumulation, however, correlated strongly with increased pathogen resistance and the resistance phenotypes in several tested lines were SA-dependent. Differently behaving lines will be used for microbiota analysis in leaves using microbial sampling and phyllotyping pipelines developed in CEPLAS Area C. This, combined with reconstitution experiments in the lab and plant survival/fitness tests in the field will inform us whether there is a detectable genetic signature for plant SA/hormone homeostasis shaping the structure of leaf microbial communities and fitness.
The project was interrupted for 12 months due to maternity leave.

C6 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

Aim of the project:
This project investigates how *Arabidopsis* adapts the systemic acquired resistance (SAR) response to different environmental conditions such as light conditions and nitrogen supply. In this context, it re-evaluates the relevance of the putative SAR signals DEFECTIVE IN INDUCED RESISTANCE1 (DIR1), azelaic acid (AzA), glycerol-3-phosphate (G3P), and methyl salicylate (MeSA) and their functional relationship with the SAR regulator pipecolic acid (Pip).

Project type:
Ph.D. project

Project start:
01.10.2013

Cooperation:
Shizue Matsubara (FZJ)

Results:
Genetic analyses with Arabidopsis mutants suggest that DIR1, AzA, and MeSA are dispensable for SAR, and that GLYCEROL INSENSITIVE1 (GLI1)-dependent G3P production contributes to the strength of SAR. In contrast to a previous report, this is independent of the time of day when the inducing inoculation occurs. Although the pathogen-inducible biosynthesis of Pip is not affected by DIR1-, AzA-, MeSA-, and G3P-signalling mutants, our data indicate that interactions between Pip and G3P signalling exist.

C7 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:
Marcel Bucher
Laura Rose
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 context, significant knowledge has been acquired regarding the rhizosphere, while similar evidences concerning the phyllosphere are lacking in several aspects.In this study, we aim to analyse how protist communities can impact the plant microbiome, by targeting major microbial 'hubs' (Agler *et al.* 2016), namely the common *A. thaliana* endophytes/pathogen genus *Albugo*.

Results:
We have developed an 18S Illumina sequencing approach to profile protists. To analyse the data, we have written and verified an analyses pipeline. First insights obtained by co-occurrence based scale free networks, indicate a major role held by green algae. Currently gnotobiotic community experiments are in progress to validate field findings.

C8 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 as well as on upper ground plant organs is crucial for plant health and immunity. Previous work within CEPLAS by the groups of Paul Schulze-Lefert and Eric Kemen demonstrated that both mutualistic and antagonistic microbe-microbe interactions are defining the plant microbiome. A subspecies of the yeast-like basidiomycete *Pseudozyma*, which belongs to the family of *Ustilaginaceae*, was identified as an epiphyte of *Arabidopsis thaliana* leaves. It is known that *Pseudozyma* can strongly influence its microbial community for example by inhibiting the growth of powdery mildews. The project aims to study the role of *Pseudozyma* in the *Arabidopsis thaliana* phyllosphere. Because of its close relation to pathogenic *Ustilaginomycetes*, also the potential role

of *Pseudozyma* as a plant pathogen and how its lifestyle influences the leaf microbiome will be studied.

Results:
Genome sequencing and annotation of *Pseudozyma* spec. has been completed. In addition, a transformation system for this fungus has been established. Competition assays demonstrated strong antibiotic activity of *Pseudozyma* spec. against several epiphytic bacteria.

Identify the genes in both host and microbe that determine interaction outcomes

We identified genomic and transcriptomic complex traits associated with lifestyle adaptations by comparative genomics of ecologically diverse bacterial and fungal species (Bai *et al.*, manuscript in press; Fesel & Zuccaro, 2016; Hiruma *et al.*, 2016; Hacquard *et al.*, 2016; Zgadzaj *et al.*, 2016; Sarkar *et al.*, unpublished). Several candidate genes important for endophytic associations (e.g., genes involved in metabolic functions, plant-microbe communication or in counteracting plant defence) were identified and several were functionally characterised (Wawra *et al.* 2016; Matei *et al.,* 2016; Jaeger *et al.*, unpublished; Widmer *et al.*, unpublished).

C9 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 start:
01.10.2013

Cooperation:
Jeff Dangl (University of North Carolina, USA)
Rob Knight (University of San Diego, USA)
Stanislav Kopriva
Ruth Ley (Cornell University, USA)

Aim of the project:
The goal of the project is the development and application of computational approaches to the analysis of genomic and phenotypic data for the study of the root and leaf microbiotas and their interactions with their plant host.

Results:
By systematic bacterial isolation procedures we have established bacterial culture collections of the leaf- and root-associated microbiotas of *A. thaliana*, capturing the majority of the taxa found in their corresponding natural host organs. Comparative phylogenomic analysis of more than 400 sequenced genomes revealed a substantial taxonomic and functional overlap between leaf and root isolates, with several differentially abundant functional categories. Further, a newly developed gnotobiotic reconstitution system for synthetic communities, together with a computational pipeline for the analysis of high-throughput sequencing data, provides a system for the study of microbial community establishment and functions under laboratory conditions (Bai *et al.*, manuscript in press).

Richard J. O'Connell
(INRA Versailles, France)
Simona Radutoiu
(Aarhus University, Denmark)
Elizabeth Satteley
(Stanford University, USA)
Julia Vorholt
(ETH Zurich, Switzerland)

Publications:

Schlaeppi K, Dombrowski N, Oter RG *et al.* (2014) *Proceedings of the National Academy of Sciences of the United States of America* 111(2):585-592.
Bulgarelli D, Garrido-Oter R, Münch PC *et al.* (2015) *Cell Host & Microbe* 17(3):392-403.
Bai Y, Muller DB, Srinivas G *et al.* (2015) *Nature* 528(7582):364-369.
Bulgarelli D, Garrido-Oter R, Munch PC, *et al.* (2015) *Cell Host & Microbe* 17(3):392-403.
Hacquard S, Garrido-Oter R, Gonzalez A *et al.* (2015) *Cell Host & Microbe* 17(5):603-616.
Bai Y, Müller DB, Srinivas G et al. (2015) Nature 528(7582):364-369.
Hacquard S, Kracher B, Hiruma K *et al.* (2016) *Nature Communications* 7:11362.
Zgadzaj R, Garrido-Oter R, Jensen DB (2016) *Proceedings of the National Academy of Sciences of the United States of America* 113(49):E7996-E8005.
Dombrowski N, Schlaeppi K, Agler MT *et al.* (2017) *ISME J* 11(1):43-55.

C10 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 start:
01.05.2015

Cooperation:
Marcel Bucher
Philipp Fesel
Gregor Langen
Debika Sarkar

Aim of the project:
Comparative genomics and transcriptome assembly of ecologically diverse fungal species to understand lifestyle adaptations.

Results:

1) *De-novo* transcriptome and genome assembly of root associated fungal endophytes including the orchid mycorrhizal *Serendipita sp., Serendipita herbamans, Chaetospermum artocarpi, Chaetospermum camelliae* were generated. Genome annotation is ongoing.
2) 85Mb Genome of an *Arabis alpina* root endophyte (Helotiales) was assembled and annotated. The response of fungal genes (*in planta*) at low phosphate was analysed and comparison of CAZymes against 52 fungal species was performed.
3) Study of tripartite plant-fungal interaction (see project C14) using RNA-Seq from split root systems of barley non-inoculated and inoculated with the pathogenic fungus *Bipolaris sorokiniana* and the endophytic fungus *Serendipita vermifera* were performed to assess local and systemic effects on plant genes.
4) Response of barley genes to the lectin FGB1 was analysed (see project C12).

C11 Functional characterization of candidate effector proteins in the Sebacinales

Researcher:
Heidi Widmer

Project leaders:
Alga Zuccaro, Stanislav Kopriva

Project type:
Associated Ph.D. project

Project start:
01.01.2015

Cooperation:
Vera Göhre

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 plant growth promotion and increased resistance to pathogens. With respect to insights into how sebacinoid fungi establishes themselves in metabolically active root cells of different hosts and how the plants are reprogrammed for enhanced performance, we are functionally characterizing candidate effector proteins in the *Sebacinales* to find answers to the following questions: Are there functionally conserved effectors between *S. indica* and *S. vermifera*? How do the sebacinoid effector proteins mediate host accommodation and mutualism?

Results:
Effector candidates were selected based on barley and *Arabidopsis* transcriptomic data and MS analysis of apoplastic fluid of *S. indica* inoculated barley roots. One of these proteins was functionally characterized and proved to be a fungal extracellular DNase. Fungal and plants overexpression lines were produced and will be further characterized. Moreover, an S. indica protein expression system with high-level protein yields 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) Nature Communications 7:13188

C12 Role of fungal 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 start:
01.08.2014

Cooperation:
Stanislav Kopriva
Jürgen Seibel
(University of Würzburg)

Aim of the project:
Beta-glucan is the most abundant cell wall polysaccharide in fungi and is perceived by the plant innate immune system as a MAMP. 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 plants *Arabidopsis* and barley. The questions posed in this project are:
1) How are different plants species/ecotypes reacting to elicitation with ß-glucan?
2) Which plant receptors are involved in the perception of beta-glucan signals?
3) How is *S. indica* avoiding recognition of beta-glucan by plants?

Results:
We could show that *Arabidopsis* WS-0 and barley Golden Promise recognize various beta-glucans as MAMPs. A GWAS analysis for *Arabidopsis* loci underlying an altered ROS-response to beta-glucan identified 6 statistically well supported peaks. SNPs and genes underlying these peaks are being interrogated fur-

ther. Selected 'extreme' ROS-response to beta-glucan differential accessions are undergoing detailed phenotyping for altered fungal colonization and the analysis of a putative receptor candidate is ongoing. We could demonstrate that the fungal lectins FGB1 and WSC1 are able to bind beta-glucan and are potentially involved in suppression of beta-glucan recognition and signaling in planta and in fungal cell wall structure.

Publications:

Fesel PH, Zuccaro A (2015) *Fungal Genetics and Biology: FG & B*.
Fesel PH, Zuccaro A (2016) *Current Opinion in Microbiology* 32:103-112.
Wawra S, Fesel P, Widmer H (2016) *Nature Communications* 7:13188.

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

Researcher:
Elaine Jaeger

Project leaders:
Gunther Döhlemann
Eric Kemen

Project type:
Associated Ph.D. project

Project start:
01.01.2015

Aim of the project:

Infection of *Zea mays* by the basidiomycete *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. The infection of seedling leaves, adult leaves and tassels induces organ-specific transcriptional changes in the pathogen as well as in the host. This project aims for a functional characterization of a leaf-specific *U. maydis* effector, which is predicted to encode an ß-L-arabinofuranosidase (Ara1).

Results:

Ara1 is required for tumour formation in leaves. Microscopic analysis shows that mutants for Ara1 are impaired in cell-cell penetration, particularly in bundle sheath cells. Enzyme assays showed that Ara1 is a specific α-L-arabinofuranosidase and by mutational analysis this activity was found to be required for virulence. Besides its tissue-specific activity, Ara1 is a maize line-specific virulence factor, a new finding in the *Ustilago* field.

C14 Cysteine proteases and their inhibitors in microbe-maize root interactions

Researcher:
Jan Schulze Hüynck

Project leader:
Gunther Döhlemann
Marcel Bucher

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.

Project type:
Associated Ph.D. project

Project start:
01.05.2016

Results:
MS analysis identified a new root PLCP in maize, CP1C, which might have distinct biochemical properties than the leaf paralogs. CP1C overexpression constructs for transient studies have been generated. A screen of 96 bacterial endophytes resulted in three candidates showing inhibition of PLCPs and seven showing activation of PLCPs. Putative candidates will be tested on root colonization assays. A sterile microcosms for maize colonization assays has been developed.

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

Researcher:
Debika Sarkar

Project leaders:
Alga Zuccaro, Marcel Bucher

Project type:
Associated Ph.D. project

Project start:
01.10.2014

Cooperation:
Michael Bonkowski

Aim of the project:
The recently sequenced *Serendipita vermifera* (MAFF 305830) is a root endophyte with wide-host spectrum beneficial effects while *Bipolaris sorokiniana* (ND90Pr) is a serious root pathogen of barley. Recent studies showed that *S. vermifera* is able to increase resistance of barley to *B. Sorokiniana*, suggesting a possible function as biological control. This project addresses two main aspects: (1) Do mutualistic and pathogenic fungi utilize similar strategies to colonize barley roots? (2) Does *S. vermifera* manipulates the plant immune system to increase resistance to pathogens and/or are there other mechanisms like antagonism in place?

Results:
RNA-seq and qPCR data of genes induced during fungus-fungus interaction suggest that *chitinase1* and *ß-1,3-exoglucanase* of *S. vermifera* might play a role in antagonism against *B. sorokiniana*. Furthermore, barley root interaction among the endophyte vs. the pathogenic fungus was analysed in a split root system. Our data showed that barley genes respond weakly to *S. vermifera* inoculation whereas a strong transcriptional response is present during *B. sorokiniana* infection. In the split root experiments, the presence of *S. vermifera* led to a significant decreased colonization of B. sorokiniana in natural soil. A systemic response was also detected and dissected.

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

Researcher:
Vera Göhre

Project leaders:
Michael Feldbrügge, Laura Rose

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).

Project type:
Associated Postdoc project

Project start:
01.10.2013

Cooperation:
Kaitlyn Courville
Lamprinos Frantzeskakis
Ronny Kellner, Eric Kemen

Results:
We have characterized the life-cycle of *T. thlaspeos* in its natural hosts, the perennial plants *Arabis alpina* and *Ar. hirsuta. T. thlaspeos* spreads systemically along the vasculature and uses conserved smut effectors such as Pep1 as well as dicot-specific effectors e.g. the NLPs. The colonization process in the model plant *A. thaliana* reflects the natural infection, and in the lab *T. thlaspeos* grows in haploid filamentous cultures. Currently, we are establishing a transformation protocol, which will enable engineering of plant beneficial pathways into the fungus.

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

Aim of the project:
To characterize the molecular basis underlying niche formation by the endophyte *T. thlaspeos* on different *Brassicacea* hosts and its impact on host microbiota. Using genetic approaches as well as association mapping, we want to answer the key questions: How does an endophyte interact with host microbiota and how does this influence host fitness? We hypothesize that *T. thlaspeos* has a significant impact on the associated microbiota. Thereby it might promote plant fitness and might show signatures of a host-microbe and microbe-microbe arms race.

Results:
To investigate *T. thlaspeos* – microbiota interactions, we have established two main approaches: first, we sampled *T. thlaspeos* infected *Brassicacea* in the field and have analysed microbial diversity; second, we have started reconstitution experiments where we pre-grow Brassicacea under axenic conditions, followed by inoculation with *T. thlaspeos* and microbial isolates we have identified as part of the microbiome in the field. With this we have established a gnotobiotic system, crucial to identify and verify underlying molecular mechanisms.

C18 The cumulative impact of environmental parameters to photosynthetic eukaryotes on molecular level

Researcher:
Ovidiu Popa

Project leader:
Oliver Ebenhöh

Project type:
CEPLAS funded via AG Ebenhöh

Aim of the project:
Plants are the most complex photoautotrophic organisms able to colonize a wide range of ecological niches for larger time periods. Adaptation to particular environments is the driving force for diversification and molecular evolution in all organisms. The domiciled ecological niche is described by a combination of abiotic and biotic constraints. Successful adaptation is the result of an evolutionary process, which optimises the molecular character of the organisms. The aim of this project is to characterise the effect of environmental factors to the genome.

Project start:
01.01.2015

Cooperation:
Ahmad Mannan (Imperial College London, UK)

Results:
A genome wide comparison of single copy genes from 80 *Arabidopsis thaliana* accessions with their orthologous genes from *Arabidopsis lyrata*, revealed a significant difference in the acquisition of mutations between functional categories and between *Arabidopsis* accessions. Further, we can observe in which genomic regions evolution acts most rapidly. Correlating the findings with environmental properties, we aim at quantifying the interplay between genome, phenotype and environment, thus in the long run making evolution predictable.

Determine reciprocal effects of host-microbe interactions on secondary metabolites

This is a new direction in Research Area C, which developed from close cooperation with Research Area D. Tryptophan-derived, indolic metabolites possess diverse functions in Arabidopsis innate immunity to microbial pathogen infection. We demonstrated the functional role and regulatory characteristics of indolic metabolism in Arabidopsis systemic acquired resistance (SAR) and its importance in maintaining a beneficial interaction with endophytes (Lahrmann *et al.*, 2015; Stahl *et al.*, 2016; Hiruma *et al.*, 2016). Based on these results a new project was initiated to explore the coordination of indole glucosinolate metabolism and ER body formation (Nakano et al., 2017). We expect to expand this field of study on metabolic interactions addressing the question: how does the (primary and secondary) metabolic status of the host affect and control complex traits during microbial interactions?

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

Researcher:
Ryohei Thomas Nakano

Project leader:
Paul Schulze-Lefert
Tamara Gigolashvili

Project type:
Postdoc project

Project start:
01.07.2015

Aim of the project:
The aim of this study is to explore the link between indole glucosinolate (IG) metabolism and ER bodies and its role in interactions with soil-borne microbes.

Results:
We have identified a potential functional link between ER bodies and IG metabolism. We have shown that PYK10, the major component of ER bodies, is a major myrosinase against IGs in *A. thaliana* roots. We also identified a tight transcriptional link between ER body system and IG metabolism, pointing to a functional engagement. PYK10 does not possess predicted basic glucosinolate-binding residues (R/K). A 3D structural modelling revealed another set of basic residues in a sequence-unrelated but structurally identical region, explaining the unpredicted my-

Cooperation:
Pawel Bednarek (Polish Academy of Science, Poland)
Christoph Böttcher (Julius Kühn-Institute, Germany)
Patrick P. Edger (Michigan State University, USA)
Ikuko Hara-Nishimura (Konan University, Japan)
Masashi Mori (Ishikawa Prefectural University, Japan)
Mikio Nishimura (National Institute for Basic Biology, Japan)
Kenji Yamada (Jagiellonian University, Poland)

rosinase activity of PYK10 and PEN2, conserved among 16 unannotated novel myrosinases. Classic and novel types myrosinases arose independently, based on maximum likelihood analysis. The study unveiled overlooked diversity of glucosinolate-myrosinase system (Nakano *et al*., 2017).

Publications:
Hiruma K, Gerlach N, Sacristan S, Nakano RT *et al*. (2016) Cell 165(2):464-474.
Nakano RT, Pislewska-Bednarek M, Yamada K *et al*. (2017) The Plant Journal: for cell and molecular biology 89(2):204-220.

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 start:
01.04.2016

Aim of the project:
This project investigates the impact of PGPRs and nitrogen-fixing and -metabolizing bacteria from the rhizosphere of *Arabidopsis* plants on the plant´s metabolome, their capacity to establish immune responses such as pathogen-induced SAR, SAR-associated defense priming, the biosynthesis of SAR-relevant defense metabolites, and signalling patterns induced by these metabolites.

Results:
An aeroponic cultivation system to grow *Arabidopsis thaliana* plants in an unstressed manner has been successfully developed in our lab. Similar to soil-grown *Arabidopsis* Col-0 plants, plants raised aeroponically were naïve and able to develop a strong biological SAR against the phytopathogenic bacterium *Pseudomonas syringae pv. maculicola*. This cultivation system can now be exploited to experimentally provide defined rhizosphere microbiomes and study the impact of these specific microbial environments on the capacities of plants to establish immune responses such as SAR.

Synthetic symbioses – shaping the microbiome to increase plant performance

The composition and functional properties of microbial communities have a significant influence on plant performance. We aim to elucidate the details of these interactions and their underlying mechanisms with the long-term goal of engineering artificial associations in major crops. As a proof of concept an additional project was recently added in this section.

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

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 signaling pathways and on environmental effects. With strong exchange with experimentalists from RA D, we start by focusing on plant exudates and bacteria of the *Rhizobium* genus to identify the relevant metabolic pathways for root colonization. We implement bioinformatics pipelines to analyze genomic features in relation to metabolic pathways and vice-versa and integrate this added knowledge into the stoichiometric model of *Rhizobium*, 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 selecting genome scale metabolic network models of different bacteria strains to reproduce their experimentally measured growth under different media condition reproducing possible root colonization environments.

Summary and Outlook

The proposed research program 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. 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. We aim to understand at multiple levels functional relationships between the plant, its associated microbes, soil metabolic activities, and the coordination between these physiological traits underlying plant health. Additionally, 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 pathogenic 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

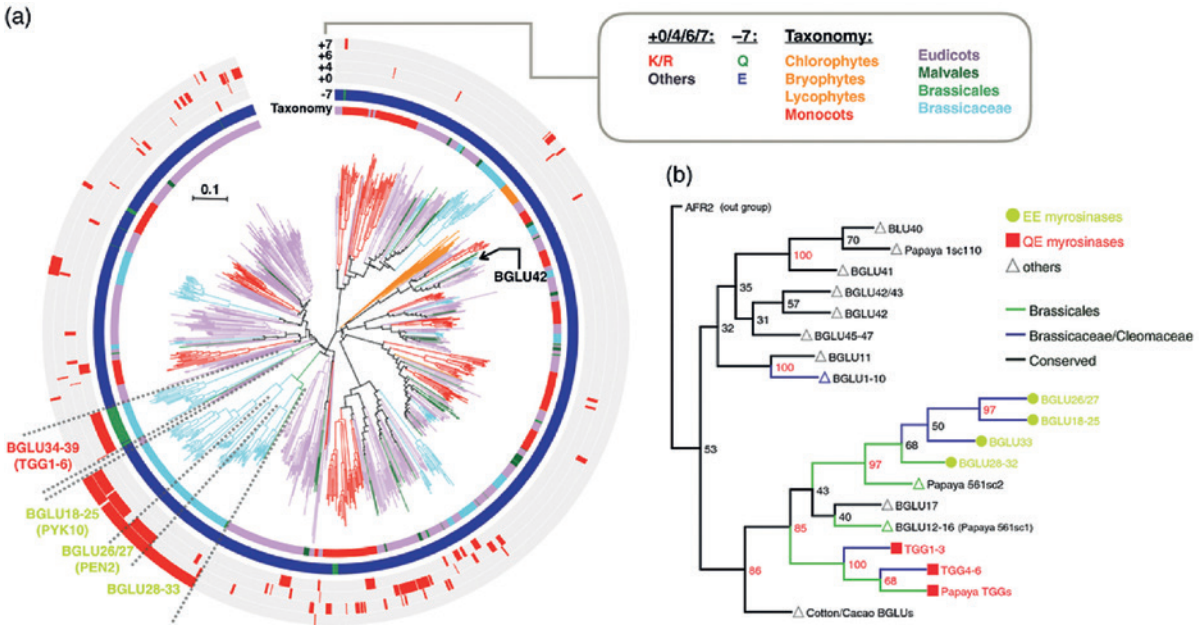
Members:
Faculty:
Ilka Axmann
Michael Bonkowski
Thomas Drepper
Oliver Ebenhöh
Ulf-Ingo Flügge
Tamara Gigolashvili
Stanislav Kopriva
Lutz Schmitt
Vlada B. Urlacher
Jürgen Zeier

Early career researchers:
Dorian Baumann
Dennis Dienst
Nikolas Ditz
Henning Frerigmann
Katharina Gräfe
Jennifer Hage-Hülsmann
Richard Jacoby
Sakshi Khosa
Sarah Kranz-Finger
Anita Loeschcke
Manuela Peukert
Kalpana Shanmugarajah
Suraj Sharma
Katharina Sklorz

Plants produce a great variety of biochemically unexplored secondary metabolites with plethora of functions. The chemical diversity of plant secondary metabolites is shaped by environmental cues including 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 two main interconnected themes:

Firstly, plant-microbe interactions are studied in the context of plant nutrition and defense reactions.
Special emphasis is placed on regulatory networks leading to the synthesis of glucosinolates and tryptophan-derived indolic compounds. These secondary metabolites play an important role in plant defense including systemic acquired resistance. In addition, we are using *Arabidopsis* ecotypes and a genome-wide association study to identify genes responsible for the observed genotype-specific differences in the rhizosphere microbiome. Candidate genes as well as the differences in root exudates in the selected *Arabidopsis* ecotypes and mutants will be further characterised, and their impact on plant-microbe interactions will be studied. For these types of experiments, a microbe collection has been established which covers the entire range of root-derived and sequenced bacteria strains provided by RA C.

Secondly, signalling molecules involved in plant-microbe interactions are identified and characterized and key compounds in synthetic microbal communities are produced.
This approach intends to understand reciprocal metabolic signalling in plant-microbe interactions. Moreover, further components of regulatory modules controlling the biosynthesis and transport of secondary metabolites in roots and in the rhizosphere are characterized. Special emphasis is placed on the biosynthesis of terpenoids. The introduction of plant biosynthetic modules in synthetic microbes such as *Rhodobacter capsulatus* and in cyanobacteria leading to the biosynthesis of triterpenes has been successful. This milestone will have a significant impact on developing next generation agricultural products and may also lead to biotechnological and pharmaceutical innovations.



Lineage specific distribution of b-glucosidases and glucosinolate-binding residues across the plant kingdom. (a) Neighbor-joining tree of 962 ß-glucosidases from 52 plant species with genome sequences deposited to the Phytozome database. (b) ß-Glucosidases from Brassicales and Malvales were subjected to maximum likelihood analysis. (Nakano RT, Pislewska-Bednarek M, Yamada K, Edger PP et al (2017) PYK10 myrosinase reveals a functional coordination between endoplasmic reticulum bodies and glucosinolates in *Arabidopsis thaliana*. The Plant Journal 89, 204–220)

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 using mutants deficient in the production of these secondary metabolites. In addition, predictive mathematical models to simulate secondary metabolite biosynthetic pathways, e.g. the biosynthesis of methionine-derived glucosinolates are established. 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. These genes are currently further investigated by multiple research groups. In addition, 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.

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
Pawel Bednarek
(Polish Academy of Sciences, Poland; MPIPZ Cologne)
Tamara Gigolashvili
Erich Glawischnig (TU München)
Ute Höcker
Stanislav Kopriva

Aim of the project:
Secondary metabolites are an important feature of plants to interact with their biotic environment. In *Brassicaceae*, glucosinolates (GLS) represent important secondary compounds and previous work indicated that GLS and their breakdown compounds might be key components for the interaction with plant growth promoting fungi. Therefore, we use GLS and related tryptophan-derived phytoalexins as a model for secondary metabolites, which might determine the assembly of the root microbiome.Thus this project aims to better understand the role of GLS regulation and their metabolism products for plant microbe interactions. The better characterised regulon might serve in the future as a good target to generate a synthetic plant with altered ability for specific plant microbe interactions.

Results:
It could be shown that GLS are not only of importance for the defense against the pathogen *Plectosphaerella cucumerina*, but GLS metabolism products serve also as a signal 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

Project start:
01.10.2014

Aim of the project:
Glucosinolates are an important class of sulphur-containing plant's secondary metabolites that play an important role in defence against pathogens. So, understanding the biosynthesis is key for deciphering plant-microbe interaction. We develop mathematical models to explain the factors governing and regulating the diversity and biosynthesis of these metabolites.

Results:
Our model can now realistically simulate different glucosinolate profiles and can help to identify which parameters determine the observed profile. We currently combine our computational analysis with bioinformatics studies, using the model as a connection between genotypic and phenotypic characteristics. Our model supports CEPLAS by i) supporting the targeted design of pathways producing specialised compounds, ii) understanding how genomic differences influence the metabolic profile and thus 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:
CEPLAS funded via AG Kopriva

Project start:
01.11.2013

Cooperation:
Oliver Ebenhöh
Karl-Erich Jaeger
Irene Klinkhammer
Achim Schmalenberger
(University of Limerick)
Lutz Schmitt
Paul Schulze-Lefert

Aim of the project:
We use natural variation in *Arabidopsis* accessions to study the basis of interaction of plants and bacteria in the rhizosphere. Enzymatic reaction of sulphates in rhizosphere is used as a measure of how the different ecotypes shape the bacterial community. The data is used for genome-wide association studies to identify genes underlying this variation. Thematically, the project is in the centre of RA D. It will identify genes underlying the ability of plants to shape their microflora. Genes involved in synthesis of secondary plant metabolites help selection of suitable compounds, enzymes, and transporters analysed in 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, including genes for P-450, terpene synthases, ABC transporters, which are being analysed in cooperation with other CEPLAS groups.

Publication:
Zgadzaj R, Garrido-Oter R, Jensen DB, Koprivova A (2016) Proceedings of the National Academy of Sciences of the United States of America 113(49):E7996-E8005.

D4 Plant secondary metabolites crucial in plant-microbe interactions

Researcher:
Manuela Peukert

Project leaders:
Stanislav Kopriva, Alga Zuccaro

Project type:
Postdoc project

Project start:
01.08.2015

Cooperation:
Sabine Metzger,
CEPLAS MS platform
Udo Seiffert (IFF Magdeburg)
Michael Kertesz
(University of Sydney)

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.

Results:
Experimental set-ups for exudate collection established and tested, showing effects of nutrient on exudate composition. Differences in response to co-cultivation with *Pseudomonas* were observed between WT *Arabidopsis* and *tt4* mutant deficient in flavonoids.

D5 Characterising plant-bacteria interactions that promote the uptake of nitrogen and sulphur from organic sources

Researcher:
Richard Jacoby

Project leaders:
Ulf-Ingo Flügge
Stanislav Kopriva

Project type:
Postdoc project

Project start:
01.06.2015

Cooperation:
Oliver Ebenhöh
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. Approximately 20 diverse bacterial isolates will be chosen from the MPIPZ's culture collection. Also, ten *Arabidopsis* accessions will be chosen according to their capacity to stimulate microbial activity. Co-cultivation experiments will be undertaken with the ~200 different combinations of 10 plants × 20 microbes, in hydroponic media where the only source of S & N is organic (delivered via protein extract). Metabolic flux pathways in top and bottom performing plant × microbe combinations will then be analysed, with mathematical expert of Prof. Ebenhöh from HHU collaborating with data analysis and interpretation. Also, genetic approaches will be undertaken to define key gene variants between contrasting genotypes.

Results:
This project has established a quantitative nutritional phenotyping strategy, which measures the capacity of MPIPZ's bacterial strains to grow upon various sources of organic N & S. This has shown that the strains exhibit a diversity of growth phenotypes across the different N & S sources. Current experiments are utilising proteomics, metabolic modelling, and plant-microbe interaction assays, aiming to pinpoint the specific microbial genes and metabolic pathways that are key for mediating plant nutrition from organic N & S sources.

D6 Regulatory and evolutionary aspects of stress-inducible plant metabolic pathways

Researcher:
Elia Stahl

Project leaders:
Jürgen Zeier
Vlada Urlacher

Project type:
Ph.D. project

Project start:
01.04.2013 – 31.03.2016

Cooperation:
Ulf-Ingo Flügge
Henning Frerigmann

Aim of the project:
The doctoral research project intended to identify new metabolites which are synthesized in plants after pathogen-infection, investigate their function in inducible plant defense, and define a regulatory network of pathogen inducible metabolite production in plants.

Results:
We showed that basal levels and induced production of tocopherols, a class of lipid-soluble antioxidants synthesized exclusively by photosynthetic organisms, contribute to *Arabidopsis* basal resistance against *Pseudomonas syringae* (Psm) by preventing oxidative damage of lipids (manuscript in preparation). In addition, we found that tryptophan-derived indole metabolism is activated in *Arabidopsis* after Psm inoculation. Indole accumulation is positively regulated by the immune regulatory metabolites salicylic acid and pipecolic acid, and contributes to non-host resistance against non-adapted pathogens. However, indolic metabolites are not required for the establishment of SAR (Stahl *et al.*, 2016).

Publications:
Stahl E, Bellwon P, Huber S (2016) Molecular Plant 9(5):662-681.
Sewelam N, Jaspert N, Van Der Kelen K (2014) Molecular Plant 7(7):1191-210.
Vogel-Adghough D, Stahl E, Návarová H (2013) Plant Signaling Behaviour 8(11):e26366.

Signalling molecules, metabolic modules, and synthetic microorganisms

Many bacteria use quorum sensing to co-ordinate their behaviour and to regulate a diverse variety of physiological processes including cell-population density. In plants, it is well established that sulphated-oligopeptides including phytosulfokines (PSKs) have growth-promoting effects. It is hypothesised that plant-associated bacteria (provided by RA C) use similar signal molecules to modulate plant growth and/or induce the formation of sulphated peptides. Plant triterpenes (e.g., marneral and thalianol) act downstream of these signalling peptides. The aim is to explore the PSK-triterpene pathway in plants and to identify bacteria acting on this pathway. Furthermore, this project aims at developing synthetic modules for the production of plant terpenoids (sequiterpenes, triterpenes and products derived thereof) in microbes. The production of triterpenes in was successfully demonstrated in *Rhodobacter capsulatus* and Cyanobacteria.

D7 Interactions of microbial endophytes with the glucosinolate metabolism in Brassicaceae

Researcher:
Katharina Sklorz

Project leaders:
Michael Bonkowski
Ulf Ingo-Flügge

Project type:
Ph.D. project

Project start:
01.03.2013

Cooperation:
Henning Frerigmann
Tamara Gigolashvili
Stanislav Kopriva,
Peter Leinweber
(Rostock University)

Aim of the project:
The projects aims to identify changes in plant root architecture and exudation in response to microbial signals.

Results:
We could show that specific signal molecules (acyl-homoserine lactones, AHLs) of gram-negative bacteria are perceived by *A. thaliana*, they induce specific phenological changes in root architecture and influence root exudation, thereby indicating a direct feed-back of plants on the rhizosphere microbial community.
Using hydroponic systems with sterile *A. thaliana*, we could identify several specific compounds released in root exudates after addition of certain AHLs compared to control plants. The unknown nature and high number of metabolites in root exudates poses analytic challenges. Further investigations using Pyrolysis-Field-Ionization MS in cooperation with Prof. Leinweber will enable us to identify the differing metabolic classes (analyses ongoing). In a second experiment effects of AHL molecules on exudation in mutants impaired in specific pathways of the glucosinolate metabolism are being investigated. In another experiment we investigated the impact of mutants of *A. thaliana* on

Sabine Metzger
(CEPLAS MS Platform)
Paul Schulze-Lefert

natural bacterial communities. The mutants lack either specific glucosinolates or camalexin or both and feedback of their exudates on microbial community composition is investigated by phospholipid fatty acid (PLFA) profiling. The PLFA method targets the membrane composition of microorganisms and is a sensitive and quantitative indicator of soil microbial communities.

D8 Exploring the phytosulfokinetriterpene pathway in *A. thaliana*

Researcher:
Dorian Baumann

Project leader:
Stanislav Kopriva

Project type:
Ph.D. project

Project start:
02.01.2015

Cooperation:
Henning Frerigmann
Tamara Gigolashvili
Tabea Mettler-Altmann
(CEPLAS MS Platform)
Sabine Metzger
(CEPLAS MS Platform)

Aim of the project:
We hypothesize that plants and beneficial soil bacteria communicate using signalling peptides. It is aimed to unravel the role of plant-derived peptides (phytosulfokines, PSK; root growth factors, RGF) in plant-bacteria communication and plant growth promotion. Furthermore, we will elucidate how the action of PSK is connected with the biosynthesis of the triterpenes marneral and thalianol and how plant secondary metabolites might be involved in plant-bacteria communication.

Results:
We previously showed that genes involved in the biosynthesis of marneral and thalianol are induced in response to exogenous PSK. By mutant analysis it could be demonstrated that different marneral pathway derivatives might be important for PSK-induced root growth in *A. thaliana*. It could be further shown that both marneral and thalianol may have similar but not identical functions in PSK-mediated root growth stimulation. In addition, we analysed the interaction of several root-associated bacterial isolates (RA C) with the PSK-triterpene pathway *in vivo and in silico*. Candidate strains containing a putative bacterial sulfotransferase (TPST) and presumably involved in PSK-triterpene signalling were identified by co-cultivation experiments and bioinformatic analyses.

D9 Synthetic microbes for the production of plant secondary metabolites

Researcher:
Jennifer Hage-Hülsmann

Project leaders:
Karl-Erich Jaeger, Thomas Drepper

Project type:
Ph.D. project

Project start:
01.11.2015

Aims of the project:
Synthetic modules are constructed for the production of plant terpenoids in microbes to study their biosynthesis and functional role. The role of *quorum sensing* (QS) systems for interaction of *Burkholderia glumae* with *Arabidopsis thaliana* is also studied.

Results:
We constructed a triterpenoid precursor biosynthesis module, which enabled the biosynthetic production of squalene and 2,3-oxidosqualene in the carotenogenic bacterium *Rhodobacter capsulatus*. Four model triterpene biosynthesis modules including the marneral synthase (MRN1), the thalianol synthase (THAS1), the cycloartenol synthase (CAS1) and the lupeol synthase (LUP1) from *A. thaliana* were constructed and evaluated. Triterpene formation could successfully be demonstrated in R. capsulatus strains expressing CAS1 and LUP1. *B. glumae* wild

Cooperation:
Ilka Axmann, Marcel Bucher
Ulf-Ingo Flügge
Tamara Gigolashvili
Stanislav Kopriva, Sabine Metzger
(CEPLAS MS Platform)
Lutz Schmitt, Vlada Urlacher

type reduced growth of *A. thaliana*, and one out of three QS mutants showed a significantly stronger reduction effect whereas expression of *A. thaliana* marker genes was inversely correlated.

Publication:
Binder D, Bier C, Grunberger A (2016) Chembiochem: a European journal of chemical biology 17(4):296-299.

D10 Programming triterpene biosynthesis pathways in cyanobacteria using synthetic RNA-based devices

Researcher:
Dennis Dienst

Project leader:
Ilka Axmann

Project type:
Postdoc project

Project start:
01.08.2014

Cooperation:
Thomas Drepper, Sabine Metzger
Vlada Urlacher, Pia Lindberg
(Uppsala University, Sweden)
Sven Findeiß
(University of Vienna, Austria)
André Estévez-Torres
(CNRS Paris, France)

Aim of the project:
Engineering biosynthesis of plant triterpenes in cyanobacteria (finally targeting plant growth promotion, cf. D2 and D5) and development of universal synthetic regulatory (RNA-based) modules for optimising the microbial pathway flux (also for plant-associated bacteria).

Results:
The squalene accumulating strain Δ*shc* of *Synechocystis* served as host for Co^{2+}-inducible co-expression of squalene epoxidase and two separate oxidosqualene cyclases (OSC). The corresponding mRNAs were detected; the OSC transcripts accumulated constitutively, supporting the rationale for arranging the genes separately within independent genetic modules. The conversion of squalene to oxidosqualene was demonstrated by GC-MS. An LC-MS-MS-based detection workflow has been established at University of Cologne, demonstrating synthesis of diverse triterpenes. Engineering of promoter-based logic gates for tuneable production is in progress. A platform for measuring regulatory RNA activity in *Synechocystis* has been established and is exploited for the logic gate installation. Metabolic network modelling combined with a photobioreactor setup monitoring highly controlled growth of producer strains will help to characterize and optimize the process.

D11 Functional expression and biochemical characterization of plant P450s

Researcher:
Sarah Kranz-Finger

Project leaders:
Vlada Urlacher
Karl-Erich Jaeger

Project type:
Ph.D. project

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, thalianol

Project start:
01.04.2013

Cooperation:
Ulf-Ingo Flügge
Tamara Gigolashvili
Karl-Erich Jaeger
Lutz Schmitt

hydroxylating CYP708A2 as well as their redox partner ATR 2 from *A. thaliana* were expressed in *E. coli* in functional state.
3) Marnerol pathway was established and synthesized in S. cerevisiae and applied for the biochemical characterization of CYP71A16 *in vitro*.
4) Binding studies with CYP71A16 and CYP705A12 were performed for substrate screening.
5) Bacterial CYP mutants with activity towards α-amyrin, ß-amyrin and lupeol were identified.
6) Oxygenated products of the above mentioned reactions were purified and analysed by NMR.

D12 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:
Ulf-Ingo Flügge
Lutz Schmitt
Tamara Gigolashvili

Aim of the project:
Whereas plant mono-, di- and sesquiterpene cyclases have been intensively studied in the last 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 from *A. thaliana*. Furthermore, biochemical studies on plant CYPs involved in triterpene pathway will be continued. 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:
The gene coding for marneral cyclase Mrn1 from *A. thaliana* was cloned in *E. coli* and *Pichia pastoris*.
Expression of Mrn1 in *E. coli* and P. pastoris was optimized and soluble protein observed. CYP705A12 from *A. thaliana* was expressed in *E. coli* and purified to homogeneity.

D13 In vitro analysis of selected ABC transporters from plants

Researcher:
Katharina Gräfe
Kalpana Shanmugarajah

Project leaders:
Lutz Schmitt
Andreas Weber
Karl-Erich Jaeger

Project type:
Ph.D. project

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*. Reconstitution on artificial liposomes has been initiated. In parallel, transport studies aiming to identify the natural substrate(s) of these transporters by mass spectrometry have been started. Here, we will employ plasma membrane vesicles (PMVs) containing the wild-type transporter or an ATPase deficient mutant. PMVs will be incubated with the cytosol of plant roots in the presence of ATP. After isolation of PMVs, the luminal content of wild type and ATPase

Project start:
01.06.2013

Cooperation:
Ulf-Ingo Flügge
Stanislav Kopriva

deficient mutant will be determined by MS. Differences in the MS spectra should provide first insights in to the nature of the substrate(s).

D14 *In vitro* analysis of selected ABC transporters from plants

Researcher:
Sakshi Khosa

Project leaders:
Lutz Schmitt,
Karl-Erich Jaeger

Project type:
Postdoc project

Project start:
01.01.2016

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. Further optimizations and trials in presence of nucleotides would provide insights into the structure of the protein. Simultaneously, studies employed to determine the substrate(s) of the ABC protein via mass spectrometry have been initiated. Here, the tagged wild type protein and the ATPase deficient mutant is used to create an affinity matrix and incubated with the cytosol of the plant roots in the presence of ATP. Subsequently, substrate(s) that interact with the protein can be purified and analysed by MS. Differences in the spectra of the wild type and the ATPase deficient mutant will provide preliminary information about the possible substrate(s).

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 defense compounds 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 should allow us to further exploit their bioactive potential.

1) Aarabi F, Kusajima M, Tohge T, Konishi T, **Gigolashvili T,** Takamune M, Sasazaki Y, Watanabe M, Nakashita H, Fernie AR, Saito K, Takahashi H, Hubberten HM, Hoefgen R, Maruyama-Nakashita A (2016) Sulfur deficiency-induced repressor proteins optimize glucosinolate biosynthesis in plants. *Sci Adv* 2(10):e1601087.

2) Abdallah HB, **Bauer P** (2016) Quantitative Reverse Transcription-qPCR-Based Gene Expression Analysis in Plants. *Methods Mol Biol* 1363:9-24.

3) Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, **Kemen EM** (2016) Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLoS Biol* 14(1):e1002352.

4) **Bauer P** (2016) Regulation of iron acquisition responses in plant roots by a transcription factor. *Biochem Mol Biol Educ* 44(5):438-449.

5) Bemm F, Becker D, Larisch C, Kreuzer I, Escalante-Perez M, Schulze WX, Ankenbrand M, Van de Weyer AL, Krol E, Al-Rasheid KA, Mithofer A, **Weber AP,** Schultz J, Hedrich R (2016) Venus flytrap carnivorous lifestyle builds on herbivore defense strategies. *Genome Res* 26(6):812-825.

6) Bernsdorff F, Döring AC, Gruner K, Schuck S, **Bräutigam A, Zeier J** (2016) Pipecolic Acid Orchestrates Plant Systemic Acquired Resistance and Defense Priming via Salicylic Acid-Dependent and -Independent Pathways. *Plant Cell* 28(1):102-129.

7) Betti M, Bauwe H, Busch FA, Fernie AR, Keech O, Levey M, Ort DR, Parry MA, Sage R, Timm S, **Walker B, Weber AP** (2016) Manipulating photorespiration to increase plant productivity: recent advances and perspectives for crop improvement. *J Exp Bot* 67(10):2977-2988.

8) Bombarely A, Moser M, Amrad A, Bao M, Bapaume L, Barry CS, Bliek M, Boersma MR, Borghi L, Bruggmann R, **Bucher M**, D'Agostino N, Davies K, Druege U, Dudareva N, Egea-Cortines M, Delledonne M, Fernandez-Pozo N, Franken P, Grandont L, Heslop-Harrison JS, Hintzsche J, Johns M, Koes R, Lv X, Lyons E, Malla D, Martinoia E, Mattson NS, Morel P, Mueller LA, Muhlemann J, Nouri E, Passeri V, Pezzotti M, Qi Q, Reinhardt D, Rich M, Richert-Poggeler KR, Robbins TP, Schatz MC, Schranz ME, Schuurink RC, Schwarzacher T, Spelt K, Tang H, Urbanus SL, Vandenbussche M, Vijverberg K, Villarino GH, Warner RM, Weiss J, Yue Z, Zethof J, Quattrocchio F, Sims TL, Kuhlemeier C (2016) Insight into the evolution of the Solanaceae from the parental genomes of *Petunia hybrida. Nat Plants* 2(6):16074.

9) Bösch K, Frantzeskakis L, Vranes M, Kamper J, Schipper K, **Göhre V** (2016) Genetic Manipulation of the Plant Pathogen *Ustilago maydis* to Study Fungal Biology and Plant Microbe Interactions. *J Vis Exp* (115).

10) Braguy J, **Zurbriggen MD** (2016) Synthetic strategies for plant signalling studies: molecular toolbox and orthogonal platforms. *Plant J 87*(1):118-138.

11) Breiden M, **Simon R** (2016) Q&A: How does peptide signaling direct plant development? *BMC Biol* 14:58.

12) Brilhaus D, **Bräutigam A, Mettler-Altmann T**, Winter K, **Weber AP** (2016) Reversible Burst of Transcriptional Changes during Induction of Crassulacean Acid Metabolism in *Talinum triangulare*. *Plant Physiol* 170(1):102-122.

13) Brumbarova T, Ivanov R (2016) Differential Gene Expression and Protein Phosphorylation as Factors Regulating the State of the *Arabidopsis* SNX1 Protein Complexes in Response to Environmental Stimuli. *Front Plant Sci 7*:1456.

14) Brumbarova T, Le C, **Bauer P** (2016) Hydrogen Peroxide Measurement in *Arabidopsis* Root Tissue Using Amplex Red. *Bio-Protocol* 6(21).

15) Brumbarova T, Le CT, Ivanov R, **Bauer P** (2016) Regulation of ZAT12 protein stability: The role of hydrogen peroxide. *Plant Signal Behav* 11(2):e1137408.

16) **Bucher M**, Fabianska I (2016) Long-Sought Vacuolar Phosphate Transporters Identified. *Trends Plant Sci* 21(6):463-466.

17) Calderwood A, **Kopriva S,** Morris RJ (2016) Transcript Abundance Explains mRNA Mobility Data in *Arabidopsis thaliana*. *Plant Cell* 28(3):610-615.

18) Chan KX, Mabbitt PD, Phua SY, Mueller JW, Nisar N, **Gigolashvili T**, Stroeher E, Grassl J, Arlt W, Estavillo GM, Jackson CJ, Pogson BJ (2016) Sensing and signaling of oxidative stress in chloroplasts by inactivation of the SAL1 phosphoadenosine phosphatase. *Proc Natl Acad Sci U S A* 113(31):E4567-4576.

19) Chen S, Wirthmueller L, Stauber J, Lory N, Holtkotte X, Leson L, Schenkel C, Ahmad M, **Hoecker U** (2016) The functional divergence between SPA1 and SPA2 in *Arabidopsis* photomorphogenesis maps primarily to the respective N-terminal kinase-like domain. *BMC Plant Biol* 16(1):165.

20) Chen WH, Lu G, Bork P, Hu S, **Lercher MJ** (2016) Energy efficiency trade-offs drive nucleotide usage in transcribed regions. *Nat Commun* 7:11334.

21) Christopher A, Hameister H, Corrigall H, **Ebenhöh O**, Müller B, Ullner E (2016) Modelling Robust Feedback Control Mechanisms That Ensure Reliable Coordination of Histone Gene Expression with DNA Replication. *PLoS One* 11(10):e0165848.

22) Dellero Y, Jossier M, Schmitz J, **Maurino VG**, Hodges M (2016) Photorespiratory glycolate-glyoxylate metabolism. *J Exp Bot* 67(10):3041-3052.

23) Digel B, Tavakol E, Verderio G, Tondelli A, Xu X, Cattivelli L, Rossini L, **von Korff M** (2016) Photoperiod-H1 (Ppd-H1) Controls Leaf Size. *Plant Physiol* 172(1):405-415.

24) Drincovich MF, Voll LM, **Maurino VG** (2016) Editorial: On the Diversity of Roles of Organic Acids. *Front Plant Sci 7*(1592).

25) Feike D, Seung D, Graf A, Bischof S, Ellick T, Coiro M, Soyk S, Eicke S, **Mettler-Altmann T**, Lu KJ, Trick M, Zeeman SC, Smith AM (2016) The Starch Granule-Associated Protein EARLY STARVATION1 Is Required for the Control of Starch Degradation in *Arabidopsis thaliana* Leaves. *Plant Cell* 28(6):1472-1489.

26) Fernandez V, Takahashi Y, Le Gourrierec J, **Coupland G** (2016) Photoperiodic and thermosensory pathways interact through CONSTANS to promote flowering at high temperature under short days. *Plant J* 86(5):426-440.

27) **Fesel PH, Zuccaro A** (2016) beta-glucan: Crucial component of the fungal cell wall and elusive MAMP in plants. *Fungal Genet Biol* 90:53-60.

28) **Fesel PH, Zuccaro A** (2016) Dissecting endophytic lifestyle along the parasitism/mutualism continuum in *Arabidopsis*. *Curr Opin Microbiol* 32:103-112.

29) **Flügge U, Westhoff P**, Leister D (2016) Recent advances in understanding photosynthesis [version 1; referees: 3 approved]. *F1000Research2016 5*(2890).

30) **Frerigmann H**, Pislewska-Bednarek M, Sanchez-Vallet A, Molina A, Glawischnig E, **Gigolashvili T**, Bednarek P (2016) Regulation of Pathogen-Triggered Tryptophan Metabolism in *Arabidopsis thaliana* by MYB Transcription Factors and Indole Glucosinolate Conversion Products. *Mol Plant* 9(5):682-695.

31) **Frerigmann H** (2016) Chapter Four - Glucosinolate Regulation in a Complex Relationship – MYC and MYB – No One Can Act Without Each Other. *Adv Bot Res*, ed Stanislav K (Academic Press), Vol Volume 80, pp 57-97.

32) Gan X, **Hay A**, Kwantes M, Haberer G, Hallab A, Ioio RD, Hofhuis H, Pieper B, Cartolano M, Neumann U, Nikolov LA, Song B, Hajheidari M, Briskine R, Kougioumoutzi E, Vlad D, Broholm S, Hein J, Meksem K, Lightfoot D, Shimizu KK, Shimizu-Inatsugi R, Imprialou M, Kudrna D, Wing R, Sato S, Huijser P, Filatov D, Mayer KF, Mott R, **Tsiantis M** (2016) The *Cardamine hirsuta* genome offers insight into the evolution of morphological diversity. *Nat Plants* 2(11):16167.

33) Hacquard S, Kracher B, Hiruma K, Munch PC, **Garrido-Oter R**, Thon MR, Weimann A, Damm U, Dallery JF, Hainaut M, Henrissat B, Lespinet O, Sacristan S, Ver Loren van Themaat E, **Kemen E, McHardy AC, Schulze-Lefert P**, O'Connell RJ (2016) Survival trade-offs in plant roots during colonization by closely related beneficial and pathogenic fungi. *Nat Commun* 7:11362.

34) Hagemann M, Kern R, **Maurino VG**, Hanson DT, **Weber AP**, Sage RF, Bauwe H (2016) Evolution of photorespiration from cyanobacteria to land plants, considering protein phylogenies and acquisition of carbon concentrating mechanisms. *J Exp Bot 67*(10):2963-2976.

35) Hampel M, Jakobi M, Schmitz L, Meyer U, Finkernagel F, **Doehlemann G**, Heimel K (2016) Unfolded Protein Response (UPR) Regulator Cib1 Controls Expression of Genes Encoding Secreted Virulence Factors in *Ustilago maydis. PLoS One* 11(4):e0153861.

36) Hartleb D, Jarre F, **Lercher MJ** (2016) Improved Metabolic Models for *E. coli* and *Mycoplasma genitalium* from GlobalFit, an Algorithm That Simultaneously Matches Growth and Non-Growth Data Sets. *PLoS Comput Biol* 12(8):e1005036.

37) **Hay A, Tsiantis M** (2016) *Cardamine hirsuta:* a comparative view. *Curr Opin Genet Dev* 39:1-7.

38) He Z, Zhang H, Gao S, **Lercher MJ**, Chen WH, Hu S (2016) Evolview v2: an online visualization and management tool for customized and annotated phylogenetic trees. *Nucleic Acids Res* 44(W1):W236-241.

39) **Heidel AJ, Kiefer C, Coupland G, Rose LE** (2016) Pinpointing genes underlying annual/perennial transitions with comparative genomics. *BMC Genomics* 17(1):921.

40) Hiruma K, Gerlach N, Sacristan S, **Nakano RT**, Hacquard S, Kracher B, Neumann U, Ramirez D, **Bucher M**, O'Connell RJ, **Schulze-Lefert P** (2016) Root Endophyte Colletotrichum tofieldiae Confers Plant Fitness Benefits that Are Phosphate Status Dependent. *Cell* 165(2):464-474.

41) Holtkotte X, Dieterle S, Kokkelink L, Artz O, Leson L, Fittinghoff K, Hayama R, Ahmad M, **Hoecker U** (2016) Mutations in the N-terminal kinase-like domain of the repressor of photomorphogenesis SPA1 severely impair SPA1 function but not light responsiveness in *Arabidopsis. Plant J* 88(2):205-218.

42) Huang XY, Chao DY, **Koprivova A**, Danku J, Wirtz M, Müller S, Sandoval FJ, Bauwe H, Roje S, Dilkes B, Hell R, **Kopriva S**, Salt DE (2016) Nuclear Localised MORE SULPHUR ACCUMULATION1 Epigenetically Regulates Sulphur Homeostasis in *Arabidopsis thaliana*. *PLoS Genet* 12(9):e1006298.

43) Hussner A, **Mettler-Altmann T, Weber APM,** Sand-Jensen K (2016) Acclimation of photosynthesis to supersaturated CO_2 in aquatic plant bicarbonate users. *Freshwater Biology* 61(10):1720-1732.

44) Hyun Y, **Richter R,** Vincent C, Martinez-Gallegos R, Porri A, **Coupland G** (2016) Multi-layered Regulation of SPL15 and Cooperation with SOC1 Integrate Endogenous Flowering Pathways at the *Arabidopsis* Shoot Meristem. *Dev Cell* 37(3):254-266.

45) **Jacoby RP**, Che-Othman MH, Millar AH, Taylor NL (2016) Analysis of the sodium chloride-dependent respiratory kinetics of wheat mitochondria reveals differential effects on phosphorylating and non-phosphorylating electron transport pathways. *Plant Cell Environ* 39(4):823-833.

46) Jaegle B, **Uroic MK**, Holtkotte X, **Lucas C,** Termath AO, Schmalz HG, **Bucher M, Hoecker U**, **Hülskamp M**, Schrader A (2016) A fast and simple LC-MS-based characterization of the flavonoid biosynthesis pathway for few seed(ling)s. *BMC Plant Biol* 16(1):190.

47) **Kopriva S, Gigolashvili T** (2016) Chapter Five - Glucosinolate Synthesis in the Context of Plant Metabolism. *Adv Bot Res*, ed Stanislav K (Academic Press), Vol Volume 80, pp 99-124.

48) **Koprivova A, Kopriva S** (2016) Sulfation pathways in plants. *Chem Biol Interact* 259(Pt A):23-30.

49) **Koprivova A, Kopriva S** (2016) Sulfur metabolism and its manipulation in crops. *J Genet Genomics* 43(11):623-629.

50) **Koprivova A, Kopriva S** (2016) Hormonal control of sulfate uptake and assimilation. *Plant Mol Biol* 91(6):617-627.

51) Korte J, Alber M, Trujillo CM, Syson K, Koliwer-Brandl H, Deenen R, **Köhrer K**, DeJesus MA, Hartman T, Jacobs WR, Jr., Bornemann S, Ioerger TR, Ehrt S, Kalscheuer R (2016) Trehalose-6-Phosphate-Mediated Toxicity Determines Essentiality of OtsB2 in *Mycobacterium tuberculosis In Vitro* and in Mice. *PLoS Pathog* 12(12):e1006043.

52) Le CT, Brumbarova T, Ivanov R, Stoof C, Weber E, Mohrbacher J, Fink-Straube C, **Bauer P** (2016) ZINC FINGER OF ARABIDOPSIS THALIANA12 (ZAT12) Interacts with FER-LIKE IRON DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT) Linking Iron Deficiency and Oxidative Stress Responses. *Plant Physiol* 170(1):540-557.

53) Le-Huu P, Petrović D, Strodel B, **Urlacher VB** (2016) One-Pot, Two-Step Hydroxylation of the Macrocyclic Diterpenoid ß-Cembrenediol Catalyzed by P450 BM3 Mutants. *ChemCatChem* 8(24):3755-3761.

54) Lehmann MM, Wegener F, Barthel M, **Maurino VG**, Siegwolf RT, Buchmann N, Werner C, Werner RA (2016) Metabolic Fate of the Carboxyl Groups of Malate and Pyruvate and their Influence on delta(13)C of Leaf-Respired CO_2 during Light Enhanced Dark Respiration. *Front Plant Sci* 7:739.

55) Mackinder LCM, Meyer MT, **Mettler-Altmann T,** Chen VK, Mitchell MC, Caspari O, Freeman Rosenzweig ES, Pallesen L, Reeves G, Itakura A, Roth R, Sommer F, Geimer S, Mühlhaus T, Schroda M, Goodenough U, Stitt M, Griffiths H, Jonikas MC (2016) A repeat protein links Rubisco to form the eukaryotic carbon-concentrating organelle. *Proc Natl Acad Sci U S A* 113(21):5958-5963.

56) Mai H-J, **Bauer P** (2016) From the proteomic point of view: Integration of adaptive changes to iron deficiency in plants. *Current Plant Biology* 5:45-56.

57) Mai HJ, Pateyron S, **Bauer P** (2016) Iron homeostasis in *Arabidopsis thaliana:* transcriptomic analyses reveal novel FIT-regulated genes, iron deficiency marker genes and functional gene networks. *BMC Plant Biol* 16(1):211.

58) Maillard A, Sorin E, Etienne P, Diquelou S, **Koprivova A, Kopriva S,** Arkoun M, Gallardo K, Turner M, Cruz F, Yvin JC, Ourry A (2016) Non-Specific Root Transport of Nutrient Gives Access to an Early Nutritional Indicator: The Case of Sulfate and Molybdate. *PLoS One* 11(11):e0166910.

59) Matei A, **Doehlemann G** (2016) Cell biology of corn smut disease-*Ustilago maydis* as a model for biotrophic interactions. *Curr Opin Microbiol* 34:60-66.

60) Matsubara S, Schneider T, **Maurino VG** (2016) Dissecting Long-Term Adjustments of Photoprotective and Photo-Oxidative Stress Acclimation Occurring in Dynamic Light Environments. *Front Plant Sci* 7:1690.

61) **Matuszyńska A**, Heidari S, Jahns P, **Ebenhöh O** (2016) A mathematical model of non-photochemical quenching to study short-term light memory in plants. *Biochim Biophys Acta* 1857(12):1860-1869.

62) Misas-Villamil JC, van der Hoorn RA, **Doehlemann G** (2016) Papain-like cysteine proteases as hubs in plant immunity. *New Phytol* 212(4):902-907.

63) Mulki MA, **von Korff M** (2016) CONSTANS Controls Floral Repression by Up-Regulating VERNALIZATION2 (VRN-H2) in Barley. *Plant Physiol* 170(1):325-337.

64) Naranjo Arcos M.A. and **Bauer P** (2016) Iron Nutrition, Oxidative Stress, and Pathogen Defense. Nutritional Deficiency, Dr. Pinar Erkekoğlu (Ed.), InTech, DOI: 10.5772/63204.

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

66) **Ökmen B, Doehlemann G** (2016) Clash between the borders: spotlight on apoplastic processes in plant-microbe interactions. *New Phytol* 212(4):799-801.

67) Pires MV, Pereira Junior AA, Medeiros DB, Daloso DM, Pham PA, Barros KA, Engqvist MK, Florian A, Krahnert I, **Maurino VG**, Araujo WL, Fernie AR (2016) The influence of alternative pathways of respiration that utilize branched-chain amino acids following water shortage in *Arabidopsis. Plant Cell Environ* 39(6):1304-1319.

68) **Ploch S, Rose LE**, Bass D, **Bonkowski M** (2016) High Diversity Revealed in Leaf-Associated Protists (Rhizaria: Cercozoa) of Brassicaceae. *J Eukaryot Microbiol* 63(5):635-641.

69) Pokhilko A, Zhao J, **Ebenhöh O**, Smith MC, Stark WM, Colloms SD (2016) The mechanism of varphiC31 integrase directionality: experimental analysis and computational modelling. *Nucleic Acids Res* 44(15):7360-7372.

70) Redkar A, **Doehlemann G** (2016) EdU Based DNA Synthesis and Cell Proliferation Assay in Maize Infected by the Smut Fungus *Ustilago maydis. Bio-protocol* 6(6):e1761.

71) Redkar A, **Doehlemann G** (2016) *Ustilago maydis* Virulence Assays in Maize. *Bio-protocol* 6(6):e1760.

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

73) Rhee SY, **Parker JE,** Mockler TC (2016) A glimpse into the future of genome-enabled plant biology from the shores of Cold Spring Harbor. *Genome Biol* 17:3.

74) Rovenich H, **Zuccaro A**, Thomma BP (2016) Convergent evolution of filamentous microbes towards evasion of glycan-triggered immunity. *New Phytol* 212(4):896-901.

75) Ruhe J, Agler MT, Placzek A, Kramer K, Finkemeier I, **Kemen EM** (2016) Obligate Biotroph Pathogens of the Genus Albugo Are Better Adapted to Active Host Defense Compared to Niche Competitors. *Front Plant Sci 7*:820.

76) Sadowsky A, **Mettler-Altmann T**, Ott S (2016) Metabolic response to desiccation stress in strains of green algal photobionts (Trebouxia) from two Antarctic lichens of southern habitats. *Phycologia* 55(6):703-714.

77) Samodelov SL, Beyer HM, Guo X, Augustin M, Jia KP, Baz L, **Ebenhöh O**, Beyer P, Weber W, Al-Babili S, **Zurbriggen MD** (2016) StrigoQuant: A genetically encoded biosensor for quantifying strigolactone activity and specificity. *Sci Adv* 2(11):e1601266.

78) Schlüter U, Denton AK, **Bräutigam A** (2016) Understanding metabolite transport and metabolism in C_4 plants through RNA-seq. *Curr Opin Plant Biol* 31:83-90.

79) **Schlüter U, Weber APM** (2016) The Road to C_4 Photosynthesis: Evolution of a Complex Trait via Intermediary States. *Plant and Cell Physiology* 57(5):881-889.

80) Schneider A, Steinberger I, Herdean A, Gandini C, Eisenhut M, Kurz S, Morper A, **Hoecker N**, Ruhle T, Labs M, **Flügge UI**, Geimer S, Schmidt SB, Husted S, **Weber AP**, Spetea C, Leister D (2016) The Evolutionarily Conserved Protein PHOTOSYNTHESIS AFFECTED MUTANT71 Is Required for Efficient Manganese Uptake at the Thylakoid Membrane in *Arabidopsis*. *Plant Cell* 28(4):892-910.

81) **Schuler ML, Mantegazza O, Weber AP** (2016) Engineering C_4 photosynthesis into C_3 chassis in the synthetic biology age. ***Plant J*** 87(1):51-65.

82) Smith RW, Helwig B, Westphal AH, Pel E, Horner M, Beyer HM, Samodelov SL, Weber W, **Zurbriggen MD**, Borst JW, Fleck C (2016) Unearthing the transition rates between photoreceptor conformers. *BMC Syst Biol* 10(1):110.

83) Somssich M, Bleckmann A, **Simon R** (2016) Shared and distinct functions of the pseudokinase CORYNE (CRN) in shoot and root stem cell maintenance of *Arabidopsis*. J Exp Bot *67(16):4901-4915.*

84) Somssich M, Je BI, **Simon R**, Jackson D (2016) CLAVATA-WUSCHEL signaling in the shoot meristem. *Development* 143(18):3238-3248.

85) **Stahl E**, Bellwon P, Huber S, Schlaeppi K, Bernsdorff F, Vallat-Michel A, Mauch F, **Zeier J** (2016) Regulatory and Functional Aspects of Indolic Metabolism in Plant Systemic Acquired Resistance. *Mol Plant* 9(5):662-681.

86) Szappanos B, Fritzemeier J, Csorgo B, Lazar V, Lu X, Fekete G, Balint B, Herczeg R, Nagy I, Notebaart RA, **Lercher MJ**, Pal C, Papp B (2016) Adaptive evolution of complex innovations through stepwise metabolic niche expansion. *Nat Commun* 7:11607.

87) Taniguchi M, **Weber AP**, von Caemmerer S (2016) Future Research into C_4 Biology. ***Plant Cell Physiol*** 57(5):879-880.

88) Vijayakumar V, Liebisch G, Buer B, Xue L, Gerlach N, Blau S, Schmitz J, **Bucher M** (2016) Integrated multi-omics analysis supports role of lysophosphatidylcholine and related glycerophospholipids in the *Lotus japonicus-Glomus intraradices* mycorrhizal symbiosis. *Plant Cell Environ* 39(2):393-415.

89) 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 doi: 10.1111/micc.12345

90) Vuolo F, Mentink RA, Hajheidari M, Bailey CD, Filatov DA, **Tsiantis M** (2016) Coupled enhancer and coding sequence evolution of a homeobox gene shaped leaf diversity. *Genes Dev* 30(21):2370-2375.

91) Wang Q, Hasson A, Rossmann S, **Theres K** (2016) Divide et impera: boundaries shape the plant body and initiate new meristems. *New Phytol* 209(2):485-498.

92) **Wawra S, Fesel P, Widmer H,** Timm M, Seibel J, Leson L, Kesseler L, Nostadt R, Hilbert M, Langen G, **Zuccaro A** (2016) The fungal-specific ß-glucan-binding lectin FGB1 alters cell-wall composition and suppresses glucan-triggered immunity in plants. *Nat Commun* 7:13188.

93) Weiss M, Waller F, **Zuccaro A**, Selosse MA (2016) *Sebacinales* - one thousand and one interactions with land plants. *New Phytol* 211(1):20-40.

94) Welchen E, Schmitz J, Fuchs P, Garcia L, Wagner S, Wienstroer J, Schertl P, Braun HP, Schwarzländer M, Gonzalez DH, **Maurino VG** (2016) d-Lactate Dehydrogenase Links Methylglyoxal Degradation and Electron Transport through Cytochrome c. *Plant Physiol* 172(2):901-912.

95) Xu J, **Bräutigam A**, Li Y, **Weber AP**, Zhu XG (2016) Systems analysis of cis-regulatory motifs in C_4 photosynthesis genes using maize and rice leaf transcriptomic data during a process of de-etiolation. *J Exp Bot*.

96) Yanai I, **Lercher MJ** (2016) Forty years of The Selfish Gene are not enough. *Genome Biol* 17:39.

97) Zgadzaj R, **Garrido-Oter R**, Jensen DB, **Koprivova A, Schulze-Lefert P**, Radutoiu S (2016) Root nodule symbiosis in *Lotus japonicus* drives the establishment of distinctive rhizosphere, root, and nodule bacterial communities. *Proc Natl Acad Sci U S A* 113(49):E7996-E8005.

Publications

CEPLAS
ANNUAL
REPORT
2016

Plant Metabolism and Metabolomics Platform

Coordination:
Dr. Tabea Mettler-Altmann,
Dr. Sabine Metzger

Support:
Maria Graf,
Elisabeth Klemp,
Katrin Weber,
Dr. Vera Wewer

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. The team of Tabea Mettler-Altmann is specialised on metabolites of the central metabolism, whereas Sabine Metzger's team provides expertise on metabolites of the specialised metabolism. Both our laboratories are involved in teaching as well. We contribute to yearly practical courses for undergraduate students, for example in the Quantitative Biology Programme. We further organise specialised CEPLAS scientific courses for doctoral researchers and postdoctoral fellows. In these courses, participants not only learn the basics of metabolite extraction, quantification and statistical analysis but get real hands-on experience with our analytical instruments. Additionally, we supervised Bachelor theses.

Metabolites of the central metabolism

The following seven high-through-put methods are established on our instruments for various matrices now and their application led to six peer-reviewed publications in 2016:

- A **GC-TOF method** for the relative quantification of organic acids, amino acids and sugars in the facultative CAM plant *Talinum triangulare* (Brilhaus *et al.*, 2016), ten different submerged aquatic plants (Hussner *et al.*, 2016), the red alga *Cyanidioschyzon merolae* (Rademacher *et al.*, 2016), the green algae *Trebouxia* isolated from two different Antarctic lichens (Sadowsky *et al.*, 2016), a C_3-photosynthesis species and two C_3-C_4-photosynthesis species of the genus *Moricandia* (Schlüter et al. 2016), and blood plasma from *Canis lupus* (Vollmer *et al.*,2016).
- An **UHPLC-DAD method** for the absolute quantification of amino acids in the red alga *Cyanidioschyzon merolae* (Rademacher *et al.*, 2016).
- An **HPLC-MS/MS method** for the separation and absolute quantification of phosphorylated sugar alcohols such as glycerol-3-phosphate in *Arabidopsis thaliana* (collaboration with Ziba Ajami-Rashidi and Jürgen Zeier, HHU) and phosphorylated sugars e.g. the isomeric hexose-phosphates glucose-1-phosphate, glucose-6-phoshpate and fructose-6-phosphate in higher plants and green algae.
- A **GC-TOF method** for the relative quantification of the triterpenes thalianol and marneral in roots of *Arabidopsis thaliana* (collaboration with Dorian Baumann, UoC).
- A **GC-TOF method** for the absolute quantification of fatty acid methyl esters (FAMEs) in *Arabidopsis thaliana* seeds and seedlings.
- An **UHPLC-TOF method** for the relative quantification of the glycolipids ustilagic acid derivatives and mannosylerythritol lipids (MELs) in the fungus *Ustilago maydis* (collaboration with Kerstin Schipper, HHU).
- An **EA-IRMS method** for the quantification of total carbon, total nitrogen and the carbon isotope ratio in a C_3-photosynthesis species and two C_3-C_4 photosynthesis species of the genus *Moricandia* (Schlüter *et al.* 2016) and total C and total N in ten different submerged aquatic plants (Hussner *et al.*, 2016).

Metabolites of the specialised metabolism

Our Laboratory in Cologne is equipped with two LC-MS Systems. The Qtrap5500 is mostly used for targeted analyses. Whereas the MAXIS 4G as a high resolution mass spectrometer is predominantly used for untargeted analyses like metabolite profiling.

During the last years we established several methods for analysis of secondary metabolites like flavonoids, triterpenes or metabolites obtained from root exudates in cooperation with the different groups within CEPLAS but also with groups outside of CEPLAS.

The method portfolio has to be adapted and extended continuously. Last year we extended our methods for flavonoid analysis with a method for identification and quantification of sulphated flavonoids. Method development and establishment were part of a Bachelor thesis in our laboratory, which was successfully completed in June 2016. This method based on a neutral loss scan can also be used to identify other sulphated compounds like glucosinolates. In collaboration with the AG Bucher, AG Kopriva, the MPIPZ and the University of Bern we analysed flavonoids in different plant species (*Arabidopsis thaliana*, tomato, maize, petunia and calibrachoa).

When analysing secondary metabolites, especially in the field of profiling, the complexity of the samples and the identification of unknown metabolites are the main challenges. Therefore, we implemented an upgrade for our high resolution mass spectrometer in the beginning of 2016, which enhanced the resolution even further by a factor of 2 and increased the sensitivity by a factor of 500. This upgrade was complemented by a new software package specially designed for analysing LC-MS metabolite profiling data. With this setup, more than 2.500 analyses were done in the last year. The profiling of root exudates was performed in collaboration with Manuela Peukert (AG Kopriva) and Katharina Sklorz (AG Bonkowski).

During the development of the LC-MS method for the quantification of triterpenes we focused on optimising the HPLC separation and the choice of internal standards, while monitoring signal response linearity and matrix effects. With the resulting method we were able to quantify known triterpenes such as squalene, lupeol and cycloartenol and monitor the accumulation of additional, modified triterpenes. The method is suitable for the analysis of *Rhodobacter* and *Synechocystis* extracts and could be adapted to include other organisms. In cooperation with the AG Jaeger and the AG Axmann we analysed triterpenoids in *Rhodobacter capsulatus* and *Synechocystis* extracts. In a comparative approach we established and optimised the extraction protocol for the two organisms by combining and modifying existing protocols. Important factors that we considered were the recovery rate of triterpenes, solubility in different solvents and protection from oxidation.

Identification of substrates of an ABC-transporter is the focus in a cooperation with the AG Schmitt. Here we started to develop a method to measure the metabolites transported into vesicles. Questions that have to be addressed in this project are the incubation time required for transport, how to break up the vesicles, how much sample material is needed and how sensitive the system is. To answer these questions, we started with the test system PDR5 as the transporter and ketoconazole as a substrate.
Our laboratory also has access to quantitative element analysis with an ICP-MS. We established an extraction protocol to measure elements not only in small amounts of plant tissue (AG Bucher, AG Kopriva) and algae (AG Melkonian), we were also successful to determine iron of metalloproteins in different types of *Drosophila tissue* (collaboration with MPI for Biology of Ageing).

Publications:

Brilhaus D, Bräutigam A, Mettler-Altmann T, Winter K, Weber AP (2016) Reversible Burst of Transcriptional Changes during Induction of Crassulacean Acid Metabolism in *Talinum triangulare*. *Plant physiology* 170(1):102-122.

Hussner A, Mettler-Altmann T, Weber APM, Sand-Jensen K (2016) Acclimation of photosynthesis to supersaturated CO_2 in aquatic plant bicarbonate users. *Freshwater Biology* 61(10):1720-1732.

Rademacher N, Kern R, Fujiwara T, Mettler-Altmann T, Miyagishima SY, Hagemann M, Eisenhut M, Weber AP (2016) Photorespiratory glycolate oxidase is essential for the survival of the red alga Cyanidioschyzon merolae under ambient CO_2 conditions. *Journal of experimental botany* 67(10):3165-3175.

Sadowsky A, Mettler-Altmann T, S Ott (2016) Response of the metabolite profile in the context of desiccation tolerance of Antarctic lichens from southernmost habitats. Phycologia 55(6): 703-714

Schlüter U, Bräutigam A, Gowik U, Melzer M, Christin PA, Kurz S, Mettler-Altmann T, Weber AP (2017) Photosynthesis in C_3-C_4 intermediate Moricandia species. *Journal of experimental botany* 68(2):191-206.

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 doi: 10.1111/micc.12345

Plant Metabolism and Metabolomics Platform

CEPLAS
ANNUAL
REPORT
2016

Promotion of Early Career Researchers

Discover plant sciences – research internships for undergraduates

CEPLAS awards fellowships to undergraduate students in Cologne and Düsseldorf to foster interest of excellent and talented students in plant sciences at an early career stage. Each year twelve fellowships are offered to 3rd semester B.Sc. Biology and Biochemistry students. In addition, students with a background in mathematics, physics or computer science but interest in plant biology are highly welcome to join one of the interdisciplinary CEPLAS groups.
During their 3-week research projects, students receive full-time contracts. After successful completion of the project including a written report, students are awarded with a research certificate.
The success of the programme is reflected in the fact that many participants continue to work or even start their Bachelor thesis in the lab where they did their internship or in neighbouring CEPLAS labs.

Bachelor programme in Quantitative Biology

In fall 2015, the first students began their studies in the Bachelor programme in Quantitative Biology, a joint study programme between the Universities of Cologne and Düsseldorf. To attract students, we advertised the programme online, during regular lectures and information events, with experimental demonstrations, flyers, posters and booklets. In addition, the programme was promoted by student representatives and via social media.
Within the first year of the programme (qualification phase), students were trained in Bioinformatics, Mathematical Modelling, Biostatistics, Physical Biology of the Cell, Systems Biology and Synthetic Biology.
The emphasis of the first courses was on quantitative methods which were taught within typical biological settings. By now all students of the first cohort have moved on to the research phase of the programme which will complete their undergraduate studies. In this phase, students should be able to use their newly acquired quantitative skills to tackle complex biological research questions.
In January 2017, we evaluated the programme's modules and the organisation of the programme to have a basis for adjustments, if needed.
Apart from minor modifications in the chronology of the mathematical modules, the feedback we received was nearly exclusively positive or very positive.
The second cohort that started in October 2016 consists of twelve students, eight from Heinrich Heine University and four from University of Cologne.
In the following year, current students will participate advertising the programme to attract future students via peer-to-peer communication.
Experiences from the first two application procedures and cohorts has shown that in addition to formal grades, mainly the motivation of the students should be considered as a criterion for admission to the programme.

In addition, individual achievements, such as active participation at the international iGEM competition in synthetic biology will be acknowledged in the process of admission to the programme.

Promotion of Early Career Researchers

CEPLAS Graduate School

The CEPLAS Graduate School offers a 3-year structured Ph.D. programme in a stimulating research environment focusing on plant science. The highly collaborative Ph.D. research projects cover topics including molecular biology, biochemistry, genetics, and developmental biology of plants as well as computational and theoretical biology and plant-microbe interactions.
Accompanying the research projects, the CEPLAS Graduate School offers a comprehensive training programme in scientific and transferable skills. Workshops covering advanced methodologies and concepts allow the doctoral researchers to broaden their scientific knowledge. Transferable skill courses prepare the young scientists for their future career paths.

Development of the programme

As of December 2016 the CEPLAS Graduate School has 33 members. Apart from 29 regular doctoral researches, 4 doctoral researchers are associated members, i.e. are financed by other sources than CEPLAS but participate in the structured programme. In 2016 the Graduate School accepted 2 new Ph.D. students, one regular and one associated member.
Moreover, we were happy that the first three CEPLAS students graduated in 2016: Elia Stahl, Armin Sadat Khonsari and Anna Matuszyńska.

Speaker Graduate School:	Ute Höcker
Coordinator Graduate School:	Esther Jawurek (Sira Groscurth and Petra Fackendahl during maternity leave)
PhD representatives:	Meike Hüdig, Michael Thielen (deputy)
Current number of doctoral researchers:	33
Proportion of female scientists:	67%
Internationality:	33% international students from 6 countries (China, India, Iran, Italy, Spain, Switzerland)

CEPLAS Postdoc programme

CEPLAS offers a comprehensive scientific and career development programme for postdoctoral researchers. The programme aims at preparing our postdoctoral fellows for their next career step in or outside academia. Programme components are scientific interaction and training, mentoring, coaching and networking with industry.

Development of the programme

In 2016 the first transcripts were issued to alumni postdocs who have fulfilled the curriculum of the postdoc programme. The postdoctoral programme transcripts comprise a description of the programme and list all achievements during the CEPLAS postdoctoral phase such as publications, participation at training courses, conferences, CEPLAS events, and other activities and accomplishments.
At the end of 2016, the postdoc programme had 23 active programme members. Four postdocs joined CEPLAS, four left the cluster. The majority of our currently 14 alumni is currently working in non-university research institutions, three in a company. Four postdocs took up a position abroad. To remain connected after their membership with CEPLAS, alumni can join the CEPLAS LinkedIn Group. The CEPLAS LinkedIn Group has been set up in 2016 by current postdocs to bring the CEPLAS community even closer together, providing a platform to share job announcements, ideas and information related to plant sciences and broaden the members' networks.

A step towards independence - research grants for CEPLAS postdocs
In 2016 three CEPLAS postdocs, received highly respected fellowships.

- **Wilma van Esse** (MPIPZ, member of von Korff group) was awarded a three-year VENI grant scholarship from The Netherlands Organisation for Scientific Research (NWO) to conduct independent research. Wilma's research focuses on the trade-off between different yield components in barley at the molecular and genetic level with the aim to improve cereal crop yield. Wilma started her project beginning of 2017 at Wageningen University, Netherlands in close collaboration with Maria von Korff.

- In July 2016, **Richard Jacoby** (UoC, member of Flügge/Kopriva groups) started his 2-year Marie Skłodowska-Curie Individual Fellowship. He investigates which specific bacterial strains are most effective in enhancing plant uptake of nitrogen (N) and sulphur (S) from organic sources, as well as which specific *Arabidopsis* accessions are the most receptive hosts for these interactions.

- **Filipa Tomé** (HHU at MPIPZ, member of von Korff group) received an Alexander von Humboldt research fellowship. Filipa's project deals with the genetic and environmental control of inflorescence development and floret fertility in barley and wheat. Filipa will start the fellowship in summer 2017.

Promotion of Early Career Researchers

Speaker Postdoc Programme:	Rüdiger Simon
Coordinator Postdoc Programme:	Juliane Schmid
Postdoc representatives:	Filipa Tomé, Luise Brand (deputy)
Current number of postdoctoral researchers:	23
Proportion of female scientists:	65%
Internationality:	65% international postdocs from 10 countries (Australia, China, France, India, Italy, Japan, Netherlands, Portugal, Switzerland, USA)

Early career researchers' trainings and activities

Training programme

Each year the CEPLAS research programme is complemented by a broad variety of **scientific courses** to train the (post)doctoral researchers in additional methods and keep them up to date on the latest techniques.
The **career training programme** includes career-relevant transferable skill courses and workshops to further train CEPLAS early career researchers for their future career.
Especially, the Ph.D. curriculum includes five mandatory courses, *Good Scientific Practice, Scientific Presentation, Scientific Writing* and two additional courses free of choice. Postdocs are encouraged to participate in any courses, which fit their needs.

Workshop overview*

Scientific training:
- CRISPR/Cas9
- Protein Crystallization & X-ray Structure Analysis
- Introduction to Statistical Data Analysis
- Data Mining – Gene Expression Analysis
- Advanced Methods for Fluorescence Imaging
- Analysis of Primary Metabolites by GS-MS
- Scientific Writing using LaTeX
- Data Analysis
- Data Visualization
- Einführung in R
- Gentechnische Arbeiten in gentechnischen Anlagen

Career training:
- Career Planning – how to find your perfect job (incl. individual consultations)
- Optimizing Writing
- Get into Teaching
- Leadership Skills
- Presenting Science
- Negotiation Training
- Managing R&D Projects
- Academic Writing
- Proposal Writing
- Grant Writing - Focus on Natural and Life Sciences
- English Presentation Skills
- Time- and Self-Management
- Führungsqualifikationen
- Einführung in den gewerblichen Rechtsschutz
- Karriere aktiv gestalten
- Teamleitung und Teamentwicklung
- Gespräche wirkungsvoll führen

*Workshops attended by early career researchers in 2016.

In 2016 we launched a comprehensive survey among the young researchers which included questions on scientific qualification, career exploration and career awareness as well as job search self-efficacy. As a response to this assessment, the new workshop programme for 2017 was set up. Among others it will include courses on statistical analysis (e.g. R-programming), synthetic biology, quantitative genetics and a follow-up course of the CRISPR/Cas9 lecture to focus on recent developments of the technique.

Networking with industry

CEPLAS Speed Dating

A highlight was the "Speed Dating with Industry" event, which took place in April at the "Haus der Universität" in the city of Düsseldorf. The event was jointly organised by Günter Strittmatter, several early career researchers from CEPLAS and the CEPLAS office with the aim to directly expose young scientists to career opportunities outside of academia and to give them the opportunity to expand their scientific network. Twelve representatives from various organisations and with a variety of different backgrounds including scientists, project managers, group leaders, and patent attorneys accepted the invitation to spend an interactive day with the young scientists of CEPLAS.
At the beginning of the event, our guests briefly introduced themselves and their organisations. Thereafter the young scientists had the opportunity to talk individually with each guest for 10 minutes ("Speed dating"). Discussions between young scientists and guests were continued during lunch, coffee breaks and a joined dinner in the city of Düsseldorf. Young scientists very much appreciated that our guests were very open and shared their insights into career opportunities and life paths outside academia. This event also extended and strengthened the CEPLAS network with cooperation partners. Due to the positive feedback the Speed dating event will be organised again in April 2017.

It was an interesting day meeting with enthusiastic scientists from different disciplines that can potentially become our future colleagues. An excellent day to explain to young motivated scientist their opportunities in industry."
Paul Maris (PhD), Breeding Director Cut Flowers Dümmen Orange

"I really enjoyed the meeting. Very well organised, good atmosphere, diverse and interesting conversation on career development as well as sharing work-life balance experience."
Dr. Susan Gabriels, Scientist Phytopathology, Monsanto

Excursions to industry

In mid February, a group of 14 Ph.D. students and postdocs visited the company "metanomics" in Berlin. The group was accompanied by Rüdiger Simon, speaker of the CEPLAS Postdoc programme. Metanomics was initially founded as a joint venture between scientists from the Max Planck Institute of Molec-

ular Plant Physiology and BASF AG and is now a member of the international BASF Plant Science platform. After receiving insights into the company which specialises in metabolite profiling, our early career researchers had the opportunity to present their projects to a group of metanomics scientists. Short talks about the different research divisions of metanomics were followed by stimulating topic table discussions. A guided tour through the large facility park and the greenhouses was also offered in the course of this interesting visit.

An excursion to BayerCropScience was planned for summer 2016 but had to be postponed due to the negotiations of Bayer with Monsanto. In late September 2016, ten CEPLAS researchers visited Phytowelt Green Technologies GmbH in Cologne. The group was accompanied by Ute Höcker, speaker of the CEPLAS Graduate School. Phytowelt Green Technologies as it exists today was founded in 2006 as a spin-off from the Max Planck Institute for Plant Breeding Research. The R&D facilities are situated at Cologne BioCampus. The service and research processes of the company bridge white and green technology including an expertise on secondary plant metabolites and their production in microorganisms as well as the improvement of plants as renewable resource for bioenergy and biomaterials by protoplast fusion. The group received an insight into the main research activities of Phytowelt and all researchers had the opportunity to briefly present their work in an "elevator pitch". The excursion was completed by a visit to the company's poplar field next to the BioCampus. CEPLAS young researchers were given the opportunity to see tetraploid poplar lines with increased biomass – a vivid result of Phytowelt's experience in protoplast fusion.

In January 2017, a group of early career researchers will visit KWS SAAT AG, a big plant breeding company located in Einbeck. A second excursion to WeGrow, a relatively young company founded as spin-off of the Universtiy of Bonn with focus on breeding and cultivating kiri trees, and a third excursion is currently being planned.

Young Researchers Retreat

In 2016, the annual young researchers retreat took place at the international conference centre "Auf dem heiligen Berg" in Wuppertal. More than 90 principal and associated investigators, postdoctoral and doctoral researchers participated in the two-day event. The programme was divided into various sessions, each chaired by one of the young researchers who were also responsible to briefly summarise the focus of the respective research area. Early doctoral and postdoctoral researchers introduced their projects in short talks while advanced Ph.D. researchers and postdocs presented their progress in longer presentations, each followed by fruitful discussions and constructive feedback. Our guest speakers from the German Centre for Integrative Biodiversity Research (iDiv), Nicole van Dam and Christian Wirth enriched the discussions and gave an insight into the research at iDiv.
Moreover, we included three workshops in this year's programme. The workshop *Positive Feedback*, taught by a professional coach, addressed all participants including PIs. It stressed the importance of positive feedback for high performance and gave hints on how to give, claim and receive positive feedback. In addition, we organised two other workshops that were directed towards the young researchers only: *Insights into academic careers* and *Insights into a career in industry*. The first one was led by several CEPLAS principal investigators who explicitly shared their personal career paths and pointed out strategies for a successful career in academia. The second workshop was hosted by two researchers and one recruiter from Bayer CropScience AG, giving insight into a career in industry. They focused on the application procedure and every-day work in a company as compared to academia. Both workshops were highly interactive and were very well received by doctoral and postdoctoral researchers.

Support for individual research stays abroad – the CEPLAS mobility fund

CEPLAS highly encourages (post)doctoral researchers to broaden their horizon by spending time in other labs in Germany and abroad, with the aim to acquire new expertise and to advance their research projects through collaboration. With support from the CEPLAS mobility fund, several early career researchers visited partnering labs at the University of Toronto, the Sainsbury Laboratory and the University of Bologna. Two research stays at the University of California, San Diego, and the University of California, Los Angeles will take place early in 2017.
The mobility fund also provided financial support for two PhD students to participate at workshops/summer schools abroad. Moreover, it funded research stays of three incoming international master students from Italy, Netherlands and China at one of the CEPLAS institutions.

In 2016, the following exchanges were supported:

- Franklin Villegas, University of Wageningen, Netherlands
  Visit to CEPLAS
- Francesco Tacchino, University of Pavia, Italy
  Visit to CEPLAS
- Stephan Wawra, UoC
  Visit to John Innes Centre, UK
- Priyanka Mishra, UoC
  Visit to Summer School in Valencia, Spain
- Agatha Walla, HHU/MPIPZ
  Visit to James Hutton Institute, UK
- Qianwen Ge, Jiliang University, China
  Visit to CEPLAS
- Kumari Billakurthi, HHU
  Visit to University of Toronto, Canada
- Ryohei Thomas Nakano, MPIPZ
  Visit to University of Bologna, Italy

CEPLAS
ANNUAL
REPORT
2016

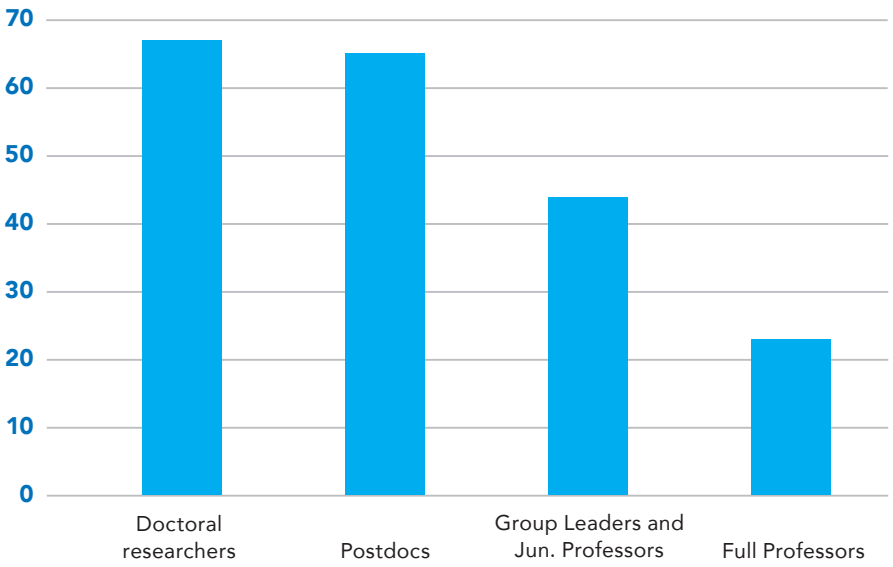
CEPLAS Equal Opportunity Programme

The CEPLAS Equal Opportunity Programme aims to increase the proportion of women in leading positions. To that end, CEPLAS focuses specifically on promoting the careers of female scientists during the Ph.D., postdoctoral and group leader phases.
Furthermore, CEPLAS promotes a family-friendly work environment and offers support to assist male and female scientists with balancing career and family life.

Female scientists in CEPLAS – key data

The equal opportunity monitoring of the German Research Foundation indicates that female scientists are still underrepresented in leading positions. The proportion of women at different career levels shows a severe dropout rate after the Ph.D. phase: only 24% of the faculty positions in biology are filled with women (source: Chancengleichheits-Monitoring 2016, DFG). The reasons for this are diverse. Balancing family and career (e.g. having/raising children, elderly care) is a barrier women encounter much more frequently than men. Moreover, the lack of numerous female role models provides little encouragement to pursue a still male-dominated and competitive scientific career path.
In CEPLAS the proportion of female (49%) and male (51%) scientists is almost balanced. However, when comparing the percentage of women at different career stages the number of female scientists in group leader positions or at (junior) professor level still needs to be improved. Nevertheless, the proportion of CEPLAS female scientists is higher at almost all career stages when compared to the general data from the Faculties of the Mathematical and Natural Sciences of UoC and HHU (UoC 2015: 43% Ph.D., 50% postdocs, 55% group leaders and junior professors, 19% professors; HHU 2014: 40% Ph.D., 33% Habilitation, 55% junior professors and 15% professors; source: Gender Datenreport 2016, UoC and Rechenschaftsbericht der Zentralen Gleichstellungsbeauftragten 2015, HHU).

Proportion of female researchers within CEPLAS at different career levels.



Equal opportunity measures

CEPLAS Women in Plant Sciences Day

It is generally observed that more faculty positions are filled with men than with women, which apparently is caused by two main reasons: i) balancing family and career is more difficult and challenging for women; thus, a lack of support (e.g. child care, advanced training, flexibility in work-life balance) is one major obstruction; ii) men tend to be better negotiators, which may help them to acquire a permanent position. Furthermore, women might underestimate the importance of networking. To specifically address the needs of women, we developed a new event, which is only open to female scientists working within CEPLAS. In 2016, the "CEPLAS Women in Plant Sciences Day" focused on "Strategies for a Successful Career in Science". Four modules were offered, taught by professional coaches:

- "Work-Life-Integration" - How to combine private life and professional aspire?
- "Mind full or mindful?!" - An Introduction to Mindfulness-Based Stress Reduction
- "Power Games of Men and Women in the Scientific Context"
- "Authentic Networking for Women in Academia"

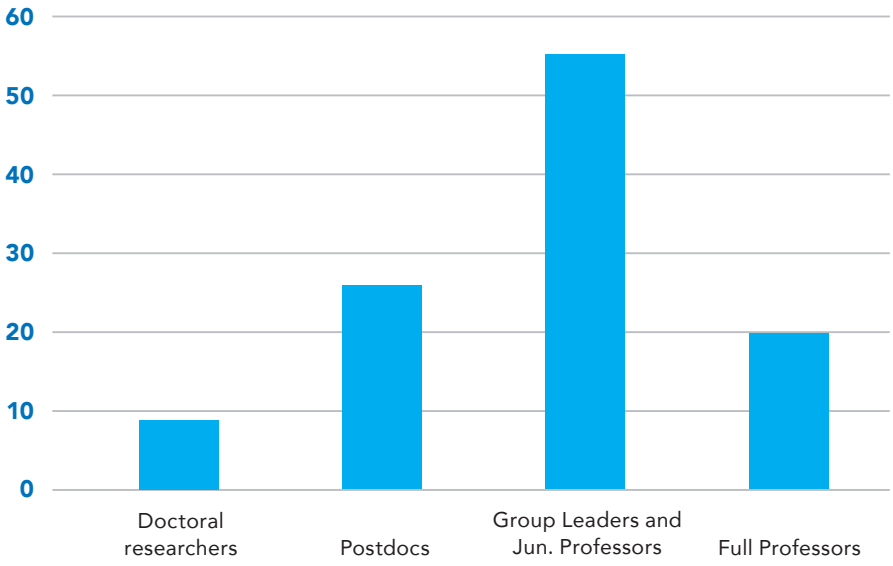
Especially the module "Power Games of Men and Women in the Scientific Context" by Dr. Peter Modler was highly appreciated by the participants. Peter Modler highlighted typical women and men stereotypic behaviours and communication forms by using role plays.
Following the different modules, the event was completed by a networking session. Guided by one of the trainers the participants were able to improve their networking abilities using newly presented methods.
Due to the positive feedback we received from the participants, the "Women in Plant Sciences Day" will take place again in 2017. Planned topics are repartee and negotiations (budget, salary etc.).

Family support

Helping hands programme

In CEPLAS, a large proportion of scientists at different career levels have young children. To support members with young children and during pregnancy or parental leave, CEPLAS established the helping hands programme. In 2016, the programme was opened to CEPLAS fellows caring for relatives in need. The CEPLAS scientists are able to apply for financial support for additional scientific and technical personnel to ensure continued operation of their research projects and to allow a better balancing of family obligations and a research career.

Proportion of parents at different career levels with children under 12 years.



Child-Care during CEPLAS events and parents-child room

During all CEPLAS events we offer child-care in cooperation with the Family Support Services of the Universities. This offer is widely accepted by parents and their children. We are happy that increasingly more male researchers are also taking advantage of this opportunity.

At the Universities Cologne and Düsseldorf parents have access to a parents-child room. Both rooms are equipped with child-care needs and computer desks. However, to be more flexible in the future we are planning to buy a "KidsBox" in 2017. The "KidsBox" is a mobile children's room, enabling spontaneous child-care in usual university rooms or other locations.

CEPLAS Equal Opportunity Programme

Equal Opportunity Board

The CEPLAS Gender Board was established in 2014, including one female professor from each partner institution. The Gender Board's responsibilities cover helping hands applications and special requests for gender funds. However, since 2016 the CEPLAS Equal Opportunity Programme has not only addressed gender-related issues but also diversity. Therefore, the committee was renamed "Equal Opportunity Board". Furthermore, the committee was newly formed to include a junior professor (Ilka Axmann, HHU), a female professor (Ute Höcker, UoC) and a male professor (Benjamin Stich, MPIPZ) as well as the coordinator of the Equal Opportunity Office.

Outreach Activities

Public outreach

CEPLAS Symposium 2016

On May 23 and 24, the fourth CEPLAS Symposium took place at Heinrich Heine University Düsseldorf. Unlike previous years, this meeting was split into a public day and an internal day, for CEPLAS members only. The first day started with overview presentations of CEPLAS research area coordinators on their research areas. In addition, one speaker from each research area gave a scientific presentation on a selected topic. The programme continued with keynote lectures given by Philippe Vandenkoornhuyse (University of Rennes) and Barry Scott (Massey University).
At the subsequent poster session at the Botanical Garden, CEPLAS early career researchers had the chance to present their projects to the members of the SAB and other CEPLAS members. The day concluded with a BBQ for all CEPLAS members and guests.

Poster session in the Botanical Garden Düsseldorf.

Exhibition "Nutzpflanzen: gestern | heute | morgen" at the Botanical Garden Düsseldorf

In June, the new crops section at the Botanical Garden Düsseldorf was re-opened with the exhibition "Nutzpflanzen: gestern | heute | morgen" The large exhibition shows the development from wild towards domesticated crop species, their usage and cultural history. CEPLAS contributes to the exhibition with a section on (energy) plants of the future. From July to September, several guided tours were offered by Peter Westhoff and Andreas Weber, e.g. on the history of crop plants and the role of crops in a global context.

Public lecture series: "Vom Urweizen der Steinzeit zu den Genpflanzen der Zukunft"

As in previous years, we organised our public lecture series: "Vom Urweizen der Steinzeit zu den Genpflanzen der Zukunft" also in 2016.
This year the lecture series took place at the "Haus der Universität" in Düsseldorf. Peter Westhoff and Andreas Weber gave a comprehensive overview covering the beginning of agriculture and the methods of plant breeding as well as the challenges of the future.
The mixed audience was very interested and appreciated the opportunity for subsequent discussions and questions. For 2017 we are working on a new concept of the lecture series in collaboration with the Botanical Garden Düsseldorf.

CEPLAS Research and Education

The CEPLAS Research and Education programme has been developed in collaboration with members of the Center for Teacher Training (Zentrum für LehrerInnenbildung, ZfL) and the Institute for Didactics of Biology (Institut für Biologiedidaktik) at University of Cologne as well as several CEPLAS partner schools.
This learning initiative 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.
In a three-week laboratory phase, CEPLAS scientists familiarised student teachers with current plant molecular biology research and basic lab work. After the lab phase, the students developed teaching concepts and experimental settings, which are applicable in biology lessons in secondary schools.

These concepts where then tested in a six-day internship with highly motivated pupils. The students greatly benefitted from this programme as they could gather first teaching experience and at the same time trained themselves in current research.
At the closing event in April, pupils presented their findings in talks to CEPLAS members, students, teachers, and their peers. In addition, pupils prepared posters to disseminate major results of their research in classroom work.

Satellite Workshop on C_4 Photosynthesis

In August CEPLAS hosted the *Satellite Workshop on C_4 Photosynthesis*. The workshop was embedded in the *17th International Congress on Photosynthesis Research* held in Maastricht.
Multiple renowned national and international speakers presented the current state of research in the C_4 photosynthesis field. Among the speakers were several CEPLAS faculty members as well as some early career researchers. The latter had in addition the opportunity to present their projects at a poster session in the Botanical Garden Düsseldorf.

Competence Area "Food Security"

In November 2016, CEPLAS started to establish a new Competence Area (CA) on "Food Security" initiated at the University of Cologne. CAs are part of the Institutional Strategy of the University of Cologne.
Based on the FAO definition, Food Security is existing, „when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food which meets their dietary needs and food preferences for an active and healthy life". The impact of global climate change and an increasing world population are major issues in food security. But also social, economic and political aspects play an important role.
This Competence Area will facilitate knowledge transfer between disciplines by organising international conferences, workshops or summer schools. Additionally, it benefits from collaboration with internal and external research institutions, non-governmental partners and partners from industry. The Competence Area also attributes great importance to the involvement of Food Security in teaching at the university, e.g. by offering lecture series to sensitise future multipliers for that topic.

Political outreach

CEPLAS recognises the need to communicate the contributions of fundamental science on complex scientific problems to solving societal challenges not only to lay audiences but also to policy makers. Moreover, CEPLAS wants to draw the attention of decision makers in politics to the role of plant sciences, in and for Germany as a research location and within the framework of Germany's national strategy responding to global challenge of food security and sustainability.

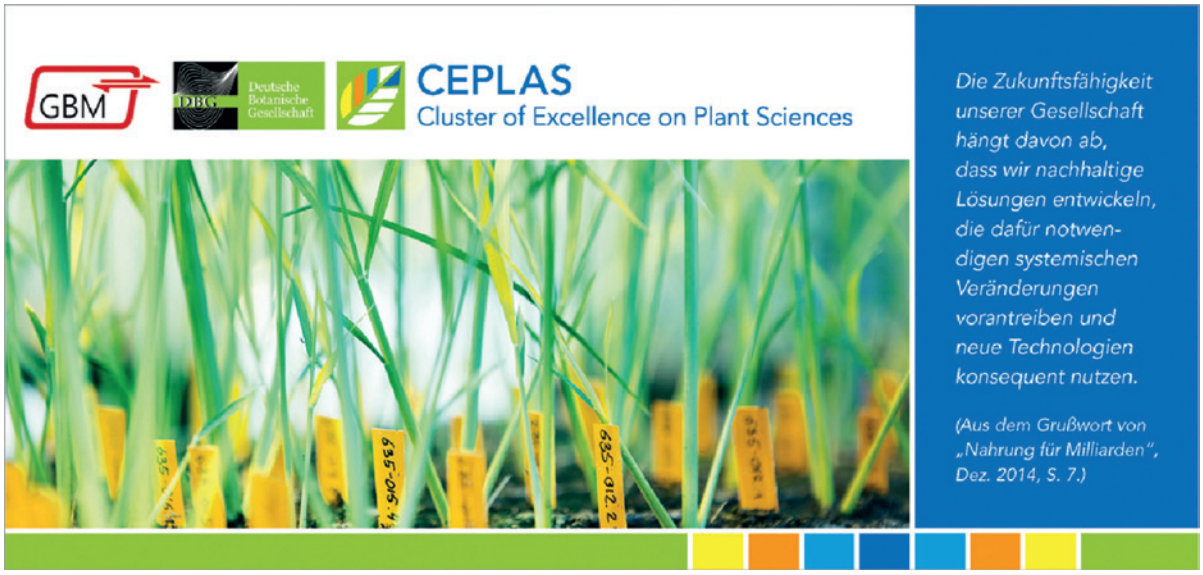
CEPLAS therefore liaises with members of the German Parliament and Ministries to inform on plant science topics such as the potential of novel breeding methods.

In 2016, several individual meetings with members form the German Parliament took place in Berlin. As a result, Andreas Weber was invited to a meeting between researchers and politicians on "Neue Züchtungsmethoden - Genome Editing" and contributed with his long-standing expert knowledge.

Together with the German Society for Plant Sciences (DBG) and the section "Plant Biochemistry/Molecular Biology of Plants" of the Society for Biochemistry and Molecular Biology (GBM) CEPLAS invited members of the German Parliament, the Federal Ministry of Education and Research (BMBF) and the Federal Ministry of Food and Agriculture (BMEL) for a parliamentary breakfast in Berlin in October.

The aim of this meeting was to emphasise the importance of plant sciences in Germany and the need for a national strategy to tackle the global challenges. Members of different political parties followed the invitation and took the chance to discuss with CEPLAS, DBG and GBM representatives.

Andreas Weber, Karl-Josef Dietz, president of the DBG, and Stephan Clemens, representative of the GBM, gave introductory speeches to open up the discussion.



CEPLAS in the media

- Rheinische Post 30.11.2016: Gymnasiastinnen forschen im Uni-Labor.
- Press release of the Heinrich Heine University 27.10.2016: Humboldt-Professur: Erfolg für Vorschlag aus Düsseldorf, Jülich und Köln.
- Press release of the Heinrich Heine University 14.10.2016: Richtfest beim Forschungsgebäude der Lebenswissenschaften.
- Press release of the Heinrich Heine University 28.09.2016: Prof. Maria von Korff-Schmising ernannt.
- Press release of the Heinrich Heine University 16.09.2016: Dem Duft der Pflanzen auf der Spur.
- Westdeutsche Zeitung 11.08.2016: Exzellenzcluster Ceplas: Forschung an der Uni soll exzellent bleiben.
- Video feature transgen.de 24.06.2016: Jenny fragt: Fotosynthese – Warum wollen Forscher sie verändern?
- Video feature Hyperraum TV 19.06.2016: Das Evolutionsmodell der Photosynthese und die Optimierung von Nutzpflanzen.
- Rheinische Post 17.06.2016: Genpflanzen? Nein danke.
- Press release of the University of Cologne 25.05.2016: Exzellenzcluster CEPLAS sucht Partnerschulen in Köln und Düsseldorf.
- Press release of the Heinrich Heine University 04.05.2016: „NutzPflanzen – Pflanzen nutzen: Vom Urweizen der Steinzeit zu den Genpflanzen der Zukunft.
- Press release of the Heinrich Heine University 02.05.2016: Prof. Dr. Benjamin Stich zum W3-Professor für Quantitative Genetik und Genomik der Pflanzen ernannt.
- Press release of the Heinrich Heine University 07.04.2016: NRW-Wissenschaftsministerin Schulze besucht Heinrich-Heine-Universität Düsseldorf.
- Video feature Hyperraum TV 27.03.2016: Alles Pflanze: Forschung, Genetik und Ertrag.
- Video feature Hyperraum TV 27.03.2016: Talk: Gentechnik-Debatte bringt Pflanzenforschung in Misskredit.
- Press release of the Heinrich Heine University 24.02.2016: Invasion von Zellen durch pathogene Bakterien – Schlüssel zum Verständnis zur Herkunft der Eukaryoten?
- Press release of the Heinrich Heine University 22.02.2016: Eine neue Rolle für Vitamin B6.
- Pflanzenforschung.de 17.02.2016: Eine Frage der Balance – Verbindung zwischen Vitamin B6 Komplex und Stickstoffmetabolismus entdeckt.
- Pflanzenforschung.de 09.02.2016: Neues zum Langzeitschutz von Pflanzen – Die Rolle des Indol-Metabolismus bei systemisch erworbenen Resistenzen.
- Köln Nachrichten 19.01.2016: Spitzenforschung mit umgekehrten Vorzeichen.
- Press release of the University of Cologne 15.01.2016: Spitzenforschung an die Schulen.

Outreach Activities

CEPLAS
ANNUAL
REPORT
2016

Technology Transfer and Cooperation Management

Compendium on application-relevant aspects within CEPLAS projects

The goals and status of research in each of the CEPLAS projects were summarised in a compendium, with a specific focus on application-relevant aspects and opportunities for cooperation with external partners from industry ("Opportunities for Public-Private Partnerships", available upon request). The document has been distributed to a variety of companies and is now the basis for information of potentially interested companies in this area and discussions with them on possible cooperation.

Establishing and maintaining contacts with industry

Companies in the field of plant breeding and biotechnology have been constantly informed about the status and new developments within the CEPLAS programme by providing them information material and by personal discussions. Visits of companies by CEPLAS scientists led to Metanomics in Berlin, and to Phytowelt in Cologne.
In addition, CEPLAS has again organised an information event for representatives from industry and other areas outside of academia to present the CEPLAS research programme and to stimulate discussions on potential applications of research results and cooperation. The event took place on February 16, 2016, at the "Haus der Universität", in Düsseldorf. This year, it was organised together with the "Forschungsdialog Rheinland", a cooperation of Universities, Universities of Applied Sciences, Forschungszentrum Jülich, Deutsches Luft- und Raumfahrtszentrum and the Chamber of Commerce and Industry. After the welcome addresses by the president of the HHU, Prof. Dr. Anja Steinbeck, and the managing director of the Chamber of Commerce and Industry Düsseldorf, Klaus Zimmermann, presentations were given by Andreas Weber, Martin Lercher and Stijn Spaepen on the organisation and selected research fields of CEPLAS. Following these presentations, the approximately 40 external participants had the opportunity for further discussions with CEPLAS scientists.

Funding in the framework of the BMBF programme "Plant Breeding Research for Bioeconomy"

A total of 13 project proposals have been submitted by CEPLAS scientists in the framework of the BMBF call "Plant Breeding Research for the Bioeconomy".

Three of these 13 applications have included partners from industry. Overall, 106 applications for funding of projects have been submitted to this BMBF programme. In a comprehensive peer reviewing process, 6 of the 13 proposals including scientist from CEPLAS have been awarded the requested grant (success rate: 46%; in 4 out of these 6 cooperative projects, CEPLAS scientists are the project coordinators); these also include two of the three proposals with participation of industry partners (industry partners are Saaten-Union Biotech GmbH and KWS SAAT SE). In total, the BMBF programme will fund 22 projects, over a period of 3 years (success rate: 21%). CEPLAS scientists will receive a total of ca. € 4.5 Mio additional funding by the BMBF programme, starting in 2016. Research in the funded projects with participation of CEPLAS scientists will address the optimisation of photosynthetic efficiency, the effect of microbiotic soil biodiversity on plant performance and the improvement of cell wall composition of plants for use in bioeconomy. The number of projects selected for funding and the volume of funding is seen as a great success of CEPLAS scientists in the evaluation process of the BMBF programme, demonstrating the scientific excellence of CEPLAS research, on the one hand, and also its relevance for potential application in plant breeding, on the other hand.

Database

In the context of establishing an overall database for information on CEPLAS projects, a concept for the structure of integrating data on application-relevant aspects and cooperation with companies has been developed.

Support in career planning of young researchers

A so called "Speed Dating" event has been organised for early career researchers on April 08, 2016, with 14 representatives from companies and other non-academic professional areas. In presentations by the external participants and in short discussions with each of the external participants, (post)doctoral researchers could get an impression on professional career opportunities outside academia, and could also obtain first-hand answers for their questions in this context.
In addition, G. Strittmatter has individually given advice to early career scientists on their professional career planning and on designing job applications for companies.

Contacts with politicians

Discussions with politicians at various levels had a strong focus on the impact of plants sciences and their results in plant breeding for the development of crop varieties that can stand the future challenges for agriculture. For this purpose, information documents were created providing a scientific evaluation of "New Breeding Technologies" that can be used in the political debate on the future legal framework for the use of these technologies in breeding and research.

Planning of CEPLAS II

In the planning of CEPLAS II, cooperation with industry partners in research as well as in educational programmes plays a significant role. One goal is to establish a comprehensive "Strategic Partnership" that covers various elements of cooperation in research and education with a long-term perspective, beyond the typical project-wise cooperation that have already been established in previous years. Discussion with potential partners for such a "Strategic Partnership" have been initiated.

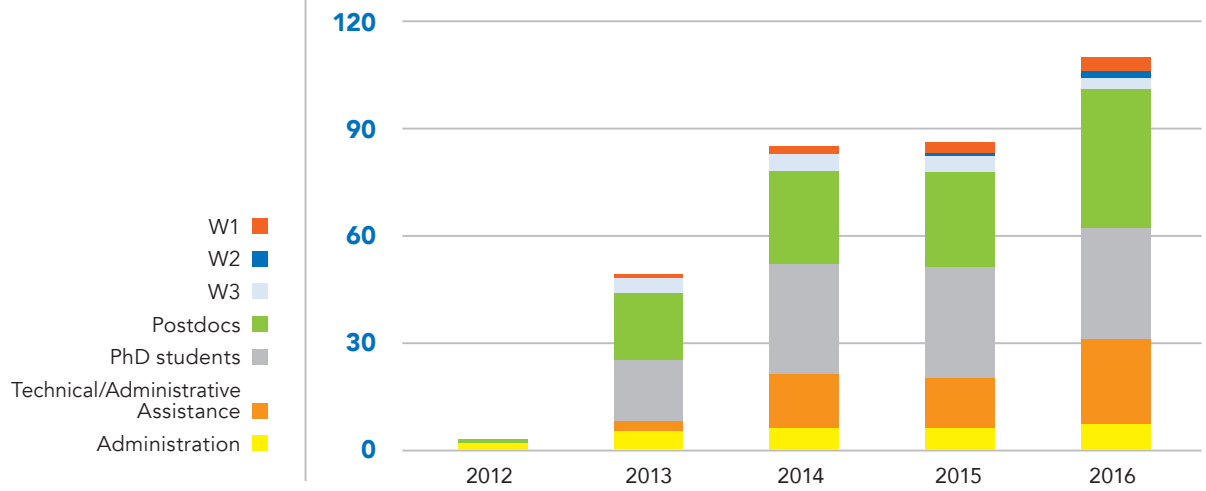
Key Figures

Staff

Internal staffing

Since the beginning of the Cluster end of 2012, a continuous increase in personnel can be recorded. Concerning early career researchers, the was one central turn-over in 2015 when the first cohort of Postdocs had finished and a new cohort started. For doctoral researchers, cohorts started in 2013 and 2014 each with a funding duration of three years.

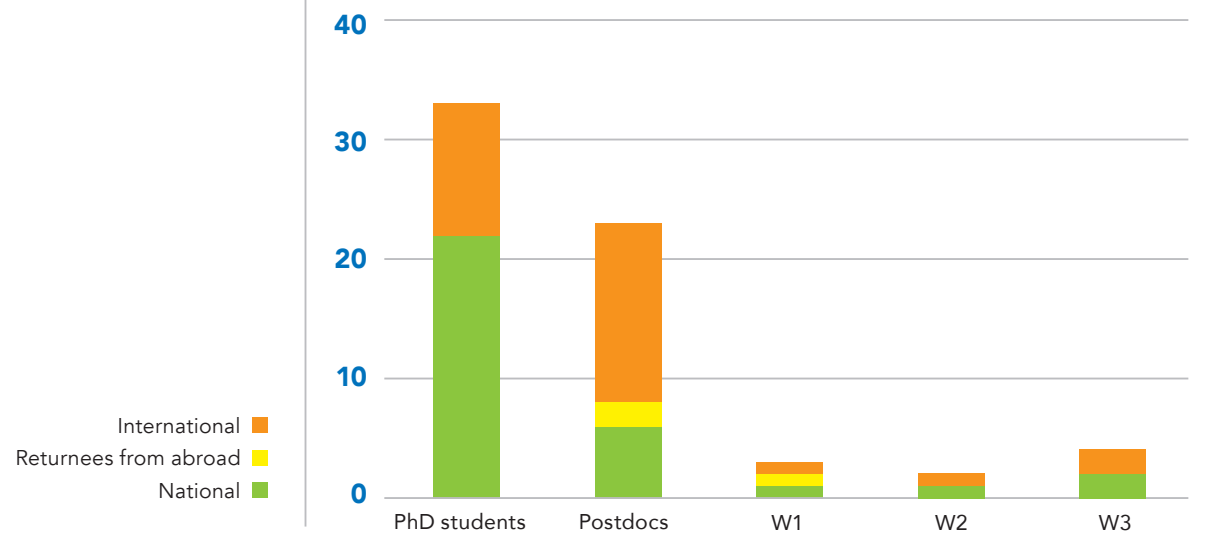
Employment figures from 2012 – 2016 (record date November 1st)



International researchers@CEPLAS

CEPLAS offers a highly international, diverse research environment. In our early career programmes between 33 % (doctoral researchers) and 65 % (postdocs) have an international background. Moreover, we succeeded in appointing several international researchers and/or returnees to our faculty positions.

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



New faculty 2016

With the appointment of Benjamin Stich, we finally succeeded in filling all faculty positions that were announced in the context of CEPLAS.

Benjamin Stich
Position: W3 Quantitative Genetics and Genomics of Plants
Start: 01.05.2016

Research focus

Most traits of agronomic importance are quantitative traits, i.e. the phenotypic observations cannot be assigned to distinct classes but follow a continuous distribution. This is caused by a polygenic inheritance as well as the importance of genotype*environment interaction for such traits.
The work of the Institute for Quantitative Genetics and Genomics of Plants aims to identify the causes of natural phenotypic variation of crop plants on a molecular level, in order to attain the ultimate goal of our work - the prediction of phenotypic performance under various environmental conditions. This requires combined efforts on creating novel plant material, exploiting the possibilities of *omics technologies, and developing innovative biostatistical procedures.

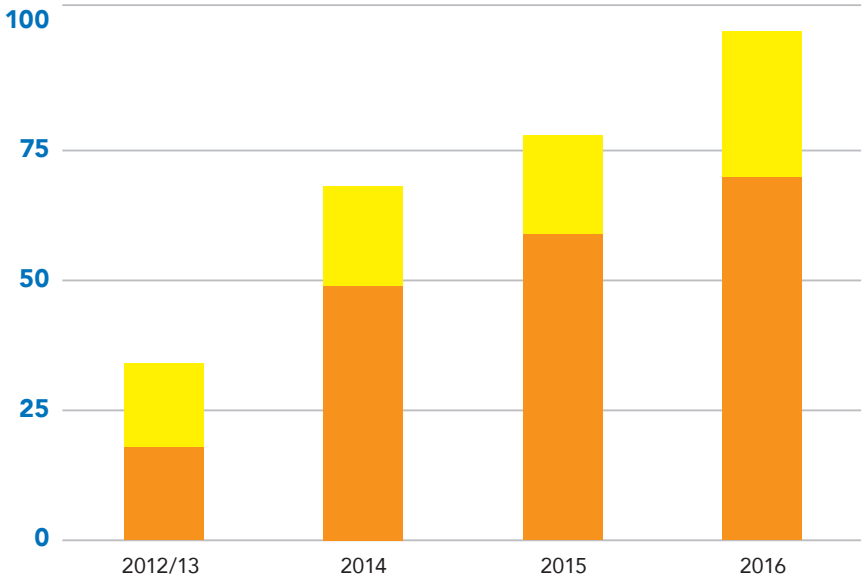
Short CV

Since 5/2016	HHU Düsseldorf Düsseldorf, Germany *Professor for Quantitative Genetics and Genomics of Plants*
2012-2016	Limagrain Pocking, Germany *Senior Maize Breeder*
2012	KWS SAAT AG Einbeck, Germany *Head Molecular Breeding Sugar Beet*
2008-2011	Max Planck Institute for Plant Breeding Research, Cologne, Germany *Independent Research Group Leader* *"Quantitative Crop* Genetics"
2006-2008	Institute for Plant Breeding, Seed Science, and Population Genetics, Hohenheim University, Stuttgart, Germany *Postdoctoral Research Associate*
2005-2006	Institute for Genomic Diversity, Cornell University, Ithaca, USA *Visiting Scientist*
2004-2006	Institute for Plant Breeding, Seed Science, and Population Genetics, Hohenheim University Stuttgart, Germany *Graduate Student*
2000-2004	Hohenheim University Stuttgart, Germany *Studies of Agricultural Biology (Diploma)*

Publications

Since the beginning of the cluster in 2012, the number of publications per year steadily increased. Moreover, each year, around 25% of publications were achieved with contributions of at least one of our early career researchers.

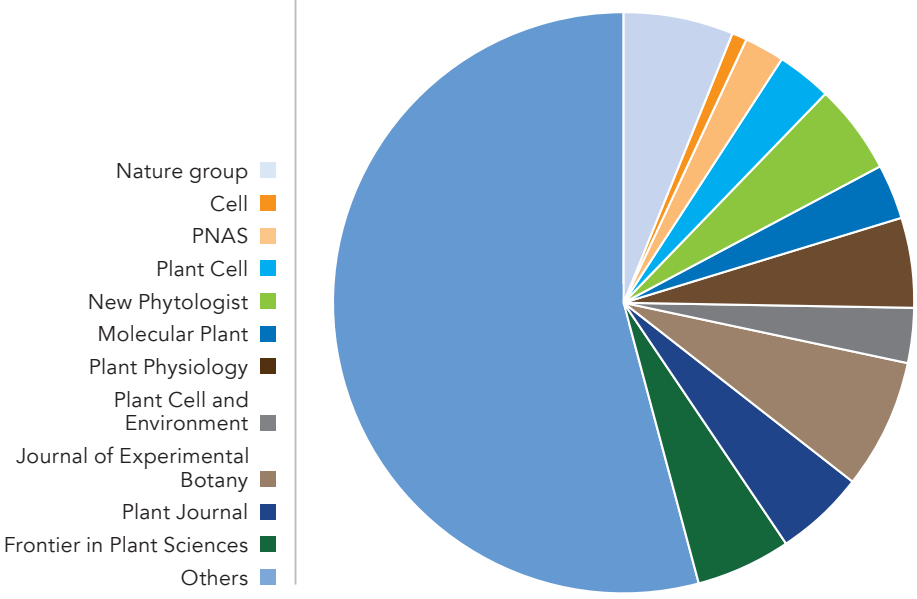
Number of publications and early career researchers' contributions (in yellow) since 2012.



Publication analysis for 2016

In 2016, CEPLAS researchers published almost 100 publications, almost 50% in high-rank journals in plant sciences.

Publication numbers 2016 for the most important journals in plant science (total n=98)

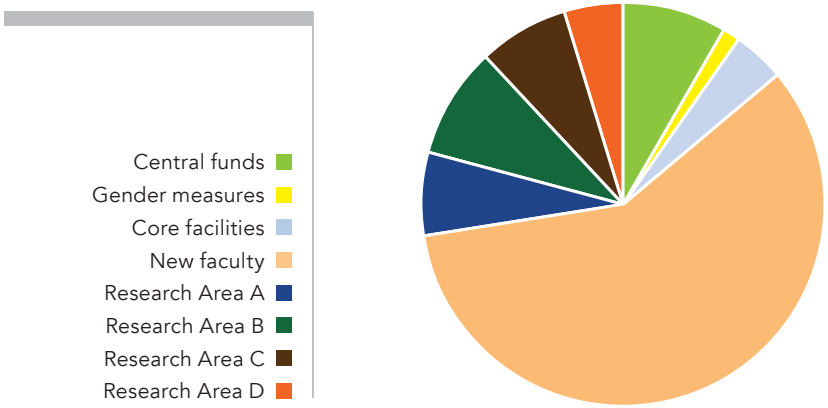


Finances

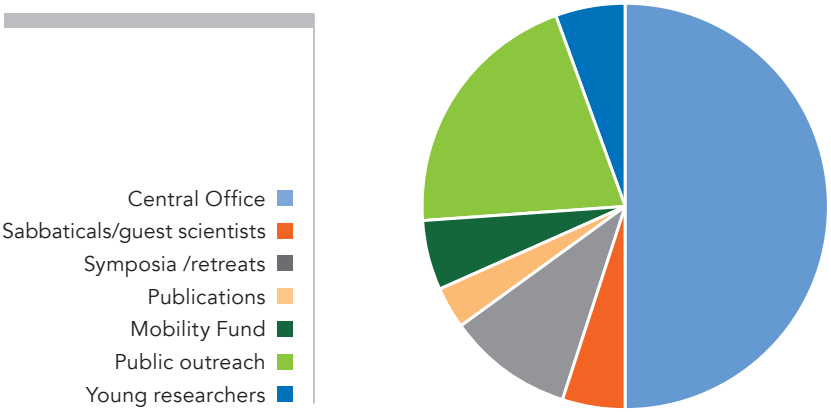
Granted funds and total spending

Total spending in 2016 (k€)

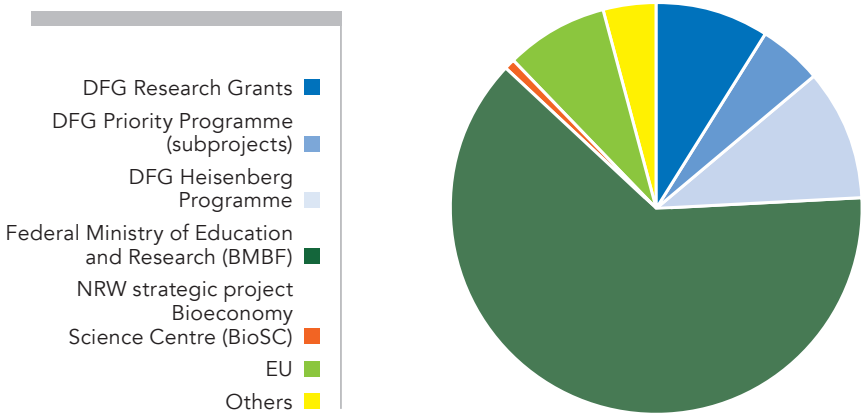
Total allowance 2016	Actual spending (calculations 03/2016)
6,107	6,090



Overview total spending in 2016 (% of total budget)



Overview central funds in 2016 (% of central funds budget k€ 529)



Fundraising from additional third-parties (total 8.6 Mio €)


3
5

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
12	Prof. Dr. Markus Kollmann	Institute of Mathematical Modelling of Biological Systems, HHU
13	Prof. Dr. Maarten Koornneef	MPIPZ
14	Prof. Dr. Stanislav Kopriva	Cologne Biocenter, UoC
15	Prof. Dr. Martin Lercher	Institute of Informatics, HHU
16	Prof. Dr. Alice McHardy	Institute of Informatics, HHU
17	Prof. Dr. Jane Parker	MPIPZ
18	Prof. Dr. Markus Pauly	Institute of Plant Cell Biology and Biotechnology
19	Prof. Dr. Laura Rose	Institute of Population Genetics, HHU
20	Prof. Dr. Lutz Schmitt	Institute of Biochemistry, HHU
21	Prof. Dr. Paul Schulze-Lefert	MPIPZ
22	Prof. Dr. Ulrich Schurr	Institute of Bio and Geosciences-2 (IBG-2), FZJ
23	Prof. Dr. Rüdiger Simon	Institute of Developmental Genetics, HHU
24	Prof. Dr. Benjamin Stich	Institute of Plant Quantitative Genetics and Genomics HHU/MPIPZ
25	Prof. Dr. Miltos Tsiantis	MPIPZ
26	Prof. Dr. Maria von Korff Schmising	Institute for Plant Genetics, HHU at MPIPZ
27	Prof. Dr. Andreas P. M. Weber	Institute of Plant Biochemistry, HHU
28	Prof. Dr. Peter Westhoff	Institute of Plant Molecular and Developmental Biology, HHU
29	Prof. Dr. Jürgen Zeier	Institute of Plant Molecular Ecophysiology, HHU
30	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. Juliette de Meaux	Cologne Biocenter, UoC
4	Dr. Thomas Drepper	Institute of Molecular Enzyme Technology, HHU
5	Dr. Tamara Gigolashvili	Cologne Biocenter, UoC
6	Prof. Dr. Georg Groth	Institute of Biochemical Plant Physiology, HHU
7	Dr. Angela Hay	MPIPZ
8	Dr. Eric Kemen	MPIPZ
9	Prof. Dr. Karl Köhrer	Centre for Biological and Medical Research (BMFZ), HHU
10	PD Dr. Veronica G. Maurino	Institute of Plant Molecular and Developmental Biology, HHU
11	Prof. Dr. Peter Nürnberg	Cologne Center for Genomics (CCG), UoC
12	Prof. Dr. Uwe Rascher	IBG-2, Plant Sciences, FZJ
13	Dr. Richard Reinhardt	Max Planck Genome Centre, MPIPZ
14	Prof. Dr. Kai Stühler	Centre for Biological and Medical Research (BMFZ), HHU
15	Prof. Dr. Klaus Theres	MPIPZ
16	Prof. Dr. Vlada B. Urlacher	Institute of Biochemistry, HHU
17	Prof. Dr. Wolfgang Werr	Cologne Biocenter, UoC
18	Prof. Dr. Matias Zurbriggen	Institut for Synthetic Biology, HHU

Contact

Central Office
Heinrich Heine University Düsseldorf
Universitätsstraße 1
Building 25.43 01.38
40225 Düsseldorf

Phone +49 (0)211- 81 10672
Email: office@ceplas.eu

www.ceplas.eu

Cluster Speaker

Prof. Dr. Andreas Weber
Institute of Plant Biochemistry
Heinrich Heine University Düsseldorf
Email: andreas.weber@hhu.de

Deputy Speaker

Prof. Dr. Stanislav Kopriva
Botanical Institute
Cologne Biocenter
Email: skopriva@uni-koeln.de