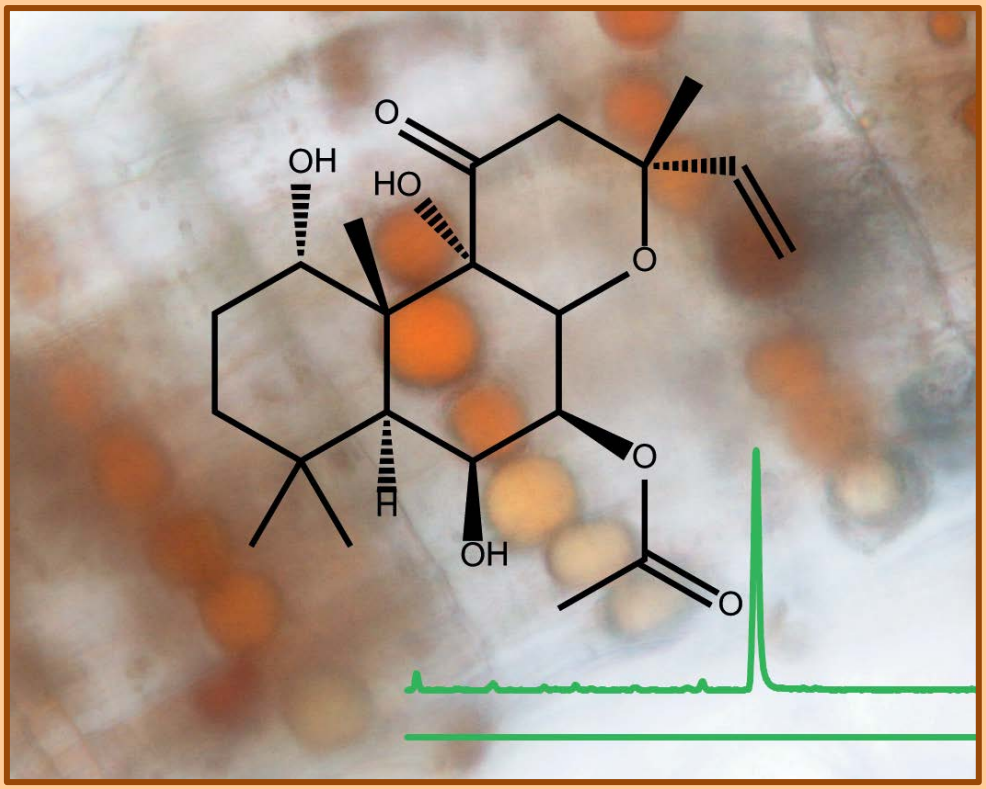
Plant Biotech Denmark
Annual meeting 2016
2 - 3 February

Faculty of Science
University of Copenhagen



PBD
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Cover photo: Cross-section of *Coleus forskohlii* root cork carrying oil body structures. The diterpene forskolin (chemical structure), which has long been recognized for its pharmaceutical properties, has been shown to be produced and accumulated exclusively in the oil body structures of this plant.

Photo: Irini Pateraki, Department of Plant and Environmental Sciences, Frederiksberg, University of Copenhagen

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Venue: University of Copenhagen, Frederiksberg Campus, Thorvaldsensvej 40, Lecture Hall A2-81.01 + Marble Hall

TUESDAY – 2 February 2016

09.30 - 10.00	*Registration, coffee/tea and croissant*	
10.00 - 10.05	**Welcome** by Henrik Brinch-Pedersen, Head of the Steering Committee, Plant Biotech Denmark	
	Session 1: Plants for Food and Feed/Plant Products Chair: Maher Abou Hachem	
10.05 - 10.50	Keynote talk within the research area 'Plants for Food and Feed': **Searching genomes and metagenomes for carbohydrate active enzymes**, by Senior researcher Bernard Henrissat, CNRS, Aix-Marseille Université, France	6
10.50 - 11.10	Selected talk within the research area 'Plants for Food and Feed': **Glycemic response and resistant starch content of ancient and genetically modified barley lines using both static and dynamic digestion models**, by PhD student Domenico Sagnelli, University of Copenhagen, Denmark	7
11.10 - 11.30	Selected talk within the research area 'Plant Products': **The evolutionary dynamics of cyanogenic glucoside biosynthesis and its genomic organisation in a biosynthetic gene cluster in legumes**, by PhD student Alexandra Öchsner, University of Copenhagen, Denmark	8
11.30 - 11.50	*Coffee/tea* and *fruit*	
	Session 2: Plant Signalling and Cellular Trafficking/Synthetic and Systems Biology Chair: Hans Thordal-Christensen	
11.50 - 12.35	Keynote talk within the research area 'Plant Signalling and Cellular Trafficking': **Membrane traffic and fusion in cytokinesis**, by Professor Gerd Jürgens, University of Tübingen, Germany	9
12.35 - 12.55	Selected talk within the research area 'Plant Signalling and Cellular Trafficking': **MicroProteins in the regulation of Flowering Time**, by PhD student Moritz Graeff, University of Copenhagen, Denmark	10
12.55 - 13.40	*Lunch*	
13.40 - 13.50	*Announcements*	
13.50 - 14.10	Selected talk within the research area 'Synthetic and Systems Biology': **Fusion of ferredoxin and cytochrome P450 enables direct light-driven biosynthesis**, by PhD student Silas Mellor, University of Copenhagen, Denmark	11
	Session 3: Plant Breeding – Quality, Productivity, Diseases and Stress/Biomass Production and Processing Chair: Stephan Wenkel	
14.10 - 14.55	Keynote talk within the research area 'Plant Breeding': **Essential RNA-based technologies and their applications in plant breeding**, by Associate Professor Guiliang Tang, Department of Biological Sciences, Michigan Technological University (MTU), USA	12
14.55 - 15.15	Selected talk within the research area 'Plant Breeding': **Cytokinin production of *Pseudomonas fluorescens* G20-18 determines the biocontrol effect against *Pseudomonas syringae* in Arabidopsis**, by Postdoc Dominik Grosskinsky, University of Copenhagen, Denmark	13

15.15 - 15.45	*Coffee/tea, cake and fruit*	
15.45 - 16.05	Selected talk within the research area 'Biomass Production and Processing': **'CPH assay': high-throughput screening of endo-glycoside hydrolases using novel chromogenic polysaccharide substrates**, by Postdoc Julia Schückel, University of Copenhagen, Denmark	14
	Session 4: Elevator talk and Poster session Chair: Mika Zagrobelny	
16.05 - 16.40	Elevator talks (3 minutes talks based on selected abstracts)	15
16.40 - 18.00	Poster session - Wine and snacks are served	24
18.00	*Dinner*	

WEDNESDAY – 3 February 2016

	Session 5: Cross Talks in Plants Chair: Simona Radutoiu	
09.00 - 09.40	**Cross Talk between Photorespiration and Nitrate Assimilation: Key to the Future of Net Primary Productivity, Food Quality, Forest Health, and Carbon Sequestration**, by Professor Arnold Bloom, UC Davis, USA	16
09.40 - 10.10	*Coffee/tea and roll*	
10.10 - 10.50	**ENSA – Engineering Nitrogen Symbiosis for Africa**, by Professor Jens Stougaard, Aarhus University, Denmark	17
10.50 - 11.30	**Structure, function and host control of the microbiota thriving at the root-soil interface**, by Dr. Davide Bulgarelli, University of Dundee, Scotland, UK	18
11.30 - 12.00	**Rice perception of symbiotic arbuscular mycorrhizal fungi requires the karrikin receptor complex**, by Dr. Caroline Gutjahr, University of Munich, Germany	19
12.00 - 12.45	*Lunch*	
	Session 6: Technologies Chair: Michael B Palmgren	
12.45 - 13.15	**Genome-editing technologies: Prospective novel techniques for agronomical crop breeding**, by Postdoc Nawaporn Onkokesung, Swedish University of Agricultural Sciences, Alnarp, Sweden	20
13.15 - 13.45	**Epigenetics in plant breeding-mechanisms and prospects**, by Professor Claudia Köhler, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden	21
13.45 - 14.15	**Tackling plant chromatin structure and its potential for plant production**, by Postdoc Iva Mozgova, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden	22
14.15 - 14.20	*Thank you for this year*	
14.20 - 14.50	*Coffee/tea, cake and fruit*	
15.00 - 18.00	Master class for registered PhD and master students	

Keynote talk within the research area 'Plants for Food and Feed'

Searching genomes and metagenomes for carbohydrate active enzymes

Bernhard Henrissat

Centre National de la Recherche Scientifique (CNRS), UMR 7257, Marseille, France

Carbohydrate-active enzymes (CAZymes) is a term that describes the enzymes that assemble and break down oligo- and polysaccharides. The term currently includes glycoside hydrolases (GHs), polysaccharides lyases (PLs), carbohydrates esterases (CEs), glycosyltransferases (GTs) and auxiliary activities (AAs) and their appended carbohydrate-binding modules (CBMs). CAZymes are amenable to a classification in clans, families and subfamilies based on amino acid similarities. This classification, which is presented and updated on a regular basis in the CAZy database (www.cazy.org), has a substantial functional predictive power that can be harnessed in particular to analyze genomes of organisms and metagenomes of microbial communities for the ability to deconstruct complex polysaccharides in particular those of plant cell walls. Various examples will be presented, whether of genomes of isolated organisms and of microbial communities.

Selected talk within the research area 'Plants for Food and Feed'

Glycemic response and resistant starch content of ancient and genetically modified barley lines using both static and dynamic digestion models

Domenico Sagnelli[1], S. Chessa[2], E. Vincze[5], W. Sorndech[6], M. Di Martino[3], J. Bao[4], A. Blennow[1], K. Hebelstrup[5]

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[3] University of Naples, Naples, Italy,
[4] Zhejiang University, Hangzhou, China,
[5] Aarhus University, Aarhus, Denmark,
[6] School of Food Technology, Institute of Agricultural Technology, University of Technology, Thailand

New types of grain can help to combat life-style related disease. *Hordeum vulgare* (*Hv*) is a major crop with nutritional and health promoting effects because of its high content of betaglucan and other bioactive compounds. *Hordeum spontaneum (Hs),* a wild ancestor of modern barley, differs from domesticated varieties and contains high-value components such as prebiotic carbohydrates that have been lost during the domestication process.

We predicted glycemic indexes (pGI) and resistant starch contents in grain models from *H. spontaneum*, a transgenic Amylose-only (AO) barley line and normal barley cultivar using both a static and a dynamic human gastro-intestinal *in vitro* model. The *in vitro* hydrolysis index (HI) values were correlated with *in vivo* GI data of barley products allowing the calculation of the pGI. Both the AO line and *H. spontaneum* had higher content of prebiotic carbohydrates; the undigested starch was 75% and 68%, respectively, of total starch content as compared to the control barley being 50%. *H. spontaneum* grain had high (15%) betaglucan content as compared to AO (4.0%) and control grains (5.0%). The pGIs of both the ancient and the AO line were lower (17.5 and 15.8, respectively) than control barley (35.0).

Our work supports the potential to include health-promoting grain of both high/full amylose and ancient germplasm origin as ingredient in grain-based products like bread and snacks.

References
Nilsson AC, Ostman EM, Knudsen KEB, Holst JJ, Bjorck IME. 2010. 140, 1932-1936.
Blennow A. Jensen SL, Shaik SS, Skryhan K, Carciofi M, Holm PB, Hebelstrup KH and Tanackovic V. Cereal Chem. 2013, 90(4):274–287
Hettiaratchi, U.P.K., Ekanayake, S. and Welihinda, J. Intern. Food Res. Journ. 2012, 19(3): 1153-1159

Selected talk within the research area 'Plant Products'

The evolutionary dynamics of cyanogenic glucoside biosynthesis and its genomic organisation in a biosynthetic gene cluster in legumes

Alexandra Öchsner, D. Lai, B. Darbani, B. L. Møller and F. Rook

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Cyanogenic glucosides are amino acid derived chemical defence compounds found in many plant species. Their biosynthesis involves two cytochrome P450 enzymes and a UDP-glucosyltransferase. We previously reported the independent evolution of the biosynthetic pathways for cyanogenic glucosides, and their genomic organisation in a gene cluster, in cassava (*Manihot esculenta*), *Sorghum bicolor*, and the model legume *Lotus japonicus*. Biosynthetic gene clusters are increasingly recognised in plant chemical defence and consist of non-homologous genes of the pathway that are co-localized in the same genomic region. In these three cases the first enzyme of the pathway is a member of the CYP79 family, which converts an amino acid precursor into an oxime. A second cytochrome P450 enzyme converts the oxime into a hydroxynitrile, followed by its glucosylation by a UDP-glucosyltransferase of the UGT85 family. In the biosynthesis of cyanogenic glucosides, the second enzyme was found to be a member of the CYP736 family in *L. japonicus*, and a member of the CYP71 family in sorghum and cassava. To investigate the evolutionary dynamics of cyanogenic glucoside biosynthesis and its genomic organisation, we analyse the pathway in other legume species.

Session 2: Plant Signalling and Cellular Trafficking/Synthetic and Systems Biology

Keynote talk within the research area 'Plant Signalling and Cellular Trafficking'

Membrane traffic and fusion in cytokinesis

Misoon Park[1], Cornelia Krause[1,3], Matthias Karnahl[1], Sandra Richter[1], Sonja Touihri[1], Farid El Kasmi[1,4], Ulrike Mayer[2], York-Dieter Stierhof[2] and Gerd Jürgens[1]

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Cytokinesis separates the forming daughter cells. Higher plants have lost the ability to constrict the plasma membrane in the division plane. Instead, TGN-derived membrane vesicles are targeted to the centre of the division plane and generate, by homotypic fusion, the partitioning membrane named cell plate. The cell plate expands in a centrifugal fashion until its margin fuses with the plasma membrane at the cortical division site. Mutant screens in *Arabidopsis* have identified a cytokinesis-specific syntaxin (aka Qa-SNARE) named KNOLLE and an interacting Sec1/Munc18 (SM) protein named KEULE both of which are required for vesicle fusion during cytokinesis. KNOLLE is only made during M-phase, targeted to the division plane and degraded in the vacuole at the end of cytokinesis. Our studies address mechanisms of KNOLLE trafficking to the plane of cell division as well as interactions of KNOLLE with different SNARE partners and with SM-protein KEULE, ensuring membrane fusion in cytokinesis.

Session 2: Plant Signalling and Cellular Trafficking/Synthetic and Systems Biology

Selected talk within the research area 'Plant Signalling and Cellular Trafficking'

MicroProteins in the regulation of Flowering Time

Moritz Graeff[1,2], Daniel Straub[1,2] and Stephan Wenkel[1,2]

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[2] Department for Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

MicroProteins (miPs) are small, single domain proteins, which functioning as negative regulators of protein complex formation and complex activity. They do so by directly interacting with other proteins, sequestering them in a non-functional sate. Many examples for this kind of protein species in plants and animals have been described. Often miPs can be found to act in developmental processes where they modulate the activity of genetic key components in these pathways. The initiation of flowering is one of the most important and complex transitions in a plants life. In the last years many genetic factors influencing this process have been identified and a revealed a very complex network of genetic regulatory circuits. However, often it still it remains unclear how these circuits are regulated and their interplay is fine tuned.

We performed a screen for potential miPs in the proteome of *Arabidopsis thaliana* and several other representative plant species and identified two small proteins, conserved among dicotyledonous plants, that affect the process of flower initiation in *Arabidopsis*. The presented data will show our characterization of the identified miPs, elucidate their role in the complex process of flowering time regulation and the underlying mechanism.

Session 2: Plant Signalling and Cellular Trafficking/Synthetic and Systems Biology

Selected talk within the research area 'Synthetic and Systems Biology'

Fusion of ferredoxin and cytochrome P450 enables direct light-driven biosynthesis

Silas Busck Mellor[1], Agnieszka Zygadlo Nielsen[1], Meike Bürow[1,2], Mohammed Saddik Motawia[1,3], Dainius Jakubauskas[1], Birger Lindberg Møller[1,3], Poul Erik Jensen[1,3]

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[3] Center for Synthetic Biology "bioSYNergy", Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

Cytochromes P450 (CYP) are involved in the biosynthesis of a massive number of specialized metabolites. In eukaryotes, the majority of CYP are inserted in the ER membrane via a single transmembrane domain. Catalysis requires an input of electrons in order to activate molecular oxygen, and these are supplied by an ER membrane bound NADPH dependent cytochrome P450 reductase (POR). Specialized metabolites produced by P450 enzymes are commonly found only in limited quantities in their natural sources, making their extraction both economically unfeasible and environmentally unsustainable. We aim to employ photosynthetic organisms as production hosts for specialized metabolites with interest to the pharmaceutical industry. Our lab has demonstrated that biosynthetic pathways can be coupled directly to photosynthetically generated reducing power by relocating the enzymes involved in the synthesis of the cyanogenic glucoside dhurrin in *Sorghum bicolor* to the chloroplast of tobacco plants[1]. In our model system two CYP enzymes acting in sequence obtain reducing power directly from photosynthesis by interacting with the small electron carrier protein ferredoxin, to drive the conversion of tyrosine into p-hydroxymandelonitrile. This project aims to optimize the electron flow needed for the activity of P450s directly from the photosynthetic electron transport chain, and eliminate the need for reductase-mediated electron transfer from NADPH to P450s via POR. To achieve this, we designed fusion enzymes between ferredoxin and cytochrome P450, which we tested using a combination of in vitro assays and LC-MS/MS analyses of compounds produced in tobacco plants transiently overexpressing the enzymes. This approach allows testing the different modules of the fusion enzyme in order to improve the partitioning of electrons between light driven P450 hydroxylations and other competitors for reduced ferredoxin in the chloroplast. This work presents a fusion between the major ferredoxin involved in photosynthesis and a model cytochrome P450, CYP79A1 from the dhurrin pathway of *Sorghum*. In vitro data shows that fusion to ferredoxin renders the cytochrome independent of added reductase when inserted in thylakoid membranes, and leads to increased allocation of electrons to cytochromes P450 in the presence of native electron sinks.

References:

1. Nielsen AZ, Ziersen B, Jensen K, Lassen LM, Olsen CE, Møller BL, Jensen PE (2013) Redirecting Photosynthetic Reducing Power toward Natural Product Synthesis. ACS Synth. Biol. 2: 308-315

Session 3: Plant Breeding - Quality, Productivity, Diseases and Stress/Biomass Production and Processing

Keynote talk within the research area 'Plant Breeding - Quality, Productivity, Diseases and Stress'

Essential RNA-based technologies and their applications in plant breeding

Guiliang Tang

Department of Biological Sciences, Michigan Technological University (MTU), USA

Genome sequencing has not only extended our understanding of the blueprints of many plant species but also revealed the secrecy of coding and non-coding genes. Here, I present a brief introduction and personal account for a few key RNA-based technologies and their development and applications for functional genomics of plant coding and non-coding genes, with a focus on short tandem target mimic, artificial microRNAs and CRISPR/Cas9. Furthermore, their multiplex technologies are discussed for functional dissection of gene networks in plant breeding.

Session 3: Plant Breeding - Quality, Productivity, Diseases and Stress/Biomass Production and Processing

Selected talk within the research area 'Plant Breeding - Quality, Productivity, Diseases and Stress'

Cytokinin production of *Pseudomonas fluorescens* G20-18 determines the biocontrol effect against *Pseudomonas syringae* in Arabidopsis

Dominik K. Großkinsky[1], *Thomas Roitsch*[1,2]

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Considering future demands in plant protection and restrictions in the use of classic pesticides, the development of alternative strategies is a major goal in plant sciences. Biological control of plant diseases by beneficial microbes offers therein a high potential for integrated plant disease management. Mechanisms contributing to such biocontrol phenomena comprise direct effects on pathogens or virulence factors and strengthening the plant. Despite the importance of phytohormones as essential plant immunity regulators, recently including also the classically growth-promoting cytokinins, their microbial production is not yet considered in biocontrol of diseases. Here we report that cytokinin production of *Pseudomonas fluorescens* G20-18 is a key determinant of biocontrol of *P. syringae* infection in Arabidopsis. Treatment with this strain strongly suppressed symptom development and spread of the pathogen, thus maintaining tissue integrity, and ultimately biomass yield. While cytokinin deficient loss-of-function mutants were impaired in controlling the infection, complemented mutants (gain-of-function), exhibiting restored cytokinin production, showed a similar biocontrol effect as the wildtype strain. The efficiency of the biocontrol effect correlated with the cytokinin levels *in planta* caused by the different bacterial strains. The analyses of Arabidopsis mutants impaired in defence pathways revealed the necessity of functional cytokinin perception in combination with other components such as salicylic acid to fully establish this biocontrol effect. This identification of microbial phytohormones to trigger biocontrol effects offers novel options for the development of successful strategies in plant protection which may be combined, based on cytokinins, with other positive effects such as increased abiotic stress tolerance and plant growth.

Selected talk within the research area 'Biomass Production and Processing'

'CPH assay': high-throughput screening of endo-glycoside hydrolases using novel chromogenic polysaccharide substrates

Julia Schückel, S. K. Kračun, W. G. T. Willats

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Endo-glycosyl hydrolases (endo-GHs) are largely responsible for degrading complex carbohydrates in plant cell walls and are important for the degradation of biomass feedstocks in biorefineries. Increasing numbers of microbial genome sequences are emerging, and from these the putative activities of large numbers of GHs can be obtained. However, there is a paucity of methods for the wide scale empirical screening of GHs against diverse substrates.

We have developed a new generation of chromogenic polysaccharide hydrogel ('CPH') substrates, which offer significant advantages over existing techniques. CPH substrates are insoluble hydrogels dyed in one of four colours that can readily be dispensed into 96-well filter plates (Figure 1) that provide the possibility to screen 96 enzymes at a time. Enzyme activity is revealed by release of soluble dyed oligomers which are collected in a corresponding 96 well plate by centrifugation or vacuum and enzyme activities are quantified spectrophotometrically. The use of 4 different colours enables the production of multiplexed assays in which substrates of different colours can be mixed in one well – theoretically enabling screening of 96 different enzymes against 96 different 4-substrate combinations. This is possible because the colours distinct absorption spectra can be resolved using simple linear regression. Synergetic or inhibitive effects can be studied using combinations of different coloured CPH-substrates in one assay.

Additionally, chromogenic biomass substrates from native and treated plant material have been developed and these allow the high-throughput profiling of enzymes and discovery of novel activities with scope of optimising industrial biomass degradation processes.

Session 4: Elevator talks + poster session

As appetizer for the poster session short talks have been selected based on submitted abstracts. Find the abstracts on the indicated pages.

Elevator talks

Cross Talk between Photorespiration and Nitrate Assimilation: Key to the Future of Net Primary Productivity, Food Quality, Forest Health, and Carbon Sequestration

Arnold Bloom

Department of Plant Sciences, University of California at Davis, Davis, CA 95616, USA

Rising carbon dioxide (CO_2) concentrations in the atmosphere inhibit the assimilation of nitrate (NO_3^-) into protein in most plants. Photorespiration, long considered a wasteful process that dissipates about 35% of photosynthetically produced carbohydrate as waste heat, actually generates reductant for NO_3^- assimilation. CO_2 inhibits photorespiration and thereby inhibits NO_3^- assimilation.

Plants vary in their relative dependence on different nitrogen (N) sources, and this explains the observed variation in ecosystem responses to elevated CO_2. At one extreme, an annual California grassland for which NO_3^- is the major N source showed a loss in net primary productivity under CO_2 enrichment, because CO_2 inhibited NO_3^- assimilation and plant organic N compounds became limiting. At the other extreme, the dominant plants in the Chesapeake Bay marsh, an ammonium-dominated ecosystem, showed a steady enhancement in photosynthesis and growth under CO_2 enrichment even after a decade of treatment. In forest soils, NO_3^- provides a small but ecologically important source of N, and so rising CO_2 contributed to the observed higher background mortality rates because declining plant protein exacerbates damage from insects and other pests as they consumed more plant material to meet their nutritional needs. Moreover, NO_3^- is the dominant N form available to crops from temperate agricultural soils, and CO_2 inhibition of NO_3^- assimilation seems to have already compromised the protein concentration of major foods.

ENSA - Engineering Nitrogen Symbiosis for Africa

Dugald Reid, Eiichi Murakami, Noor De Jong, Mette Hoffmann Asmussen, Lene Heegaard Madsen, Simona Radutoiu and Jens Stougaard

Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10. 8000 Aarhus C

ENSA uses engineering principles to establish high throughput synthetic biology approaches in plants. Legume genes required for development of symbiotic root nodules housing nitrogen-fixing rhizobia are refactored and transferred into cereals aiming at remodelling the existing mycorrhizal symbiotic pathway to enable nitrogen-fixing symbiosis in non-legume plants. At this first stage of the project a modular approach targeting three signalling modules known from legumes have been chosen for engineering and functional studies. An update of the principles and technologies that are used in the ENSA consortium (https://www.ensa.ac.uk/) will be presented and discussed.

Structure, function and host control of the microbiota thriving at the root-soil interface

Rodrigo Alegria Terrazas, Senga Robertson, Katharin Balbirnie and Davide Bulgarelli

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Plants host distinct microbial communities in the vicinity of and in association with their roots. These communities, designated the plant microbiota, engage in symbiotic relationships with their hosts that range from parasitism to mutualism. Therefore, the microbiota emerges as an extended plant phenotype capable of influencing crop yield. However, the host genetic determinants shaping the plant microbiota remain largely unknown.

My group uses barley as a model to gain fundamentally novel insights into the genetic network linking a plant genome and its associated microbiota. Using next-generation sequencing approaches, we previously characterised ~45 Gbp of the (meta)genome of the microbiota associated with wild and domesticated barley genotypes. Remarkably, this work revealed a significant effect of the plant genotype on the diversity of the microbiota, possibly representing a footprint of barley domestication.

To identify the host genetic determinants of the barley microbiota, we extended our initial investigation to a broader panel of ecologically referenced accessions. We analysed the microbiota of plant samples grown in two agricultural soils and we are defining the recruitment units of the microbiota interfering with plant growth. This discovery will be key in guiding future genetic investigations of barley-microbiota interactions. In a parallel line of research, we demonstrated that root hair development correlates with microbiota diversification, suggesting that root system architecture contributes, at least in part, to microbiota recruitment.

Collectively, our work is paving the way for the “breeding for the microbiota”, where functions of the rhizosphere microbiota could be deployed to enhance crop yield by targeting specific genes of the plants.

Rice perception of symbiotic arbuscular mycorrhizal fungi requires the karrikin receptor complex

Caroline Gutjahr[1,2], Enrico Gobbato[3], Jeongmin Choi[3], Michael Riemann[4,5], Matthew G. Johnston[3], William Summers[3], Samy Carbonnel[2], Catherine Mansfield[3], Shu-Yi Yang[1], Marina Nadal[1], Ivan Acosta[6], Makoto Takano[4], Wen-Biao-Jiao[6], Korbinian Schneeberger[6], Krystyna A. Kelly[3], Uta Paszkowski[1,3]

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Arbuscular mycorrhiza (AM) is an ancient symbiosis between plants and glomeromycotan fungi that is widespread in the plant kingdom. In terrestrial ecosystems, plants take up phosphate predominantly via the fungi and provide them with organic carbon in return. AM establishment is initiated by a bi-directional exchange of signals involving plant strigolactones and fungal chitin-derived signals called Myc factors. Following recognition AM fungi form a hyphopodium at the root surface, enter the root cortex and form branched arbuscules inside cortex cells. Several plant genes have been identified that are required for intraradical colonization and arbuscule formation. However, while the initiation of colonization via hyphopodia is the first important step in symbiosis development it still remains poorly understood. We identified loss of responsiveness to AMF in the rice (*Oryza sativa*) mutant *hebiba*, reflected by the absence of physical contact and of characteristic transcriptional responses to fungal signals. Among the 26 genes deleted in *hebiba*, *DWARF 14 LIKE* is, the one responsible for loss of symbiosis. It encodes an alpha/beta-fold hydrolase that is a component of an intracellular receptor complex involved in the detection of the smoke compound karrikin. Our finding reveals an unexpected player in plant recognition of AMF and a previously unknown signaling link between symbiosis and plant development.

Genome-editing technologies: Prospective novel techniques for agronomical crop breeding

Nawaporn Onkokesung

Department of Plant Breeding, Swedish University of Agricultural Sciences, Sweden

Genetic engineering technologies i.e. transfer DNA (T-DNA) insertion and RNA interference (RNAi) has been applied for more than three decades to elucidate the functions of gene-of-interest in various plant species including agronomical crop plants such as wheat, rice, maize, tomato and potato. However, a time-consuming, and an unpredictable off-target are the major constrains of using T-DNA and RNAi approaches for plant breeding. Recently, genome editing technologies offer a novel and versatile way to precisely editing dicot and monocot plants genomes without incorporating transgenes.

We have been exploiting two genome editing techniques; transcript activator-like effector nucleases (TALENs) or clustered regularly interspaced short palindromic repeat (CRISPR)/Cas 9 systems to study functions of the candidate disease susceptibility genes in potato and barley. In order to circumvent agrobacterium-mediated plant transformation, we use transient expression approach in protoplasts to introduce permanent mutations. We have been successfully used TALENs system to mediate a precise mutation in tetraploid potato (*Solanum tuberosum* L. cv Desiree) protoplasts; however a complexity of protein engineering step and a restricted flexibility hampers a prospect of adopting TALENs system in plant breeding. CRISPR/Cas9 systems, on the contrary, offer simplicity, accessibility, versatility and efficiency for precise genome modification in monocot and dicot plants. We are currently developing standard methods for transient expression mediated by CRISPR/Cas9 systems in potato and barley protoplasts that will be used as a foundation to establish genome editing platform for monocot and dicot crop plants.

Epigenetics in plant breeding-mechanisms and prospects

Claudia Köhler

Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, Uppsala, Sweden

Epigenetic effects are connected to chemical modifications of DNA and its packaging in chromatin. In contrast to the well-known heritable genetic basis of phenotypic variation, it is poorly understood to which extend epigenetic variation can affect phenotypes. Because some epigenetic modifications can be heritable to the next generation, transgenerational epigenetic inheritance is possible and can contribute to phenotypic variability. Epigenetic modifications affect phenotypes by altering gene expression. We study epigenetic effects in the endosperm. The endosperm is only transiently required during seed development, but it is of utmost importance for human nutrition and animal feed. Epigenetic mechanisms make the endosperm sensitive to parental genome dosage, preventing hybridization between different species. This creates a major obstacle for gene pool improvements in breeding programs. I will discuss the epigenetic basis of these endosperm-based hybridization barriers as well as possibilities and prospects of overcoming them.

Tackling plant chromatin structure and its potential for plant production

Iva Mozgova

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Plant cells have the capacity to become pluripotent and establish different developmental fates in response to chemical or mechanical stimuli. Induction of ectopic organ development and regeneration of entire plants from vegetative tissue is widely used in clonal propagation of horticultural species, species preservation and as a model system for plant development. Inducing sufficient level of pluripotency in somatic cells using currently available approaches is often challenging in many plant species due to insufficient understanding of the molecular barriers that prevent it, which limits the biotechnology potential of these approaches.

The establishment and maintenance of cell fate depends on specific gene expression patterns, which need to be altered during reprogramming. Gene expression is to a large extent governed by chromatin structure – its composition and compaction level. Chromatin structure has in recent years been recognized as one of the crucial players in cell fate determination and reprogramming. The advance of next-generation sequencing (NGS) techniques has enabled the determination of genome-wide patterns of DNA methylation, nucleosome composition or histone post-translational modifications and addressing their role in cell fate specification.

In this talk, I will give an overview of the state-of-the-art methodical approaches for elucidating chromatin structure patterns. I will relate to examples on how epigenetic mechanisms of gene regulation affect cell fate determination in plants, and how approaches to modify chromatin structure can aid developmental reprograming in different plant species.

Research Areas

Abstracts that are submitted in the oral presentation category undergo a selection process and participants with selected abstracts are invited to give a 20 minute talk or a short elevator talk. The Selection committees for the six different research areas are:

Plants for Food and Feed: Healthy food and feed, fatty acids, fibres, vitamins, minerals, proteins, biofortification, flour, malt, allergens, anti-nutritional factors

Henrik Brinch-Pedersen, Aarhus University
Birte Svensson, Technical University of Denmark
Søren K. Rasmussen, University of Copenhagen

Plant Products: Starches, fibres, pharmaceuticals, specialized metabolites, biopesticides, proteins and enzymes, oil, phytochemicals, fuels, natural colors, bioplastics

Barbara Halkier, University of Copenhagen
Eva Vincze, Aarhus University
Søren Bak, University of Copenhagen

Plant Signalling and Cellular Trafficking: Cell-cell signaling, bioimaging, biomembranes, signal transduction, organism wide and community wide signaling, membrane trafficking, cellular trafficking

Jens Stougaard, Aarhus University
Anja Thoe Fuglsang, University of Copenhagen
Hans Thordal-Christensen, University of Copenhagen

Synthetic and Systems Biology: Metabolic engineering, molecular farming, omics technologies, bioinformatics, meta genomics, epigenetics

Kåre Lehmann, Aalborg University
Mathias Pribil, University of Copenhagen
Per Gregersen, Aarhus University

Plant Breeding - Quality, Productivity, Diseases and Stress: Yield, modern breeding techniques, genomics, adaptation of crops to climatic change, pathology, plant/microbe interactions, pest managements, epidemiology, disease resistance, phenomics

Torben Asp, Aarhus University
Niels Sandal, Aarhus University
Michael Lyngkjær, University of Copenhagen

Biomass production and processing: Micro and macro nutrients, plant uptake, resource use efficiency, bioenergy, carbohydrate polymers, biorefinery

Yumiko Sakuragi, University of Copenhagen
Jan K. Schjørring, University of Copenhagen
Lene Lange, Technical University of Denmark

Poster

Research Area: Plants for Food and Feed

Research Area: Plant Products

Research Area: Signalling and Cellular Trafficking

Research Area: Synthetic and Systems Biology

Research Area: Plant Breeding - Quality, Productivity, Diseases and Stress

Research Area: Biomass production and processing

Alginate protein interaction parameters as affected by degree of polymerization

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Alginate is a polysaccharide that is produced by brown algae and some bacteria. Alginate has a very strong interaction potential with β-lactoglobulin (BLG) due to the anionic properties of the polysaccharide. When alginate and BLG carries opposite charges (pH < 5), large insoluble particles with a hydrodynamic diameter measured in µM are formed. Here 3 alginates of different degrees of polymerization are characterized with respect to the interaction with BLG. The particle formation and hydrodynamic diameter as assessed by turbidity and dynamic light scattering is very dependent on pH. The particle hydrodynamic diameter is unaffected by degree of polymerization at pH 2 and 3, but differences is observed at pH 4 and 5. The apparent strength of binding, the stoichiometry and the apparent difference in enthalpy of interaction is assessed by ITC. At pH 3 high molecular weight alginate interacts with BLG with a dissociation constant (Kd) of 23 ± 3 nm and a stoichiometry of 113.8 ± 1.1, low molecular weight alginate with a Kd of 266 ± 48 nm and a stoichiometry of 15 ± 0.3. A purified alginate hexa-saccharide interacts with a Kd of 293 ± 42 µM and a stoichiometry of 1.2 ± 0.2. Similar results are obtained at pH 4 except the stoichiometry of binding which doubles. The difference in stoichiometry of binding between the polymers differs by a factor of 7.6 which corresponds closely to the difference in degree of polymerization. The Kd differ by a factor of 11.5 at pH 3 and 9.9 at pH 4. This is different from the factor of 7 that is predicted by the law of mass action. These results show the importance of consideration into alginate degree of polymerization and pH when making alginate and whey protein formations.

Interactions between probiotic *Lactobacillus acidophilus* and plant-derived oligosaccharides by adhesion and surface layer proteomics

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Prebiotics are non-digestible components in edible plants which affect the host health beneficially by stimulating selective growth of probiotic bacteria in the gastrointestinal tract (GIT). Plant-derived oligosaccharides constitute major group of probiotics. Inulin, lactulose, and galactooligosaccharides (GOS) are considered as prebiotics approved after clinical trials. By contrast, several emerging for example fructooligosaccharides (FOS), cellobiose, melibiose, and isomaltulose await approval.

Lactobacillus acidophilus NCFM is a probiotic bacterium used intensively in foods and dietary supplements, and very well studied bacterium. Probiotic adhesion to intestinal cells (HT-29 and Caco-2) and mucus layer is considered crucial for exerting health benefit to the host and some surface proteins involved in the interaction have been identified. In addition to known mucin binding proteins, some intracellular proteins of other lactobacilli may have moonlighting functions in binding to mucin.

The present study aims to investigate the effects of defined (FOS, GOS, lactulose) and emerging prebiotics (cellobiose, raffinose, melibiose, isomaltulose, polydextrose, and trehalose) on adhesion properties of *Lactobacillus acidophilus* NCFM. In addition, cellobiose and raffinose are selected for surface layer proteome analysis.

Results showed that while the adhesion to mucin and HT-29 cells was significantly changed ($p<0.05$) by growing the bacteria on cellobiose, raffinose, or polydextrose compared to grown on glucose, there was no change when grown on GOS, lactulose, melibiose, palatinose, or trehalose – growing on FOS changed the adhesion only to HT-29 cells. Surface proteome analysis revealed that growing on cellobiose or raffinose altered the abundancies of some proteins that may have roles in adhesion.

In conclusion, plant-origin indigestible defined and emerging prebiotics may affect the adhesion properties of probiotic *Lactobacillus acidophilus* so that beneficial health effects to the host may be increased.

Transcriptome sequencing in narrow-leafed lupin (*Lupinus angustifolius*): Combining short- and long-read sequencing platforms

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Narrow-leafed lupin (NLL) is a nitrogen-fixing legume with great potential to improve the EU's meager production of vegetable protein. Despite a remarkably high protein content, the use of NLL beans as food or fodder is limited by high content of anti-nutritional quinolizidine alkaloids (QAs), which make the beans bitter and toxic. QAs are thought to be made in the leaves and transported to the beans, but so far only the first pathway-specific gene is known. To gain a better understanding of the molecular basis of QA biosynthesis and transport, we used short-read sequencing (Illumina, Hiseq2500) to profile the transcriptome of 8 different tissues of a high-alkaloid NLL variety. In addition, to facilitate the assembly of the short reads, we used a single-molecule long-read sequencing platform (PacBio, SMRTbell) to sequence transcripts longer than 1Kb from an RNA pool of the different tissues. Based on the long-read sequencing, we obtained 10,661 high-quality transcript isoforms with polyA tails. About 40% of the short reads obtained by Illumina sequencing could be mapped to the reference transcript isoforms from the long-read PacBio sequencing. The rest of the short reads were assembled into 183,691 contigs with median length of 487 bp. Finally, the PacBio-derived transcript isoforms and the Illumina-derived contigs were combined to obtain a comprehensive transcriptome, which was subjected to annotation and gene expression profiling. Our transcriptomic dataset provides a solid foundation for the discovery of enzymes, transporters, and regulators that control QA levels in NLL.

Biosynthesis pathway of the unusual triterpene α-Onocerin

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This PhD project is part of TriForC (www.triforc.eu) which is an EU-funded collaborative project on establishing an integrative and innovative pipeline for the exploitation of plant triterpenes. We have focused our efforts in the biosynthesis of the unique compound α-onocerin.

α-Onocerin content was measured in different tissues of *Ononis spinosa* at different developmental stages. α-Onocerin accumulates at high levels in the roots while not detected in the leaves and stem of the plant. Further sectioning of the roots showed a gradient in α-onocerin accumulation across the root; the base of the root has highest α-onocerin content and decreases to nearly zero in the root tips.

Accordingly, RNA-seq was done on root tips and the middle section of the roots and the transcriptomes were mined for homologs of known triterpene synthesis genes.

Characterization of the candidate genes is being carried out by transient expression in *Nicotiana benthamiana*.

Degradation of potent Rubisco inhibitor by selective sugar phosphatase

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Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyses the conversion of atmospheric carbon dioxide into organic compounds in photosynthetic organisms. Alongside carboxylating the five-carbon sugar ribulose-1,5-bisphosphate (RuBP), Rubisco produces a small amount of xylulose-1,5-bisphosphate (XuBP), a potent inhibitor of Rubisco. The AAA+ protein Rubisco activase removes XuBP from the active site of Rubisco in an ATP-dependent process. However, free XuBP rapidly rebinds to Rubisco, perpetuating its inhibitory effect. Here, we combine biochemical and structural analyses to show that the CbbY protein of the photosynthetic bacterium *Rhodobacter sphaeroides* and *Arabidopsis thaliana* is a highly selective XuBP phosphatase. We also show that CbbY converts XuBP to the non-inhibitory compound xylulose-5-phosphate, which is recycled back to RuBP. We solve the crystal structures of CbbY from *R. sphaeroides* and *A. thaliana*, and through mutational analysis show that the cap domain of the protein confers the selectivity for XuBP over RuBP. Finally, in vitro experiments with CbbY from *R. sphaeroides* reveal that CbbY cooperates with Rubisco activase to prevent a detrimental build-up of XuBP at the Rubisco active site. We suggest that CbbY, which is conserved in algae and plants, is an important component of the cellular machinery that has evolved to deal with the shortcomings of the ancient enzyme Rubisco.

Natural diversity of cucurbitacins in Cucurbits

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Plants have evolved a large diversity of triterpenes to adapt to the environment. Many of the triterpenes are beneficial to the pharmaceutical and agricultural sectors. Cucurbitacins are highly unsaturated and oxygenated triterpenes which exhibit different bioactivity like anticancer, antidiabetic and insecticidal based on the structural diversity. In this project, we aim to explore the natural diversity of cucurbitacins in different species and varieties of the Cucurbits.

Cucurbitacins consist of 20 types of variants and are known for their bitter taste in the fruit. To obtain the most variable accessions regarding cucurbitacin production, 10-day old seedlings from around 100 wild and cultivated accessions were screened by LC-MS. To our surprise, there is no significant difference on cucurbitacin production between the wild bitter accession and cultivated sweet accessions of four species tested. This indicates a different cucubitacin regulatory mechanism between seedlings and fruits. To determine which part produces the most cucurbitacin, 10-day old seedlings were dissected into four different tissues. Our results showed that different cucurbitacin variants are present in different tissues. For example, cucurbitacin E was abundant in roots whereas cucurbitacin B was abundant in cotyledon. This difference might come from their different biological function.

The most variable accessions and tissues will be further processed for bioprospecting.

Plant derived diterpenoids alter crucially the structure and physical-chemical properties of phospholipid membranes

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Coniferous trees are able to release resins for defense mechanisms that contain diterpenoids. Among other diterpenoids, neoabietic acid (NA) and dehydroabietic acid (DAA) are the main resin acids (RA) of the Oleoresin. They are accumulated for defense mechanisms and damage the tissue of the target. Interactions between RAs and lipids are likely one major reason for their toxicity. To correlate the membrane interactions with the chemical structure and toxicity of RAs, two natural RAs and a synthetic one derived from DAA have been thoroughly studied on cell membrane models. Differential light scattering and Cryo-Transmission Electron microscopy gave insight on the structural impact of these RAs on DPPC vesicles: RAs lead to the formation of tubular structures. The results suggest that RAs have a fluidizing effect on DPPC membranes. Moreover, the localization of the RAs in DPPC monolayers was studied using Neutron Reflectivity (NR). NR results suggest a perturbation of the head group area by polar RAs ranging into the lower tail region, whereas more hydrophobic RAs cause sterol-like effects.

Effect of *Azospirillum brasilense* strains on *Lotus japonicas* root growth and development

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Azospirillum brasilense, a nitrogen-fixing bacteria, is broadly present in soils around the world. Strains of this bacterium can live isolated in the soil or in the rizosphere of a wide plant families. The beneficial effects of *Azospirillum* on some crops, such as maize or wheat are well documented and have been mainly associated with the increase of root system and therefore, the enlarged area available for mineral uptake. The beneficial effect of *Azospirillum* on crops proved to be, however from time to time inconsistent, and environment dependent (1). On the other hand, the molecular mechanisms behind the beneficial or less favourable interactions with the different hosts are largely unknown (2).

We set to better understand the molecular basis of the interaction between *Azospirillum* and the model legume host, *Lotus japonicus*. Specific growth conditions in which *Azospirillum* has a detrimental effect on the legume host have been identified. We are currently using such defined conditions to better understand the molecular mechanism involved in the different responses mounted by the two interacting partner leading to contrasting responses in the host. We will present here our progress on this study based on *Lotus* mutants analyses and host gene expression investigations.

1. María I. Saubidet et al. (2002) *Plant Soil* 245: 215–222.
2. Guido V Bloemberg & Ben JJ Lugtenberg. (2001) *Curr Opin Plant Biol* 4:343–350.

Characterisation of the novel *Lotus japonicus* symbiotic mutant EXO422

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The symbiotic interaction between legume plants and soil bacteria, collectively referred to as rhizobia, results in the formation of root nodules, within which rhizobia convert atmospheric nitrogen into forms biologically available to the plant. In return, the rhizobia are provided with carbon sources. Although well studied, the mechanisms and signaling pathways that allow the plant to co-ordinate rhizobia infection and nodule organogenesis remain incomplete.

To further investigate these mechanisms, a symbiotic mutant isolated from a random mutagenesis screen of *Lotus japonicus* Gifu is being characterised. The EXO422 mutant forms large–uninfected nodules when inoculated with the *Mesorhizobium loti* R7A *exoU*. In contrast, Gifu plants form only small-uninfected nodule primordia. Infection thread formation by *exoU* is similarly impaired on both Gifu and mutant plants. Wild-type *M. loti* R7A, forms less infection threads and shows reduced nodulation rates on EXO422 compared to Gifu. The symbiotic phenotypes indicate that EXO422 is impaired in the signaling that co-ordinates the infection process and nodule development. EXO422 shows additional phenotypes including the formation of short roots that harbour many root hairs. These phenotypes are likely related to differences in ethylene production. Furthermore, EXO422 forms spontaneous nodules in the absence of rhizobia. Rough mapping of EXO422 indicates that the mutation responsible for the large–uninfected nodule phenotype in association with *exoU* is located at the top of chromosome 2. Mapping of the spontaneous nodule phenotype indicates the responsible mutation is at the end of chromosome 4.

Rab5 activation mediates ancient pre- and post-invasive plant innate immunity

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Pre- and post-invasive plant innate immunity is highly effective in preventing proliferation of filamentous pathogens acting either before or after host cell entry. Already early land plants had evolved defence structures resembling encasements found in extant plants. The encasement hinders close contact between the pathogen and the host cell cytosol and is therefore likely to have played a crucial role for early land plants to overcome attack by filamentous pathogens. Here we show that VPS9a, an ancient regulator of eukaryote endomembrane trafficking, is required for timely encasement formation in response to attack from a non-adapted powdery mildew in Arabidopsis. Interestingly, the encasement facilitates both pre- and post-invasive immunity, and acts in concert with the previously well-described pathways defined by PEN1 and PEN2. Moreover, the encasement provides defence to an adapted powdery mildew fungus, which was considered to have overcome pre-invasive immunity. We speculate that VPS9a has played a conserved role in organizing the encasement response and thereby contributed to immunity of land plants, all since they evolved some 450 Mya. As symbiotic associations with beneficial fungi are considered critical for the terrestrial colonization by plants, we propose that the encasement has enabled early land plants to decide between fungal friend and foe.

The powdery mildew fungus influences barley ROR2-mediated immunity

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The *Blumeria graminis* (*Bg*) fungi cause powdery mildew disease on different grasses including barley and wheat. The pathogenic fungi initiate their attack on the plant with a penetration attempt at the cell wall and plasma membrane. However, most of these attempts get negated in non-host plants. In a screen for mutants, which affected the non-host penetration resistance of the *Bg* f. sp. *hordei* (*Bgh*) in *Arabidopsis thaliana* the gene PEN1 was discovered. An orthologous gene, ROR2, of the PEN1 was isolated from the host *Hordeum vulgare* (Barley).

These genes encode syntaxins, required for full penetration resistance by host and non-host plants. To mediate successful penetration, the fungi and other pathogens interfere with the host plants cell immunity machinery by the help of effectors, which are small molecules specialised in interacting with one or more plant protein/s. Immunity regulating plant proteins are the primary potential target of the effectors. In *Bgh* over 500 effector-candidates have been identified.

An effector-candidate from *Bgh* was shown to interact with a barley ubiquitin E3 ligase in correlation to susceptibility of the *Bgh*. This interaction is dependent on the ROR2 gene's functionality. Data suggests the interaction between ROR2 and the E3 ligase alters the penetration resistance to *Bgh* and ROR2 localization in the plant cells.

The main focus of my project is to investigate the interaction of these three proteins *in planta* and further demonstrate the specific amino acid residues or protein domains necessary for this interplay.

Volatiles from the burnet moth *Zygaena filipendulae* (Lepidoptera) and associated flowers, and their role in mating communication

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Emissions of volatiles play an important role in communication between male and female insects. The burnet moth *Zygaena filipendulae* contains the cyanogenic glucosides linamarin and lotaustralin, which can be degraded to the volatiles HCN, acetone and 2-butanone. Linamarin and lotaustralin have previously been shown to be transferred from male to female during mating and hypothesized to be involved in mating communication. In this study, the volatile emissions from different ontogenetic stages of *Z. filipendulae*, and from flowers inducing mating were measured using head space solid-phase micro-extraction (SPME) GC-MS. All *Z. filipendulae* life-stages emit HCN, acetone and 2-butanone. Virgin females show higher emissions than mated females, while mated males have higher emissions than virgin males. HCN emission was only rarely detected in the course of male-female copulation. This indicates a role of the cyanogenic glucoside derived volatiles during female calling as well as during male courtship behaviour, but not as defence during copulation. Males rejected for mating by a female were accepted after injection of linamarin or lotaustralin, demonstrating that cyanogenic glucosides are important for female assessment of the fitness of the male. Analysis of emissions from males and females as well as from flowers used during mate calling resulted in identification of putative pheromones in *Z. filipendulae*.

The role of phased siRNAs in *Lotus japonicus* development and nodulation symbiosis

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Legumes such as pea, beans and the model trefoil *Lotus japonicus* (*L. japonicus*) develop nitrogen fixing root nodules in the presence of compatible rhizobial bacteria. Phased small interfering RNAs (phasiRNA) play paramount roles in the regulation of plant developmental processes including legume nodulation symbiosis. However, only one group of phasiRNAs, the TAS3 derived phasiRNAs (tasiR-ARFs), has been characterized in the model legume *L. japonicus.* The aim of this project was to investigate if phasiRNAs and their respective targets are involved in legume development and symbiosis with rhizobial bacteria. To this end, we screened a *L. japonicus* degradome sequencing dataset for loci generating populations of phased 21 nt sequences. In parallel we identified an allelic series of mutants impaired in the putative *L. japonicus* ortholog of the Arabidopsis DICER-like gene *DCL4*, and confirmed the molecular dependence of selected phasiRNAs on this gene. To test whether phasiRNAs had a potential role in *L. japonicus* development, we investigated the impact of *DCL4* function on developmental and symbiotic phenotypes. Using loss-of-function mutants in other *L. japonicus* genes involved in phasiRNA biogenesis, namely *ARGONAUTE 7* (*AGO7*), *SUPPRESSOR OF GENE SILENCING 3* (*SGS3*), *RNA-DEPENDENT RNA POLYMERASE 6* (*RDR6*), we attempted to dissect the role of tasiR-ARFs as well as other phasiRNA groups in development and symbiosis in this species. Aiming to understand a systemic necrosis phenotype observed in *dcl4* mutants at advanced stages of plant development, we further screened these plants for signs of abnormal immunity status or hormone homeostasis.

A phospholipid uptake system in the model plant *Arabidopsis thaliana*

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Plants use solar energy to produce lipids directly from inorganic elements and are not thought to require molecular systems for lipid uptake from the environment. Here we show that *Arabidopsis thaliana* Aminophospholipid ATPase10 (ALA10) is a P4-type ATPase flippase that internalises exogenous phospholipids across the plasma membrane, after which they are rapidly metabolised. ALA10 expression and phospholipid uptake are high in the epidermal cells of the root tip and in guard cells, the latter of which regulate the size of stomatal apertures to modulate gas exchange. ALA10 knockout mutants exhibit reduced phospholipid uptake at the root tips and guard cells and are affected in growth and transpiration. The presence of a phospholipid uptake system in plants is surprising. Our results suggest that one possible physiological role of this system is to internalise lysophosphatidylcholine, a signalling lipid involved in root development and stomatal control.

Assembly of symbionts and endophytes at the legume root interface

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Legumes have the capacity to recognize and benefit from a broad spectrum of microbes establishing root nodule symbiosis (RNS), arbuscular mychorriza (AM) symbiosis, and endophytic association. Besides these beneficial associations, legumes are also hosts for various epiphytic or pathogenic microorganisms; therefore they provide a valuable system for studying host interactions with microbial communities. The existence of a common genetic program for RNS and AM was demonstrated. Furthermore, corresponding components from monocots can perform the same function in legumes, demonstrating the establishment of this genetic program before the divergence of monocots and dicots. In addition to this, *Lotus* spontaneous nodule cells can be colonized, and infected intracellularly by Nod-factor defective rhizobia, indicating that genetic components of an ancient infection/colonization program still operates in *Lotus*. We are taking advantage of the large panel of resources available for *Lotus japonicus* to study the accommodation of symbiotic and endophytic microbes inside roots and nodules. Analyses based on a binary interaction established between *Lotus* and a nonsymbiotic soil bacterium allowed identification of host and microbial components involved in the process of nodule colonization. In addition, pyrosequencing of the bacterial 16S rRNA gene provided clues on the role of legume genes in the establishment of root and nodule inhabiting or associated bacterial microbiota. The current status and the main findings from our studies will be presented.

Proper AMSH3 deubiquitinase activity is required for defense

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The first layer of plant defense is activated by plasma membrane-bound receptors that recognize conserved microbial molecules. Some receptors are constitutively internalized to an endosomal compartment and either recycled to the PM or sent for degradation via multivesicular bodies (MVBs) that ultimately fuse with the lytic vacuole. Ubiquitination and deubiquitination of the PM receptors appears to control these processes.

We identified two unique alleles of *AMSH3* in a screen for suppressors of programmed cell death triggered by loss of *SYP121* and *SYP122*. *AMSH3* is a protein multi-domain JAMM/MPN type deubiquitinase (DUB) that interacts with ESCRT-III components and is essential for proper vacuolar trafficking via MVBs (Katsiarimpa et al. (2011) TPC 23:3026-3040). Knock-out of *AMSH3* causes seedling lethality, thus our mutant alleles provide unique opportunities to describe *AMSH3* in defense.

We infected the *amsh3* mutants and found, as expected, that they were hyper-susceptible to the adapted pathogens, *Pseudomonas syringae* DC3000 and *Golovinomyces orontii*. This may be correlated with a misregulation of late PTI responses, as *amsh3* mutants displayed reduced sensitivity to the PAMPs elf18 and flg22 in seedling growth inhibition assays.

Interestingly, these phenotypes were correlated with a decreased K48- and K63-linked poly-ubiquitin deubiquitinase activity *in vitro*.

We are currently testing a model, where AMSH3 influences receptor traffic, and aberrant AMSH3 localization or activity results in receptor misregulation and enhanced disease susceptibility.

Characterizing the role of cytokinin transport in legume nodule development

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The identification of several classes of cytokinin transporters indicates that short and long distance transport are likely required for regulating cytokinin localization and signaling. Legume nodule development provides a good system to study cytokinin transport as cytokinin is both necessary and sufficient for the cortical cell divisions observed during this process. This study will characterize the role of transporters of the Purine Permease (PUP) family during legume nodulation in *Lotus japonicus*. Transcript profiling and *pPUP-GUS* reporter gene studies showed several *LjPUPs* are expressed during nodule development, especially *PUP1* and *PUP3*, in nodules. We have isolated *PUP1*, *PUP2*, *PUP3* and *PUP4* single mutants with Lotus Endogenous Retrotransposon1 (LORE1) insertions, which will be used for constructing multiple mutants and further phenotypic and functional analyses. Lines overexpressing PUP will be created (*35Spromoter-PUP*) for additional analyses. We will also identify the subcellular localization of LjPUPs through fusion proteins composed of LjPUP-reporter and performing transport assays in tobacco microsomes. To explore the role of LjPUPs in cytokinin homeostasis and signalling, *LjPUPs* and cytokinin signalling genes both in *pup* and wide type will be assayed. Together, it is expected the functional analysis of genes of the PUP family will clarify the role and effects of cytokinin transporters during nodule development in *L. japonicus* and may have implications for the understanding of regulation of cytokinin homeostasis in other plants.

Systems biology approach to lycophytes: Selaginella mollendorffi

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Expression atlas efforts, such as AtGenExpress for Arabidopsis, provide invaluable resource for a studied organism [1]. Currently, the common method used to determine the expression values of genes across tissue types and treatments has been microarrays. This method however has several disadvantages as the initial design of the array is expensive and often large amount of genes is absent from the microarray. However, recent advances within data analysis and sequencing have enabled the use of RNA sequencing (RNA-Seq) data to determine co-expression, with almost the same precision as microarrays [2].

We have generated an expression atlas for Selaginella moellendorfii, a model species for Lycophytes, and important ingredient of Chinese medicine. It belongs to the first group of plants containing vascular tissue, and believed to have developed its root system independently,

The tissue atlas includes all major tissue types and environmental perturbations, such as cold, heat and time series. We have used the data to predict more than 8000 new transcripts. Held together with data from other evolutionary important species, this enables us to show how multiple pathways evolved and duplicated over time.

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Using protein scaffolds to redirect photosynthetic reducing power for biosynthesis of natural products

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Photosynthesis in plants provides ATP and NADPH as well as carbon sources for primary metabolism. Cytochrome P450 monooxygenases (P450s) in the plant endoplasmic reticulum (ER) are essential in the synthesis of many bioactive natural products, powered by single electron transfers from NADPH. We have recently demonstrated that it is possible to break the evolutionary compartmentalization of energy generation and P450-catalysed biosynthesis, by relocating an entire P450 dependent pathway to the chloroplast and driving the pathway by direct use of the reducing power generated by photosystem I in a light-dependent manner [1]. This demonstrates the potential of transferring pathways for structurally complex high-value natural products and directly tapping into the reducing power generated by photosynthesis to drive the P450s using water as the primary electron donor.

Current work is directed towards exploring different strategies to optimize channeling of product formation. One approach to ensure co-localization of the enzymes in the thylakoid membrane is the fusion of the enzymes to components of the Twin-arginine translocation pathway – TatB and TatC. These are membrane anchored and have inherent self-organizing properties which will allow us to recruit the enzymes into close proximity and thereby reduce metabolic load. An alternative approach is scaffolding which aims at more efficient channeling of the substrate and intermediates. For this we utilize the protein-protein binding properties of PDZ domains for building a modular synthetic scaffold. These are around 80-90 amino acids and bind to the C-terminus of their target proteins (~10 aa) with different specificity. By making fusion proteins of the required enzymes with the ligand peptides, we can spatially recruit them in a desirable manner. We expect that both strategies should reduce formation of intermediaries, limit cross-talk between signaling pathways, improve substrate channeling and consequently increase product yields.

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Establishing and screening a *Lotus japonicus* LORE1 mutant population

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Lotus retrotransposon 1 (LORE1) can be activated during tissue culture, giving rise to plant lines with active LORE1 elements. In the founder line for the LORE1 mutant collection, a LORE1 element is active in the male germline, giving rise to independent stable insertions in the founder line progeny. On average, five new *LORE1* copies are found in each line. *LORE1* has an exonic preference, which results in a high incidence of knockout mutations in genes (Fukai *et al.*, 2010, Urbanski *et al.*, 2012). At present ~85.000 *LORE1* lines have been developed. The total fraction of genes targeted with exonic insertions was 65%, and 81% of the genes with exonic lengths larger than 1 kb were targeted. The insertion data, plant line information and seed stocks are freely available at the LORE1 resource website http://carb.au.dk/lore1. We have performed forward screening of several thousand lines from this population. A number of mutant lines with phenotypes such as light green leaves, variegated leaves (leaves with white sectors), symbiotic mutants, root mutants, dwarf mutants and leaf shape mutants were found. The mutants were confirmed in the next generation. Among ~5,500 LORE1 lines, we identified ten families segregating plants with a pale leaf phenotype. Consulting the database with annotated insertions, we found candidate insertions for 6 of the lines. Co-segregation of the insertions with the mutant phenotypes was confirmed using genotyping PCR or sequence specific amplified polymorphisms (SSAP) analysis. We have also found many new alleles of known symbiotic genes through both forward and reverse genetics.

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Spatially confined protein targeting to the thylakoid membrane – a CURT1A-based approach

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Thylakoids of land plant chloroplasts are composed of grana stacks and stroma lamellae, substructures that confer a characteristic three dimensional ultrastructure to the membrane system and support a lateral heterogeneity with respect to the distribution of photosynthetic complexes. Upon changes in environmental conditions thylakoids can undergo various structural rearrangements which account for the high plasticity of the membrane system. In Arabidopsis thaliana, the CURT1 protein family was shown to be involved in grana formation by facilitating membrane bending in the grana margins. The degree of membrane bending is thereby correlated with the amount of CURT1 being present in the thylakoid membrane. While a lack of CURT1 proteins leads to a general loss in thylakoid membrane curvature the accumulation of CURT1A causes a hyper-membrane bending phenotype. Here, we present approaches to monitor and dissect the CURT1 complex composition and its assembly dynamics and how to exploit the properties intrinsic to the CURT1 proteins to target enzymes in a scaffold-like manner into spatially confined domains of the thylakoid membrane, the grana margins.

Synthetic Biology as a tool for pathway elucidation - Case study: Carnosic acid-related diterpenes

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Synthetic biology approaches achieving the reconstruction of specific plant natural product biosynthetic pathways in dedicated microbial “chassis” have provided access to important industrial compounds (e.g. artemisinin, resveratrol, vanillin). However, the potential of such production systems to facilitate elucidation of plant biosynthetic pathways has been underexplored. Here we report on the application of a modular terpene production platform in the characterization of the biosynthetic pathway leading to the potent antioxidant carnosic acid and related diterpenes in *Salvia pomifera* and *Rosmarinus officinalis*. Four new cytochrome P450 enzymes are identified (CYP76AH24, CYP71BE52, CYP76AK6 and CYP76AK8), the combined activities of which account for all the oxidation events leading to the biosynthesis of the major diterpenes produced in these plants.

In addition to revealing an important plant natural product biosynthetic pathway, this work highlights the usefulness of synthetic biology approaches in the elucidation of plant natural product biosynthetic pathways and develops yeast as a tool to exploit the wealth of next-generation sequencing data that is constantly being produced through transcriptomic, genomic, or metagenomic studies. The approach described here will be useful to numerous researchers studying terpene biosynthesis in plants and microorganisms.

Towards implementation of precise genome editing in plants

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Previously, generation of desired knock out mutants has relied on random mutagenesis. With the introduction of sequence-specific nucleases, it has become possible to perform targeted engineering of the plant genome, ranging from controlled removal of undesirable traits or introduction of desirable genes. However, realization of the potential at the organismal level relies on efficient heritable transfer of the generated mutations with related challenges in delivery of the reagents to the relevant cells, low recombination rates, potential cytotoxicity of the nucleases and off-target activity.

In this project we intend to knock out certain glycogens genes for generation of the putative allergenic glycoepitope, monoarabinosylated hydroxyproline, in *Nicotiana benthaminana*. We have designed vector constructs harboring the Crispr/Cas system consisting of the bacterial *Streptococcus pyogenes* or *Staphylococcus aureus* Cas9 nuclease (*Sp*Cas9 or *Sa*Cas9) which are guided to the desired sequence in the genome by a small nuclear RNA – the Crispr. The Cas9 enzyme is fused to a GFP reporter linked by the 2A autocleavage sequence allowing for detection of cells expressing Cas9. Means of delivery are agrobacterium mediated viral replicon or protoplast PEG transformation with downstream potential use of FACS (fluorescence-activated cell sorting) to identify Cas9 expresser cells. Crispr/Cas mediated gene editing in leaf cells has been obtained with the following mutation tracking and plant regeneration being major outstanding challenges to be addressed.

Advanced glycoprofiling for probing the substrate specificity range of the cell wall extensin PTM machinery

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Extensins constitute the glycoprotein component of the plant cell wall. Extensins are typically composed of SOOOOVKHY repeats, where the clustered hydroxyprolines (O) are substituted with 1-5 arabinoses and the Serine (S) is substituted with a galactose. We (Egelund et al 2007, Velasquez et al 2011 & 2014) and others have identified most or all arabinosyltransferases involved in the arabinosylation of extensins. A number of non-extensin glycoproteins, such as small peptide hormones, wall associated kinases and therapeutic proteins expressed in plants (*e.g.* Yang et al. 2012) are also subjected to extensin type arabinosylation, however, on non-clustered hydroxyprolines with chain lengths not exceeding 3 arabinoses. It remains to be shown whether or to what extend the identified extensin specific arabinosyltransferases also arabinosylates the non-extensin glycoproteins of the secretory pathway. We are addressing this by expressing the peptide hormones, PSY1 and CLV3 and the extensin EXT3 & 6 repeats, embedded into Gfp, in mutants of the identified extensin arabinosyltransferases.

Subsequent reporter glycoprotein isolation and glycoprofiling are expected to reveal whether or to what extend the arabinosylation enzyme machinery is shared for the clustered and the non-clustered arabinosylations.

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Genotyping the Danish Apple Collection

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We are currently making a genome-wide association study (GWAS) on 400 apple cultivars. Using SNP-markers and the genotyping-by-sequencing (GBS) technique we aim at mapping sugar, acid and aroma volatiles in fruits along with morphological characters. We use phenotypic data from previous studies where a large diversity in fruit quality parameters has been found.

The fundament of this study is the Danish apple cultivar collection at the Pometum (Taastrup, Denmark) and the project addresses the genetic diversity of the Danish apple collection with the ultimate aim to study associations between phenotypic traits and genotypes.

We have studied the genetic diversity of the collection using 15 microsatellite markers which previously have been used to estimate the diversity in other European apple collections. Hereby we have identified accessions with parent-offspring relationship, identified misnamed duplicates and exposed the genetic diversity of the collection. This study includes more than 600 accessions, primarily of Danish origin and the microsatellite markers have been able to distinguish all examined genotypes except for clonal colour sports.

The work has also included genotyping the S-RNase locus responsible for self-incompatibility among the accessions. For this purpose we have developed a new PCR-based high-throughput method for genotyping the S-allele locus. The method will hopefully be useful for breeders and researchers. Information is crucial for prediction of pollination in orchards and thereby fruit set. A large diversity in S-alleles has been found examining the Danish apple cultivar collection.

Targeted genome modification of barley using CRISPR/Cas9

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CRISPR/Cas9 (clustered, regularly interspaced short palindromic repeats–CRISPR-associated proteins) is the most rapidly developing genome editing method to date. The method is derived from bacterial immune systems that adapt and cleave invading viral DNA based on sequence complementarity[1]. Scientists have adopted this system to induce double-stranded breaks (DSB) which activates natural occurring repair mechanism nonhomologous end joining (NHEJ) or homology directed repair (HDR) and this can generate mutations, insertions or deletions. The CRISPR/Cas9 system uses a customized single guide RNA (sgRNA) of only 20nt which will fuse to the complementary DNA strand and direct the Cas9 nuclease to create DSB and this can be used to create sequence-specific modification in any organism of choice[2]. For unknown reasons, some sgRNA are inactive and fails to direct the Cas9. In plant transformation, it is therefore suggested to test the activity of the sgRNA in protoplast before the actual embryo transformation takes place[3].

The aim of this project is to knock-out two different genes of interest in barley by using CRISPR/Cas9 and the activity of customized sgRNAs have successfully been identified and tested in barley protoplast.

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Development of Mlo resistance in wheat against powdery mildew

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Powdery mildew is a severe disease in wheat, usually controlled by fungicides. In barley, this problem has been solved by introducing durable Mlo resistance. In hexaploid bread wheat, a similar Mlo resistance would require that all three Mlo homoeologs are mutated. To obtain this, we have two strategies; to screen a Hereford mutant population with High Resolution Melting (HRM) and to *in silico* screen a resequencing database.

The Hereford strategy: Sejet Plant Breeding developed a mutant population in the wheat cultivar Hereford. This population has been propagated to the F_4 generation. We aim to screen 2000 plants for mutations using HRM. Twenty one homoeolog-specific primer pairs with the product size of 163 - 394 bp have been developed. As the coding sequences for Ta*MloA*, *-B* and *-D* are more than 94% identical, primers were placed in the introns. Identified mutations are confirmed by sequencing; approximately one third of 73 mutations result in an amino acid change. We found missense mutations in all three homoeologs. Lines with presumably intolerant changes were selected for crossing.

The Kronos/Cadenza strategy: TILLING populations were developed in tetraploid durum wheat cv 'Kronos' and hexaploid bread wheat cv 'Cadenza' in a joint project between the University of California Davis, Rothamsted Research, The Genome Analysis Centre, and John Innes Centre (ref). They re-sequenced the exome sequence of >1,000 Kronos and >600 Cadenza mutants. We use this resource to screen for mutations in the Mlo genes in Kronos as well as Cadenza. For Kronos we found 23 mutants for MloA and 29 mutants for MloB. We selected 7 (MloA) and 9 (MloB) for crossings and future pathogen tests.

In conclusion, we are able to obtain interesting mutants by both strategies. The final prof of functional Mlo-resistance awaits the production of homozygotic triple mutants.

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Public Private Partnership for pre-breeding in perennial ryegrass: genotype performance across multiple environments points to genotype environment interactions

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Perennial ryegrass (*Lolium perenne* L.) is the main forage grass species used in Denmark and south-wards in Europe due to its superior feed quality and productivity. However, weaknesses like susceptibility to low-temperature pathogens, and inadequate growth cessation in the autumn to allow for sufficient cold hardening and winter survival, remain to be addressed.

A Public Private Partnership for pre-breeding program has been initiated with the aim to select perennial ryegrass plant material for the development of cultivars with suitable adaptation to future climates in the Nordic countries. We are exploring genetic diversity and genetic structure using a collection of 383 perennial ryegrass accessions originating from different countries. The populations have been genotyped by sequencing and genetic variation in genic regions is being assessed. The accessions are phenotyped for important agronomical traits in Denmark, Sweden, Norway, Finland, Iceland, Estonia, and Lithuania. Analysis of 22 cold adapted varieties pointed towards genotype environment interactions, and highlighted significant polymorphisms in genes previously identified as involved in cold responses. Data being generated in this project will enable us to further explore genotype environment interactions and the use of genomic selection in perennial ryegrass populations. The ability to use genomic selection for predicting the breeding value across a range of environments would significantly benefit plant breeding programs.

Initiating Genomic Selection in Tetraploid Potato

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Breeding for more space and resource efficient crops is important to feed the world's increasing population. Potatoes and other crops with storage organs in the soil produce approximately twice the amount of calories per hectare with similar or less input of nutrients and water compared to cereals. The traditional "mate and phenotype" breeding approach is costly and time-consuming; however the completion of the genome sequence of potato has enabled the application of molecular breeding technologies. Genomic selection using genome-wide molecular markers is becoming increasingly applicable to crops as the genotyping costs continue to reduce and it is thus an attractive breeding alternative.

We have used genotyping-by-sequencing to genotype 768 individuals. The individuals were randomly selected from a population of 5,000 individuals derived from a poly-parental cross generated from 18 tetraploid cultivars and breeding clones (MASPot population). Phenotypic data have been established for six agronomical important traits for the entire population.

We have generated statistical models for genomic prediction and have obtained surprisingly high predictive power with accuracies of 115%, 67%, 64%, 45%, and for starch content, chipping quality, late blight resistance, and yield, respectively. We expect, however, that the within-population predictive power is considerable higher than out-of-population, and we are currently testing an out-of-population panel. Nonetheless, our results suggest that selection of breeding material by genomic selection can be obtained with good prediction accuracies in tetraploid potato.

The biotechnology potentials of barley HvPAPhy_a as transgenes providing high phytase activities in grains and straw of mature barley plants

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The purple acid phosphatase HvPAP_a, expressed during barley grain development, is the main contributor of the phytase activity in the mature grain (Dionisio et al., 2011). The histidine acid phosphatase type phytase (HvMINPPII_a) is also expressed during seed development, but the contribution from this to the mature grain phytase activity is very low (Dionisio et al., 2007). Here we have evaluated the potential *HvPAPhy_a* CDS overexpression in barley. The promoter was the cauliflower mosaic virus 35S-promoter and barley transformation was performed via Agrobacterium. The phytase activities were measured in mature seeds, green leaves and in the dry mature straw, rachis, chaff and awns left after harvest of the seeds. Transformed barley showed phytase activity increases from 90-fold (57000 FTU/kg) in fresh green leaves, up to 29 folds (39000 FTU/kg) in mature grains and up to 33 fold (15000 FTU/kg) in the dry material as compared to non-transformed barley. Differential quantitative proteomics of transgenic fresh leaves compared to non-transformed control showed an expression of HvPAPhy_a of about 1.22 µg/g FW in line 28.4, the highest producing line. Validation of 35S::HvPAPhy_a::NOS transgene expression level was performed by qPCR with primers spanning inside the CDS. Results confirm expression differences, in grains excised 20 days after pollination and fresh leaves, of 10 and 20 fold above control, respectively. In conclusion, overexpressing of the *HvPAPhy_a* provides strongly elevated phytase activity in mature grains, in developing vegetative plant tissue, and in dead vegetative tissue. The latter indicates proteolytic resistance of the HvPAPhy_a during programmed cell death. The biotechnological advantages of HvPAPhy_a includes i) using the grains in feed, ii) use of the powdered transgenic straw either directly or as phytase enzyme extracted hereof in feed or food, iii) and last (so far non-explored) the use of the stubbles to be embedded during plowing of the soil for mobilizing the phytate-bound phosphate for plant growth.

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Targeted mutagenesis in barley

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Sequence Specific Nucleases (SSNs) are tools which are able to bind and induce double strand breaks (DSBs) at specific sites in a genome. The DSBs are repaired by the cells own DNA repair machinery and this repair frequently leads to mutations in the form of short deletions and/or insertions at the break site. Thus, if the DSB is induced in a specific gene then the repair can result in the knockout of the function of the gene.

Currently, the most widely used SSNs in plants are TALENs and the CRISPR/Cas9-system. Molecular constructs for both techniques are easy to assemble and can be designed to target almost any location in a genome. We have previously shown that TALENs controlled by the 35S-promoter can be used to induce mutations at a specific site in the barley genome (Wendt *et al.* 2013). However, in order to get inheritance of TALEN-induced mutations to the next generation, the promoter of the TALEN construct had to be exchanged with the much stronger ubiquitin promoter. Currently we are using both a TALEN and a CRISPR/Cas9 construct designed to target the same location in the barley genome. In these constructs the TALEN and the Cas9 nuclease are controlled by an ubiquitin promoter. The observed frequencies of mutations induced by the two constructs in the first generation are very similar, with 40.4% (19 plants with mutations out of 47 transgenic plants) and 40.6% (26 plants with mutations out of 64 transgenic plants) for the TALEN and the CRISPR/Cas9 constructs, respectively. We have selected several T_0-plants with mutations induced by the TALEN or the CRISPR/Cas9 constructs for further propagation and found that the progeny shows Mendelian inheritance of the mutations. Among these progeny we have identified plants homozygous for the mutations but without the T-DNA constructs, confirming that the mutations are inherited to the next generations independently of the TALEN or the CRISPR/Cas9 constructs.

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Metabolite control of flowering time in Prunus

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Dormancy is the ability of perennial plants to suspend growth in response to environmental conditions. It is well known how flowering is regulated on the genetic level in annual plants, but the transition from dormancy to flowering in perennial plants like sweet cherry (Prunus avium) is still poorly understood. Agricultural production of more than 40 million tons of stone fruit from the Prunus genus is going to be threatened increasingly over the next years, as global warming is progressing and the necessary winter chill is decreasing, especially in warmer production areas. Hence, the ability to control dormancy release and flowering in perennial plants is extremely desirable.

We aim at elucidating the role of metabolites involved in breaking dormancy and flowering in sweet cherry.
In the past, different cyanide-based products (e.g. Dormex®, AlzChem, Germany) have been used in agriculture to compensate for missing winter chill and to advance flowering, suggesting cyanide as a key player in dormancy release. Interestingly, cyanogenic glucosides, which constitute one component in an ancient plant defense system, are also known to release cyanide. The cyanogenic glucoside prunasin is present in flower buds of Prunus plants. Cyanide-based inhibition of antioxidant enzymes like catalase leads to increasing ROS levels, which are known to be important messengers in various cellular processes. When dormant sweet cherry flower buds were treated with Dormex® in controlled conditions, dormancy was released in treated buds three days earlier compared to water-treated controls. Prunasin levels were analyzed by LC-MS in these treated and non-treated flower buds over the course of 18 days from dormancy to flowering. A differential expression analysis between treated and control samples revealed promising genes candidates for flowering time in fruit trees.

High-throughput investigation of *Ramularia collo-cygni´*s effectors role in barley

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Ramularia collo-cygni (*Rcc)* developed in the last years from a well-adapted cereal endophyte into a new pathogen of barley responsible for RLS (*Ramularia* Leaf Spot) disease (1). The genetic requirements of *Rcc* isolates for the transition from endophyte to pathogen in barley are currently unknown (2). Using the genomic information of the sequenced *Rcc* to identify the timing, and key molecular and genetic components responsible for the switch from endophytic to pathogenic life style of *Rcc*, tools for barley breeding and for efficient disease diagnosis can be developed. Our hypotheses are that in the *Ramularia*-barley relationship, effectors play a role in the pathogenicity and can these molecular cues could be used to identify genetic marker usable for resistance breeding in barley. With this in mind, a strategy for High-throughput cloning and production of predicted fungal effectors, using first sequence and ligation independent cloning (4) then the *Pichia pastoris* expression system (5), have been developed and optimized. Individual *Pichia*-produced effectors were screened for contrasted responses in one tolerant and sensitive barley cultivar using ethylene production and leaf-responses as indicators for effector's action *in planta*. Effectors inducing contrasting phenotypes in the two barley cultivars have been identified and will be further tested for the stability of the observed phenotypes across a panel of sensitive and tolerant cultivars and in mapping populations.

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Rubellin D - based diagnosis marker for Ramularia leaf spot disease assessment in barley

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Ramularia collo-cygni (*Rcc*), a hemibiotroph fungi, forms initially a symptomless association with plants termed as endophyte. However, in barley the fungus switches its life cycle becoming pathogenic[1] causing a disease called as Ramularia Leaf Spot (RLS), where the symptoms spread quickly and damage the entire plant. The disease was limited earlier to Northern Europe mainly, but during the last decade it has become prominent in East America, South America and New Zealand as well[2].The molecular mechanism underlying the change in fungal lifestyle is unknown. The potential threat of the RLS makes it economically as well as environmentally important disease to diagnose at its primary stage and prevent it from damaging the barley population across the world[3].

Rcc produces a non-host specific toxin, Rubellin D, that becomes activated by light and that induces necrotic lesions on plant leaves. However, previous studies indicated that sensitive and tolerant barley cultivars to RLS might respond differently to rubellin D application[4].My project aims to better understand the role of rubellin D in RLS disease in barley. For this I have optimised and improved the method for Rubellin D production[2] and its extraction from the liquid culture. HPLC and LC-MS analysis confirmed the isolated compound is Rubellin D of high purity. Furthermore, the extracted Rubellin was tested for biological activity on barley leaves where it induced the clear necrotic phenotype with together with the differences previously observed between the sensitive and tolerant barley cultivars. For the next step, the production of a rubellin specific antibody will be initiated using complex chemistry for tagging and antibody selection using the phage display technology. The antibody will be used for barley cultivar screening and selection, and for detailed microscopical analyses of the *Rcc* during the endophytic and pathogenic stage in order to assess its use as a possible diagnostic marker for early detection of Ramularia leaf spot disease.

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The biosynthetic pathway and ecological role of hydroxynitrile glucosides in the interaction of barley and powdery mildew

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Hydroxynitrile glucosides (HNGs) are widely distributed in the plant kingdom and have diverse plant physiological and ecological functions, e.g. as translocation and storage molecules for nitrogen or as defence compounds against herbivore or pathogen attack. Barley (*Hordeum vulgare*) contains five leucine-derived hydroxynitrile glucosides: epiheterodendrin, epidermin, sutherlandin, osmaronin and dihydroosmaronin. These compounds are restricted to the leaves, where 99% are stored in the epidermal cell layer and account for 90% of the soluble carbohydrates in this cell type. Interestingly, the obligate biotrophic barley powdery mildew fungus *Blumeria graminis* f.sp. *hordei* strictly infects these cells suggesting a crucial role of HNGs in the interaction of barley and powdery mildew. Most likely, the fungus utilizes the HNGs for host recognition and as source for glucose and nitrogen. Little is known about the plant-internal transport of HNGs and the role of HNG-transporters in the interaction. We have found that HNG-biosynthesis genes are clustered within the barley genome. This cluster also harbours genes for a putative transporter of the ABC and MATE family, respectively. Comparing transcript levels in uninfected and infected leaves will provide information about their regulation and role in the interaction of barley and powdery mildew. In addition, immunolocalization of the ABC and MATE transporter will give further information about their putative role in intracellular and intercellular HNG transport, e.g. through the cytoplasm, tonoplast or to the wax layer.

Regulation of the hemoglobin/NO cycle in barley infected with powdery mildew or yellow (stripe) rust

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Nitric oxide (NO) is an important cellular signaling molecule in plants. It is involved in a range of physiological functions in plants. NO plays a particular central role in plant responses to biotic stresses, where it seems to function at different molecular levels including formation of nitrosylated proteins and cross-interference with various reactive oxygen species. Plant hemoglobins are important modulators of the NO signal, presumably by an oxidative mechanism including the direct reaction with O2. When biotrophic pathogens infect plants, the pathogen and the plant are struggling to take control over gene expression towards either compatibility or incompatibility. Our previous studies have shown that artificial up-regulation or silencing of endogenous hemoglobins in plants can modulate NO levels during pathogen infection to an extent where susceptibility levels are severely changed. We here show how barley plants infected with either powdery mildew (*Blumeria graminis f. sp hordei*) or yellow (stripe) rust (*Puccinia striiformis f. sp. hordei*) are struggling with the respective pathogen to control hemoglobin gene expression

Modification of ethylene sensitivity in ornamental plants using precise genome editing, CRISPR/Cas9

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Ethylene sensitivity has for long been of interest in improving ornamental plants e.g. Kalanchöe and Campanula. We aim to investigate changes in ethylene sensitivity in economically important ornamental plants by targeting genes in the ethylene pathway using the novel precise genome editing tool CRISPR/Cas9. The sequence specific nuclease Clustered Regularly Interspaced Palindromic Repeats / CRISPR associated protein 9 (CRISPR/Cas9) is a sequence specific DNA binding and cleaving complex which can be employed to introduce targeted double-stranded breaks (DSBs) in the host genome. The DSBs will be repaired by the non-homologous end-joining (NHEJ) repair mechanism which often results in small indels and consequently a gene knockout. The CRISPR/Cas9 system consists of a protein nuclease (Cas9) which is guided to the target sequence by a small RNA molecule (sgRNA) that recognizes a 20 bp target sequence in the genome, downstream of a protospacer adjacent motif. The sgRNA confers the sequence specificity of the CRISPR/Cas9 complex and may thus be designed to target virtually any sequence, a feature that has made it the method of choice within precise genetic engineering. Although most research with CRISPR/Cas9 has been conducted in prokaryote and mammalian cells, steps have been taken to implement the system in plants. The method has proven to function in various plant species e.g. Arabidopsis, wheat, soybean and orange which makes it plausible that this technique could be applied to ornamental plants as well. The system will be introduced using Agrobacterium tumefaciens and explant regeneration in tissue culture to create stable transformation events.

Towards identification of biomarkers that select for high biomass and grain quality

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Wheat is the third most grown crop worldwide and is used both for food and feed purposes. The use of genetic biomarkers in wheat breeding programs for selection of new lines with improved qualities has increased over the last decade. For now marker selection is mostly directed against grain quality, dwarf genes, straw height and disease resistance. However depletion of conventional fossil fuels (oil, coal and natural gas) and the use of biomass material in the chemical industry increase the demands for breeding towards crops that are more suited for biorefinery processes and production of value added products. Wheat straw has great potential for this, because mature straw yield and quality (monosugar release, cellulose and hemicellulose content) vary considerably among winter wheat genotypes, which affect the sugar accessibility of the biomass. This variation is partly genetically determined and therefor biomarkers specific for straw quality and yield can be identified. Straw yield and quality traits are not found to be linked with grain yield indicating that breeding for mature biomass traits would be feasible without compromising yield.

We aim at improving wheat breeding programs by identifying biomarkers that can select for traits important for increasing the mature biomass yield and quality (*e.g.* straw height, lodging, cell wall components, monosugars) by using a genome wide association study (GWAS).

93 winter wheat lines are grown at Sejet Plant Breeding for a 3 year period (around 50% of the lines will be grown repeatedly during the 3 year period). Cultivars are genotyped with single nucleotide polymorphism (SNP) markers (15K Illumina's Golden Gate multiplex array, Traits Genetics). Additionally they are phenotyped for yield and quality parameters of straw and grain, including plant height, lodging degree, sugar release ability (pretreatment, enzymatic hydrolysis and HPLC), and total cellulose and hemicellulose content. Significant associations between traits and SNPs are established by GWAS.

The GWAS results show significant associations for all traits analyzed from first year's data (with exception of glucose release). Markers associated with lodging and xylose release during enzymatic saccharification are especially promising, where high numbers of significant markers were found. Data show little population structure and seem diverse enough for detection of true markers.

Genetic analysis of two lodging resistance barley mutant lines *ert-c.1* and *ert-d.7*

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The *ert-c* and *ert-d* mutants are valuable lodging resistance mutants induced in barley during mid 20th century, which can be of great importance in lodging resistance breeding in barley. Recently, Bowman near iso-genic lines (NILs) carrying these *erectoides* loci have been developed by backcrossing, and the introgression regions have been defined using a SNP array. In the current study, allelic tests were conducted to substantiate the identities of *ert-c.1*, *ert-d.7* and their corresponding Bowman NILs BW305(*ert-c.1*) and BW306(*ert-d.7*). Genetic mapping was also carried out in five F_2 populations of BW305 x Bowman, BW305 x Barke, BW306 x Bowman, BW306 x Barke and Bowman x *ert-d.7*. The results showed that BW305 and BW306 are allelic and carry the same *ert-c* locus. The *ert-d.7* mutant was determined as a double mutant with both *ert-c* and *ert-d* loci. BW306 might have lost the *ert-d* locus during generations of backcrossing. All of BW305, BW306 and the *ert-d.7* line have 2H/3H translocations. The *ert-c* locus was mapped on 3HL near the centromere and it is associated with the translocation point, but most likely outside the translocation region. The *ert-c* locus was further fine-mapped in the marker interval 2_0801 to 1_0224, and ten markers co-segregate with this locus. The *ert-d* was mapped on 7H centromere region and is associated with 2_1302. The markers closely linked or co-segregating with *ert-c* and *ert-d* could be useful in marker-assisted selection.

Physiological phenotyping by determination of phytohormone and enzyme activity signatures

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The availability of novel germplasm and cost efficient molecular techniques for whole genome sequencing, mapping and genomic selection as well as complementary techniques to image the whole plant phenome non-destructively, give access to information unimaginable just a few decades ago. However, to be able to generate cost efficient, reliable and robust non-invasive predictors for yield and quality it will be essential to link the current high-throughput genotyping and phenotyping of whole plants to the underlying physiological processes. Only the physiology of a plant integrates various levels of regulation of the expression of the genetic information as well as the impact of the environment and agricultural management.

The activities for enzymes involved in primary carbohydrate metabolism have been shown to be strongly associated with growth performance and crop yield, as well as abiotic and biotic stress responses. Since enzyme activities integrate several modes of regulation at the mRNA and protein level, the analysis of enzyme activities is very suited to analyse the physiological state of plants. This work assessed the establishment of a simple, fast and cost-effective method to determine activities for 13 key enzymes involved in carbohydrate metabolism, mainly using coupled spectrophotometric kinetic assays. These assays proved to be robust and highly suitable for the characterisation and the diagnosis of the physiological state for various plant species, including several crop species. Plant growth and development depend on a strong regulatory network of phytohormones. Only in the recent years it became evident that the central stress signalling backbone in response to pathogen infection, comprising ethylene, jasmonic and salicylic acid, is also modulated by other phytohormones. Due to extensive interactions of abscisic acid, auxin and newly also cytokinins with the stress signalling compounds, it is required to quantify spatial and temporal dynamics of the whole phytohormone spectrum, but typically established method only determine individual, single phytohormones. Based on published procedures, we developed an easy, rapid extraction and analysis method enabling determination of abscisic, indole-3-acetic, jasmonic and salicylic acid as well as eight cytokinin derivatives within the same extract and analytical run on a UHPLC-MS/MS device. Furthermore, certain phytoalexins, especially important as additional defence compounds within generalized pathogen responses and also cytokinin induced resistance, can be determined in the very same extract. Parallel determination of these phytohormones is also essential for physiological phenotyping to assess the function of various regulatory processes in plants. We successfully applied the established method to determine complex phytohormone profile in five model and crop plant species and also different cell suspension cultures.

Salt tolerance in *Nicotiana benthamiana* by overexpressing Proteinase inhibitor-II gene

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Salinity is one of the most severe environmental stresses, which impairs the crop production worldwide. Proteinase inhibitors are mainly involved in conferring the resistance against herbivores and pathogens. However, their role in abiotic stress resistance has also been reported. In order to analyze the contribution of tomato proteinase inhibitor-II gene for salt stress, *Nicotiana benthamiana* plants were transformed with tomato proteinase inhibitor-II (PI-II) under control of the rice root germin like protein 2 (OsRGLP2) promoter using *Agrobacterium tumefaciens*. T1 transgenic seedlings were subjected to stress treatments with different concentrations of sodium chloride (NaCl) (100mM, 200mM, 300mM). When analyzed, transgenic seedlings showed better growth at 100mM, 200mM and 300mM NaCl concentration as compared to wild type. Wild type seedlings exposed to 200mM and 300mM NaCl were severely affected and showed high degree of chlorosis. Transgenic seedlings growing at 200mM and 300mM NaCl showed more chlorosis than the seedlings at 100mM NaCl. Moreover, the total chlorophyll content was significantly higher in transgenic lines than the wild type. This comparative analysis of transgenic with wild type seedlings let us to suggest that proteinase inhibitors can regulate the osmotic stress.

New plant breeding techniques for bio-sustainable production of natural colors in black carrot

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Due to reports on adverse effects of synthetic colors in children (A rainbow of risks[1]), the colorant market is turning from synthetic to natural colors. Unfortunately the content of the natural color anthocyanin in *Daucus carota sativus* var. *atrorubens* (Black carrots) at current level cannot keep up with the economics of food color production. Traditional breeding approaches to increase anthocyanin content in black carrots are slow and results in limited improvements. The anthocyanin biosynthesis and regulation in black carrots is briefly described, but information from mainly fruits indicates it to be regulated by transcription factors, directly regulating the expression of the structural genes of phenylpropanoid pathway. New Plant Breeding Technologies (NPBTs) such as Zinc Finger Nucleases, Transcription Activator-Like Effector Nucleases (TALENs), Clustered Regulatory Interspaced Short Palindromic repeats (CRISPR)/Cas (CRISPR associated) and cis-/intragenesis are new alternatives to traditional breeding and conventional transgenesis. NPBTs can introduce highly specific plant genome modifications with pinpoint accuracy[2], indistinguishable from those introduced by conventional breeding and chemical/physical mutagenesis (and without unwanted side mutations or linkage drag). This has significant implications for the approval of NPBT plants by consumers and regulatory authorities, which are predicted to be leniently regulated or deregulated. In the current project, we'll use complementary DNA and RNA-based comparative genomic analysis and genetics to identify key players (genes/transcription factors) for anthocyanin synthesis in black carrots and use TALENs, CRISPR/Cas and cisgenesis to accelerate the production of anthocyanins.

1 CSPI, Food Dyes: A Rainbow of risks (https://cspinet.org/new/pdf/food-dyes-rainbow-of-risks.pdf)
2 Rationally engineered Cas9 nucleases with improved specificity, Science DOI: 10.1126/science.aad5227

Infection Biology of *Ramularia collo-cygni* in Barley

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Ramularia collo-cygni (*Rcc*) is an emerging fungal pathogen that causes the Ramularia Leaf Spot disease (RLS) in barley. It has received increasing attention during the last 20 years due to its increasing severity, infact, yield losses in barley due to *Rcc* can reach up to 35% (Walters et al., 2008). During the early life stages in barley, *Rcc* establishes an endophytic interaction becoming pathogenic only after flowering. The ability of *Rcc* to associate with a large number of wild grasses and graminaceous crop species such as wheat, oat and maize, and its capacity to adapt to different climates combined with the global change represent a big danger in barley production.

The aim of my project is to elucidate the infection biology of *Rcc* in barley monitoring the evolution of *Rcc* and its interaction with barley throughout the plant development. Different isolates of *Rcc* will be labelled with GFP (GFP-*Rcc*) to monitor the infection in real time with confocal microscopy. The infection process will be observed from seedlings to flowering (namely from endophytic to pathogenic stage) in both *Rcc* and barley wild type and in different *Rcc* isolates (mild versus aggressive) and barley cultivars (sensitive versus tolerant). A the molecular level, Identified *Rcc* effectors from transcriptome and proteome analyses performed at BRCC will be tested using *Rcc* knockout mutants.

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Proteomic analyses of the barley/endophytic/pathogenic *Ramularia collo-cygni* fungus interaction

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In tandem with the growing world population, crop production has increased during the last century as the plant breeders have developed high yielding crop cultivars. The current crop production system, with continuous growing of the same crop on large fields, tends to increase disease pressure challenging plant breeders to produce new crop cultivars that are more resistant to pathogen attack.

The fungus, *Ramularia collo-cygni* (*Rcc*) can infect cereals and grasses as an endophyte. In barley, after the flowering stage, *Rcc* can shift from being an endophyte to become a pathogen that produces the disease *Ramularia* leaf spot (RLS) with yield losses up to 35% [1]. Currently, no barley cultivar displaying total resistance against RLS, however, some cultivars are more tolerant than others. To better understand the unknown mechanism behind the shift from the endophytic to pathogenic phase of *Rcc*, different proteomic approaches have been initiated to identify plant and fungal proteins crucial for this shift. In progress we have a total proteomic analysis of *Rcc* grown in culture together with total proteomics of leaves from tolerant and sensitive barley cultivars at different time points after infection. Furthermore, a more detailed barley leaf apoplastic proteomic analysis from tolerant and sensitive cultivars at different time points after infection is on going. All the proteomics data will be combined with transcriptomic and metabolomic data to identify the molecular mechanism behind the shift from an endophyte to a pathogen of *Rcc*. Here we will present the current status of these analyses.

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Identification of genes controlling the variation in specific seed weight and grain protein content in a collection of winter barley lines

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Specific seed weight (SSW) and grain protein content (GPC) are two of the principal determinants in selection of grains. In barley, GPC is highly related to feed and malt quality in that, while higher amount is desired for feed quality, low to moderate amounts are required in malting. SSW also referred to as the bulk density of seeds (the weight of a unit volume of grain) has been found to be weakly related to overall malting quality nevertheless some maltsters believe barley with high bulk densities make good malts. Though this selection criterion may be considered a less important agronomic trait by most maltsters, to breeders it is one of the most essential in relation to seed packaging and storage. An overall increase in SSW is a requisite; this is because it offers greater packaging advantage as greater quantity may be packed within constant volume.

In this study, 101 winter barley lines grown at three trials across three different locations in Denmark for 2013 and 2014 were analyzed for GPC and SSW. Correlation between GPC (measured in %weight of seed) and SSW (measured in kg/100L) were estimated. The H^2 estimates indicated that 42% of the variation in the protein content was attributable to the genetic variation (i.e. the variation in the lines) while that of SSW was 46%. To identify the genomic regions associated with these two polygenically controlled traits, a genome-wide association study (GWAS) was performed. Best linear unbiased predictors (BLUPs) for the 101 barley lines (across all the locations, trials and years) were extracted from a mixed linear model developed for each trait taking into consideration all possible interactions between the covariates. A positive weak correlation (R=0.32, P-value = 0.001) was found in the BLUPs for GPC and SSW as against a much higher correlation (R = 0.80, p-value < 2.2e-16) in the original raw data.

Together with the BLUPs, a set of 3838 polymorphic iSelect SNP markers were used in the GWAS. Eight SNP markers were found to be significantly associated with SSW when a threshold of the $-\log_{10}$ of the P-value was set at 3. There was however no association for GPC since the genomic variance in the GPC had disappeared in the BLUPs. Five out of the 8 associated SNPs for SSW were located at the same position (22.2cM) on chromosome 2H, a region containing the photoperiod response and flowering time gene *PpdH1*. These SNPs accounted for 29-30% of the variation in SSW. Understanding the role of this gene and any others that would be found in this study to be involved in the control of SSW in barley is worth a shot.

Identifying transcripts associated with aggressiveness in wheat yellow rust by transcriptome sequencing

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Yellow rust (*Puccinia striiformis*) is currently one of the most prevalent and damaging disease on wheat, which may threaten global food security. This is emphasized by new strains adapted to warmer temperatures, and being more aggressive in general, which have spread rapidly in many wheat growing areas in recent years. More detailed knowledge is needed for understanding rust biology and epidemiology, e.g., the characteristics of aggressive isolates. Since 2011, the isolate DK09/11 of the "Warrior" race is considered 'aggressive' and spreading rapidly in Europe. In this study, progeny isolates arising from a selfing of the isolate DK09/11 on Berberis vulgaris (Rodriguez-Algaba *et al.* 2014) were selected for transcriptomic analysis. Four progeny isolates and the parent isolate DK09/11 showing different levels of aggressiveness were point inoculated on wheat leaves and harvested at three different time points (5, 7 and 9 dai) for RNA-sequencing. By using next-generation sequencing technologies, transcript expression profiles under different growth stages will be analyzed to reveal molecular mechanisms underlying aggressiveness.

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Biomaterials production utilizing plant-crafted starches

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Biopolymers, like starch, form the basis for coming generations of advanced and environmental friendly materials and prebiotic ingredients. Thermoplastic starch (TPS) is processable using conventional plastic processing techniques, such as extrusion and molding. Mechanical performance and water resistance are improved, typically by blending with synthetic polymers like polycaprolactone in Mater-Bi. Alternatively, starch designed directly in the grain could be sufficiently functionalized to provide improved plasticity, stability and water resistance. We generated over-expressor and suppressor barley lines with radically improved starch, including a water resistant amylose-only (AO, Carciofi et al 2012).

TPS prototypes were produced using conventional thermo-molding and extrusion processing using glycerol as reference plasticizer. The prototypes were tested for crystallinity, dynamic mechanical thermal analysis (DMTA), stress and strain at break tests. Control barley starch had mostly the A-type crystalline polymorph while the AO starch was both of B-type and of Vh-type. Following extrusion and equilibration at 57% R.H. both starch prototypes had mainly Vh type crystalline polymorph suggesting that all B- and A-type crystals were melted and transformed. Extruded prototypes made from the AO starch showed a 6-fold higher stress at break and 2.5-fold higher strain at break as compared to control barley starch. Our data demonstrate that AO has capability to perform similarly to conventional bioplastic blends with non-biopolymers (Malinconico et al., 2008).

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Sensing zinc deficiency: unravelling the regulation of AtbZIP19/bZIP23 transcription factors

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Deficiency of the essential micronutrient zinc (Zn) is widespread in agricultural soils, with a negative impact on crop yield and quality. Nevertheless, there is insufficient knowledge on the regulatory and molecular mechanisms underlying the plant response to inadequate zinc nutrition. This information should contribute to the development of plant-based strategies for improved nutrient-use-efficiency traits in crops. Previously, the transcription factors (TFs) ZIP19 and bZIP23, part of the Arabidopsis bZIP small sub-group F[1], were identified as essential regulators of the response to zinc deficiency[2]. The defining characteristic of the F group is the presence of a conserved Cys- and His-rich motif at the N-terminal of the basic region[1,3]. His/Cys-rich regions are known to be able to bind divalent transition metals and being involved in regulatory functions[4]. Therefore we propose that cytosolic free Zn^{2+}, via a direct and reversible binding to the His/Cys-rich motif, may act as a signal of the cell zinc status modulating bZIP19/23 activity[5]. We present our recent results within this project, aiming at determining the plant zinc deficiency signalling mechanism anchored in the bZIP19/23 TFs.

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Characterisation of key elements in hexose-phosphate metabolism

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Central metabolism of carbohydrates comprises the core reactions that connect all other parts of plant metabolism, such as photosynthesis, respiration, nitrogen-assimilation and formation of structural cell components and secondary metabolites. Consequently, carbohydrate metabolism is essential for plant development and stress tolerance, and it defines the major components of many harvested plant products. Our aim is to characterise key regulatory enzymes of hexose-phosphate metabolism. We will focus on F2KP (Fru-6-P, 2-kinase/Fru-2,6-bisPase) and PFKs (phosphofructokinases) from *A. thaliana*. F2KP is a bifunctional enzyme, which phosphorylates and dephosphorylates the C-2 position of Fru-6-P. The catalytic regions of F2KP reside at the C-terminal half of the amino acid sequence and is homologous to mammalian enzymes (Nielsen *et al*., 2004). In addition, F2KP in higher plants comprises a long N-terminus, containing several predicted functional domains. We aim to characterise the molecular function of these motifs. PFKs catalyse the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate. In *A*. *thaliana*, the PFKs comprise 5 cytosolic and 2 plastidic isoforms (Mustroph *et al*., 2007, Nielsen et al 2004). Our studies will aim to characterize the kinetics of selected *A*. *thaliana* PFKs and their impact on metabolism as revealed by KO-mutants.

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Low silicon *Brachypodium* mutant as a tool for investigation of silicon effects on cell wall composition

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Silicon functions as a beneficial element in plants by improving their resistance towards biotic and abiotic stresses. However, silicon may have a negative impact on the biocatalytic and thermal degradation of lignocellulosic biomass to be used for bioenergy purposes. Solutions to reduce silicon either by engineering of biomass or by development of downstream separation methods are therefore targeted. On this background, the objective of our research is to understand silicon deposition mechanisms and interactions with cell wall components in grass species in order to improve the quality of lignocellulose biomass.

We have isolated and characterized *Brachypodium* mutants defective in the silicon transport protein *bdLsi1-1*. The silicon concentration in *bdLsi1-1* mutants was on average 0.15% of the dry matter, showing over 80% reduction compared to wild type plants. Comprehensive microarray polymer profiling (CoMPP) revealed several alterations in both pectin and hemicellulose fractions. The most pronounced modifications were observed in leaves and leaf sheaths relative to stem and seeds. Also the composition of the lignin fraction appeared to be affected. Using the mutant lines as a valuable tool, further studies of silicon deposition and interactions with cell wall components in the mutant lines, as well as the consequences for enzymatic degradability of cell walls are ongoing.

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