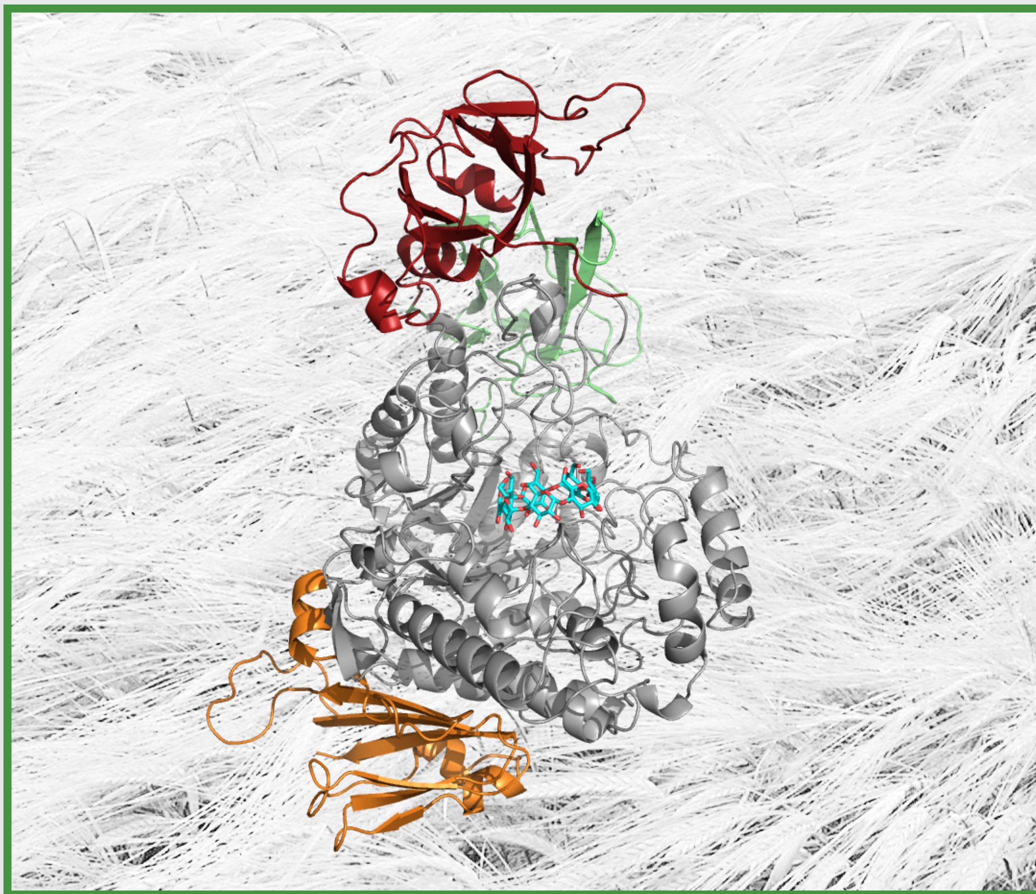
Plant Biotech Denmark Annual meeting 2012 February 2-3

Faculty of Science
University of Copenhagen



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Cover photo: Overall structure of the starch debranching enzyme Barley Limit Dextrinase with the competitive inhibitor β-cyclo dextrin superimposed in the active site.
N-domain, red; CBM48, green; Catalytic domain, gray; C-domain, orange; β-cyclodextrin, shown as sticks.
Work by: Malene Bech Vester-Christensen, Marie S. Møller, Maher Abou Hachem, Birte Svensson (Department of Systems Biology, Technical University of Denmark), Anette Henriksen (Carlsberg Laboratory)

Programme

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9.00 - 9.30	*Registration, coffee/tea and croissant*	
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Programme

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FRIDAY- February 3, 2012

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Key Note Talk

The Arabidopsis Somatic Embryogenesis Receptor-like Kinases; why do we need them in signaling?

Catherine Albrecht, Christoph Bücherl, Walter van Dongen, Wilma van Esse, Colette ten Hove, Marije aan den Toorn, Sacco de Vries

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The Arabidopsis SERK family of receptor-like kinases consists of 5 members. Although all exhibit kinase activity, no ligands are known to bind to the extracellular domains. Instead, the mode of action appears to be an association with others RLKs such as BRI1 or FLS2, both being receptors that do bind ligands. Other then SERK3, also known as BAK1, null mutants do not show a phenotype. When combining mutations of different members, a variety of phenotypes are seen that range from defective tapetum formation, impaired brassinolide signaling, accelerated cell death, impaired PAMP signaling and impaired abscission. There appears to be marked specificity residing in the protein structure of the individual members. We are interested in the question why diverse signaling pathways require the same SERK combination, often operative in the same cells. We use a combination of molecular-genetic and biochemical tools to eventually provide input for a more quantitative modeling approach to help answer this question. The results suggest that higher-order oligomeric complexes of main receptors and SERKs are pre-assembled in different cells.

1. Wilma van Esse, Prety Surendran, Adri Westphal, Boudewijn van Veen, Cathy Albrecht, Jan-Willem Borst, Sacco de Vries. Quantification of the BRI1 receptor in planta. *Plant Physiol* **2011** 156: 1691-1700
2. Catherine Albrecht, Freddy Boutrot, Cécile Segonzac, Benjamin Schwessinger, Selena Gimenez-Ibanez, John P. Rathjen, Delphine Chinchilla Sacco de Vries and Cyril Zipfel. Brassinosteroids inhibit pathogen-associated molecular pattern–triggered immune signaling independent of the receptor kinase BAK1. *Proc Natl Acad Sci USA* **2011** (in press) www.pnas.org/cgi/doi/10.1073/pnas.1109921108

Elucidation of a Secondary Metabolite Transport Pathway

Andersen T G[1], Nour-Eldin H H[1], Burow M[1], Jørgensen M E[1] , Madsen S R[1], Olsen C CE[2], Hedrich R[3] ,Geiger D[3] and Halkier B A[1]

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[3] University of Würzburg, Institute for Molecular Plant Physiology and Biophysics, Julius-von-Sachs-Platz 2, D-97082 Würzburg, Germany

Remodeling membranes and regulating lipid pumps – understanding the molecular mechanisms of vesicle biogenesis.

Merethe Mørch Frøsig, Thomas Günther-Pomorski and Rosa L. López Marqués

Faculty of LIFE Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark.

P4-ATPases play a critical role in the biogenesis of transport vesicles in the secretory and endocytic pathways and P4-ATPase activity is held responsible for the creation and maintenance of membrane phospholipid asymmetry. P4-ATPases form a stable complex with beta-subunits and current evidence strongly suggests that this complex is able to transport phospholipids from the outer to the inner leaflet of biological membranes. In *S. cerevisiae* two kinases named Fpk1p and Fpk2p have been found to regulate and activate two plasma membrane P4-ATPases, Dnf1p and Dnf2p.

The aim of this project is to understand the regulation by phosphorylation of P4-ATPases from higher eukaryotes using yeast as a host for heterologous expression of plant kinases and P4-ATPases. A yeast strain without kinase-induced phospholipid transport at the plasma membrane has been created, and several Arabidopsis kinases have been cloned. Recent results strongly suggest that plant P4-ATPases are regulated in a similar way as yeast P4-ATPases. We are also currently looking into novel phenotypes of knock-out yeast strains related to the kinases and P4-ATPases.

These results will be further analyzed in order to understand the similarities and differences between P4-ATPase regulation in unicellular and multicellular organisms. It is known that P4-ATPases and their specific kinases are linked to different aspects of lipid metabolism via protein networks. Thus, the project will also provide a better insight on how the biophysical and biochemical properties of membranes are controlled, both in yeast and plants.

Key Note Talk

Plant Ionomics

David E. Salt, Professor and 6th Century Chair

School of Biological Sciences, University of Aberdeen, Aberdeen, UK.

Understanding how organisms control their ionome or mineral nutrient and trace element composition, could have a significant impact on both plant and human health. Furthermore, associating the genetic determinants that underlie natural ionomics variation with the landscape of the individuals that carry these genotypes will provide insight into the genetic basis of adaptation and speciation. We have coupled the natural variation present in *Arabidopsis thaliana* and rice with high-throughput mineral nutrient and trace element profiling to determine the biological significance of connections between an organisms genome and its ionome. We have used PCR-based positional cloning, DNA microarray based approaches, QTL and genome-wide association mapping, and whole genome re-sequencing to identify polymorphic loci defining genes that control the ionome. Association of naturally polymorphic loci with the landscape is also starting to reveal the genetic architecture underlying potential adaptation to the environment. We have developed a publicly searchable online database containing ionomic data on *A. thaliana* leaf tissue and seed, and rice grain. In *A. thaliana* this includes over 1,000 accessions and mapping lines, and T-DNA insertion lines defining knockouts in 2,429 genes. In rice this includes over 2,000 rice varieties and mapping lines (www.ionomicshub.org). The database is being updated regularly.

Understanding manganese deficiency in barley

Pai Pedas[1], Bianca Naumann-Busch[2], Søren Husted[1], Poul Erik Jensen[2], Jan K. Schjørring[1]

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Future sustainable improvement in the productivity of cropping systems depends on novel resource-efficient plant genotypes designed to match site-specific soil and climatic conditions. Deficiency in essential mineral micronutrients such as manganese, iron, zinc, copper and boron is a significant problem for crop productivity in major parts of the world. Manganese deficiency is a serious problem in Northern Europe, especially on alkaline and porous soils as these oxidative conditions are reducing the bio-availability of Mn. Interestingly, barley (*Hordeum vulgare*) genotypes display a marked difference in their ability to tolerate growth at low manganese concentrations, a phenomenon designated as differential manganese use-efficiency.

More than 30 enzymes are activated by manganese and a major role is found for the water splitting complex in photosystem II. We have previously found differences in how barley genotypes differing in manganese-efficiency are controlling and optimizing their photosynthetic performance when exposed to manganese deficiency (Husted et al 2009). Induction of manganese deficiency led to a decline in the photosynthetic quantum yield efficiency for both genotypes, although faster in the manganese-inefficient genotype. Leaf tissue and thylakoid membrane manganese concentrations were reduced under manganese deficiency, but no difference between the two genotypes was observed and no visual deficiency symptoms were developed, suggesting that the total manganese foliar content is insufficient to describe the functional active amount of manganese.

The aim of the study is to analyze the general effects on photosynthetic performance of manganese deficiency in barley and to decipher the genotypic differences between two contrasting barley genotypes. To achieve this we employed chlorophyll fluorescence measurements in combination with an array of biochemical analyses as well as mass spectrometric proteome analysis. The obtained results will be presented at the conference.

References:

Husted S., Laursen K.H., Hebbern C.A., Schmidt S.B., Pedas P., Haldrup A., Jensen P.E. (2009). Manganese deficiency leads to genotype-specific changes in fluorescence induction kinetics and state transitions. *Plant Physiology* 150: 825-833

mRNA decay in kinase-mediated responses to pathogens

Milena Roux[1], Kristoffer Palma[2], Magnus Rasmussen[1], Laura Arribas[1], John Mundy[1] & Morten Petersen[1]

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Plants have evolved multi-layered defence responses, activated upon recognition of invading pathogens. One layer includes trans-membrane receptors that recognize evolutionarily conserved microbe-associated molecular patterns (MAMPs). Signalling via MAP kinases from these receptors leads to reprogramming of gene expression and production of host proteins for thwarting pathogenic intruders. MAMP-activated MAP kinase 4 (MPK4) regulates the expression of a subset of defence genes via a WRKY transcription factor. However, how plant MAP kinases regulate defence genes is still poorly understood. Recently we found an *in vivo* association in *Arabidopsis* between MPK4 and PAT1, a component of the mRNA decapping machinery. Interestingly, *pat1* mutants exhibit the same phenotypic characteristics found in *mpk4* mutants, namely dwarfism and increased resistance toward bacterial pathogens. These data strongly suggest that MPK4 and PAT1 function together to regulate defense responses. mRNA decapping represents a critical step in eukaryotic mRNA turnover, and MPK4 is a regulatory node controlling transcriptional reprogramming via transcription factors. Thus, linking MPK4 to mRNA decay offers another efficient mechanism for this MAP kinase to regulate the rapid changes required to instigate defense responses. We are using genetics and biochemistry to probe the function of PAT1 and mRNA decapping in plant innate immunity, research that remains largely unexplored. The mRNA regulatory machinery offers an attractive target for viral attack, and is abused for the proliferation of diverse viruses including HIV. It is possible that plant viruses employ similar tactics to subvert defenses, and we are studying the role of mRNA decay in viral defense. Genetic approaches in *Arabidopsis* should improve our understanding of processes that cause pathogenesis in both animals and plants, providing novel strategies and selective targets for therapeutic and agronomic intervention.

Key Note Talk

Towards a Reference Sequence of the Bread Wheat Genome

Mario Caccamo, Head of Department

The Genome Analysis Centre, Norwich Research Park, Norwich, UK

The application of advanced genomics to improve breeding techniques in grass crops will play a key role in securing affordable and nutritious food for an increasing human population. In particular bread wheat (*Triticum aestivum)* is the most widely traded agricultural commodity. Bread wheat, however, has one of the largest and most complex genomes yet to be sequenced. As a result of several recent hybridization events the bread wheat genome is allohexaploid (6n=42, AA,BB,DD) where each subgenome accounts for ~5-6 Gb of a total size estimated at ~15-16 Gb. Although genome size varies in grasses due to the expansion of retroelements, gene order is conserved along large chromosomal segments enabling comparative methods between related species. Several research groups across the work working under the coordination of the International Wheat Genome Sequencing Consortium (IWGSC, www.wheatgenome.org) are committed to establish a high quality, gold-standard reference sequence of the wheat genome, anchored to the genetic maps that will provide high resolution links between the traits and variations with the sequence features and polymorphisms underlying them. In this presentation I will report on progress and achievements in this project and focus on a recent initiative to obtain high-quality sequences on the individual chromosome arms for the reference variety Chinese Spring. These sequences facilitate the anchoring of the physical contigs and provide useful sequencing data to the breeding community. These sequences have been generated using next-generation sequencing technologies and will allow the placement of most of the wheat genes to chromosomes.

The sequences generated for each chromosome arm are assembled using the latest software tools. One of the main challenges when working with the bread wheat genome is the repeat content and the size of the target (~500Mb for the largest chromosome arms). Recent advances in sequence assembly algorithms opened up new opportunities but the ability to generate de novo assemblies from short reads for large eukaryote genomes remains a challenge. Most of the current assembly tools struggle to deal with the massive datasets generated by the next-generation sequencing technologies. Some of the recent assembly algorithms have been designed to offer efficient alternatives to represent these datasets in main memory, but in general the results are assemblies with large numbers of contigs. In the second part of the talk I will review the state-of-the-art of the assembly algorithms.

Implications of high-temperature events and water deficits on protein profiles in wheat (*Triticum aestivum* L. cv. *Vinjett*) grain

Susanne Jacobsen[1], Fen Yang[1], Huawei Li[1,2,3], Anders D. Jørgensen[1], Ib Søndergaard[4], Christine Finnie[1], Birte Svensson[1], Dong Jiang[3], Xiao Wang[1,2,3] and Bernd Wollenweber[1]

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Increased climatic variability is resulting in an increase of both the frequency and the magnitude of extreme climate events. Therefore, cereals may be exposed to more than one stress event in the growing season, which may ultimately affect crop yield and quality. Effects are reported of interaction of water deficits and/or a high-temperature event (32°C) during vegetative growth (terminal spikelet) with either of these stress events applied during generative growth (anthesis) in wheat. Influence of combinations of stress on protein fractions (albumins, globulins, gliadins and glutenins) in grains and stress-induced changes on the albumin and gliadin proteomes were investigated by 2-DE and MS.

The synthesis of individual protein fractions was shown to be affected by both the type and time of the applied stresses. Identified drought or high-temperature-responsive proteins included proteins involved in primary metabolism, storage and stress response such as late embryogenesis abundant proteins, peroxiredoxins and a-amylase/trypsin inhibitors. Several proteins, e.g. heat shock protein and 14-3-3 protein changed in abundance only under multiple high temperatures.

Reference:

Yang, F., Jørgensen, A.D., Huawei, L., Søndergaard, I., Finnie, C., Svensson, B., Jiang, D., Wollenweber, B., Jacobsen, S. (2011). Implications of high-temperature events and water deficits on protein profiles in wheat (*Triticum aestivum* L. cv. *Vinjett*) grain. *Proteomics* 11: 1684-1695

Establishment of retrotransposon-mutagenized population of model legume *Lotus japonicus* and high throughput, deep sequencing-based insertion site identification.

Dorian Urbański, Anna Małolepszy, Stig Uggerhøj Andersen, Jens Stougaard

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Targeted gene knockout by homologues recombination is an unavailable technology in plant genetic studies. Lack of such powerful tool implicates usage of mediate techniques like random mutagenesis or indirect methods like gene silencing. While chemical or physical mutagens, causing single base changes, are simple to apply but tedious to identify, easily traceable insertional mutagenesis becomes an attractive counterpart. Retrotransposons are ubiquitous genetic elements that are able to replicate via an RNA intermediate and insert a new copy into another genomic locus, potentially resulting in a random gene knock-out. Retrotransposons were used as natural mutagens in previous studies in rice (*Tos17*)(Miyao *et al*. 2003) or *Medicago tuncatula* (*Tnt1*)(Tagede *et al*. 2008). In our study on model legume plant *Lotus japonicus* (Lotus), we have taken advantage of inducible de-repression of Lotus retrotransposon 1 (LORE1), a member of small family belonging to Ty3-*Gypsy* class of LTR retro-elements. LORE1 has a unique feature of being transcriptionally active in gametophyte (Fukai et al. 2010), producing a small number of new insertions only during transition to a new generation.

We are currently using a founder line with active LORE1 to generate a large, mutagenized population of *Lotus japonicus* Gifu. For robust identification of novel insertion sites we are using a 2D pooling strategy and taking advantage of the known LORE1 LTR sequence to simultaneously amplify LORE1 flanking sequence tags (FSTs) in dozens of pooled plants. Illumina deep sequencing technology, an extremely high degree of multiplexing and custom-designed bioinformatic pipeline led us to establishing high throughput detection method for robust identification of new insertions. Study on a test set of 3744 plants, enabled identification of 8935 novel insertions with an average of 2,4 per plant. Novel LORE1 copies do not show tendency to cluster and are evenly distributed along Lotus chromosomes. Moreover, genes were preferentially targeted and insertions in exons were 5.7 times more frequent than in intergenic regions, with 23% of all new insertions localized in CDSs. In a current stage, the project is being scaled up to saturate Lotus genome to a level where 95% of all the genes are knocked out by the LORE1 insertions. Project outcome is publicly available and open for the community. The list of LORE1 insertions can be browsed at http://carb.dk/resources.asp

References:
Miyao, A., Tanaka, K., Murata, K., Sawaki, H., Takeda, S., Abe, K., Shinozuka, Y., Onosato, K., Hirochika, H. (2003) Target Site Specificity of the Tos17 Retrotransposon Shows a Preference for Insertion within Genes and against Insertion in Retrotransposon-Rich Regions of the Genome. The Plant Cell 15, 1771-1780.
Tadege, M., Wen, J., He J.,Tu, H., Kwak, Y., Eschstruth, A., Cayrel, A., Endre, G., Zhao, P.X., Chabaud, M., Ratet, P., Mysore, K.S. (2008) Large-scale insertional mutagenesis using the Tnt1 retrotransposon in the model legume Medicago truncatula. The Plant Journal 54, 335-347.
Fukai, E., Fukai, E., Umehara, Y., Sato, S., Endo, M., Kouchi, H., Hayashi, M., Stougaard, J., Hirochika H. (2010) Derepression of the Plant Chromovirus LORE1 Induces Germline Transposition in Regenerated Plants. *PLoS Genet* 6(3), e1000868.

Why using *Brachypodium distachyon* as a model?

Richard Sibout, Group Leader

Institut Jean-Pierre Bourgin (UMR1318) INRA-AgroParisTech, Centre de Versailles-Grignon Route de St-Cyr (RD10) 78026 Versailles France

Recent years have seen a growing interest in grasses not only as food and feed source, but also as dedicated feedstocks for bio-energy, biomaterials and green chemicals. Grasses have fundamental differences in development and physiology compared to dicots, to which the model species Arabidopsis belongs. For instance, seed, shoots, root architecture and cell wall composition are strikingly different. Researchers have recently adopted *Brachypodium distachyon* as a new grass model. This species is closely related to wheat and fulfills all criteria for a powerful model for systems biology in grasses. In accordance with the recent publication of *Brachypodium distachyon* genome, many resources and tools have been developed. A T-DNA consortium aims to produce 40 000 lines and chemically induced mutant populations have been produced to identify allelic mutants by TILLING. More than 2000 natural accessions are available and a few labs developed Recombinant Inbred Line populations. Thus, the nascent Brachypodium community contribute to the development of genomic toolboxes that will greatly accelerate food, energy and stress-related research in grasses. Attractive features of Brachypodium for the production of bioenergies partly derived from lignocellulosic cell walls will be shown during this presentation to illustrate why Brachypodium could be used as a model.

Desorption electrospray ionization mass spectrometry imaging of cyanogenic glucosides distribution in plant tissue

Nanna Bjarnholt, Bin Li, Camilla Knudsen, Natascha Krahl Hansen, Kirsten Jørgensen, Christian Janfelt, Birger Lindberg Møller

Department of Plant Biology and Biotechnology, University of Copenhagen

Imaging of the tissue- and cell-specific localization of proteins and transcripts by e.g. GFP-staining, immunolocalization or *in situ*-PCR is an important tool in plant science, widely used to assign gene and protein function. Being able to image the localization of metabolites is equally desirable for investigations of metabolic pathways; however this type of imaging is not as well-developed and the methods may only work for compounds with specific functional groups. We have previously developed a method for imaging the distribution of various metabolites in leaves and flower bud cross sections using desorption electrospray ionization mass spectrometry (DESI). This method involves imprinting the plant tissue on porous Teflon and imaging the metabolite distribution in this imprint by DESI. For imprinting the tissue is pressed, whereby the cells are disrupted and the contents transferred to the Teflon surface. We now show that the process allows reliable imaging of cyanogenic glucosides (CGs) distribution although the cell disruption causes the compounds to be intermixed with large amounts of specific CG hydrolyzing β-glucosidases. This is demonstrated by imaging distributions of CGs and the related compounds rhodiocyanosides in leaves of *Lotus japonicus* TILLING mutants with and without active β-glucosidases. The method was further employed to image the age-dependent lengthwise distribution of the CG dhurrin in etiolated sorghum seedlings, and the accumulation of the CGs linamarin and lotaustralin in the outer cell layers of cassava tubers. All images were verified by metabolite extractions and LC-MS analyses or by comparisons with previously published results. This approach can be used to visualize metabolite distribution of relevance in e.g. plant-pathogen interactions, developmental studies etc., and to identify overall co-localization of enzymes with potential products or substrates. In this study the method was also used for visualizing the enzymatic degradation of CGs and rhodiocyanosides and formation of degradation products by wounding leaves prior to imprinting.

Recent strategic initiatives at Aarhus University and ERC grant (European Research Council grant)

Jens Stougaard, Professor

Department of Molecular Biology and Genetics, Aarhus University

Recent strategic initiatives at University of Copenhagen (Copenhagen Plant Science Center)

Poul Erik Jensen, Professor

Department of Plant Biology and Biotechnology, University of Copenhagen

Center for Dynamic Molecular Interactions, DynaMo - a Center of Excellence funded by the Danish National Research Foundation,

Barbara Ann Halkier, Professor

Department of Plant Biology and Biotechnology, University of Copenhagen

PUMPKIN (Center for membrane pumps in cells and disease), Continuation of existing Danish National Research Foundation research center

Thomas Günther-Pomorski, Associate Professor

Department of Plant Biology and Biotechnology, University of Copenhagen

Center for Biosustainability - The Novo Nordic Foundation Center

Morten Nørholm, Senior Scientist/Project leader

DTU Biosustain, Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability

Agriculture and the food versus fuel dilemma-critical issues and technologies

Richard A. Dixon, Professor

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In a report by the United States Departments of Energy and Agriculture entitled "*Breaking the Barriers to Cellulosic Ethanol*", it was noted that "cellulosic biomass is an attractive energy feedstock because it is … abundant, domestic, [and] renewable". Furthermore, it may not compete with the food supply in the same was as does ethanol from corn. Plant cell walls constitute the majority of lignocellulosic biomass by weight, and are converted to liquid biofuels via sugar release and subsequent fermentation. However, features of plant cell walls such as the presence of lignin reduce access of enzymes and chemicals to hemicellulose and cellulose, thus reducing the efficiency of enzymatic hydrolysis of biomass to release sugars. This phenomenon is referred to as recalcitrance. Breaking the recalcitrance barrier is critical for lignocellulosic liquid biofuels to become cost effective. A second factor that limits the competitiveness of lignocellulosic biofuels is the cost of transportation from the site of harvest to the refinery. Increasing biomass density directly reduces such costs, as a greater mass of biomass can be accommodated in the same volume. Combining reduced recalcitrance with increased biomass density, and additional improvements in yield, abiotic stress tolerance, and water and nitrogen use efficiency will lead to the development of lignocellulosic feedstocks that can revolutionize the biofuels industry.

A recent report by the National Research Council entitled "Renewable Fuel Standard: Potential Economic and Environmental Effects of U.S. Biofuel Policy" concluded that the United States is unlikely to meet the targets for lignocellulosic ethanol mandated by the Renewable Fuel Standard, largely due to cost issues. These can only be overcome by innovative technology advances. Here, we describe research that illustrates the feasibility of both reducing the recalcitrance barrier and increasing biomass density in dedicated bioenergy crops. These studies have moved beyond the proof-of-concept stage to encompass field trials in elite germplasm. Furthermore, they are also revealing novel basic insights into lignin biosynthesis and cell wall assembly to inform further technology development.

Challenges in assessing health benefits of phytochemicals: the story of 'super-broccoli

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Natural Products and Health Programme, Institute of Food Research, BBSRC, UK

Epidemiological evidence from prospective cohort studies and retrospective case-control studies supports a role of certain diets in reducing the risk of development and progression of diseases such as cancer and cardiovascular disease. However, identification of the responsible bioactive phytochemicals within the complex plant matrix and assessing their health benefits has proven challenging. Studies using cell and animal models are used extensively to assess bioactivity and get an insight of molecular mechanisms that mediate the health effects, but extrapolating these to humans requires caution. Well-designed human intervention studies are needed to provide high quality evidence for health benefits of dietary components derived from plants. We have developed a 'super-broccoli', high in glucosinolates (GSLs) that delivers increased levels of isothiocyanates, degradation products of GSLs thought to mediate the health benefits of cruciferous diets. Introgression of parts of the genome from a wild broccoli ancestor, *Brassica villosa*, confers 3-4 fold increase in aliphatic glucosinolates compared to standard broccoli cultivars. We have used this broccoli in human studies to determine bioavailability and have been undertaking human intervention studies to assess health benefits and identify molecular mechanisms using transcriptomic and metabolomic approaches.

RNAseq of medicinal plants – elucidation of natural product pathways

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The study of the biosynthesis of plant alkaloids at the enzyme and gene level has greatly advanced in recent years. A number of genes are available from the monoterpenoid indole-, tetrahydrobenzylisoquinoline-, and structurally related to both of the previous classes, the terpenoid-isoquinoline alkaloid biosynthetic pathways. To date, however, only partial understanding of the formation of medicinal natural products at the enzyme and gene levels has been attained. The explosive increase in understanding of biology over the past two decades has been enabled by work on model genetic organisms. The study of selected species-specific medicinal natural products, however, requires investigation of those plant species that harbor all or most components of the focal biosynthetic pathway. Detailed genetic and biochemical information on these highly specialized species is often missing. Having comprehensive medicinal plant transcriptomes would greatly advance research on medicinal plant species. We now seek to generate and use transcriptome data to understand the complete formation, storage and regulation of plant-derived medicinal compounds at the enzyme and gene level. Results will be presented from efforts to date to produce deep transcriptome datasets from members of the Papaveraceae and to interrogate the datasets for candidate alkaloid biosynthetic genes.

Use of Chemoinformatics for the Elucidation of Phytochemicals' Role in Health and Disease

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Similar to pharmaceuticals, edible and medicinal plants contain compounds that act as modifiers of biological functions. However, the level of complexity is increased by the simultaneous presence of a variety of components, with diverse chemical structures and numerous biological targets. Nowadays, it is widely recognized that systems chemical biology has the potential to increase our understanding of how small molecules interact with biological systems. A fruitful strategy to approach and explore this field is, therefore, to borrow informatics methods that are well established in pharmaceutical research for the elucidation of phytochemicals' role in health and disease.

We have recently initiated a project at CBS/DTU, where we used text mining to construct a unique database with state-of-the-art information concerning food and its molecular components. During the talk, I will present the approach we followed for developing the database and I will highlight applications of chemoinformatics in linking the phytochemical space with the human proteome.

Yeast artificial chromosomes for discovery and production of pharmaceuticals and nutraceuticals

Jørgen Hansen, Director of Research

Evolva Biotech A/S, Denmark

That the majority of current pharmaceuticals have their root in Nature is well known. It is also clear, however, that the potential of the natural kingdoms in this respect is largely untapped, and f. x. green plants alone are expected to contain an immense amount of valuable chemicals. Evolva uses combinatorial biochemistry to discover novel small molecules with activity on disease targets, and to establish novel or improved production pathways for valuable compounds. We do this by simultaneous expression in yeast of multiple (50-200) natural source genes from artificial chromosomes (eYACs). The process is combinatorial, so a very large number of gene combinations are tested. In some cases this means that novel metabolic pathways can be created by chance, leading to novel compounds. In other cases it means that improved or novel production pathways are created, leading to tractable manufacturing processes for f. x. nutraceuticals. The presentation will contain examples of both.

Food security in the context of climate change: targets for the next decade

Bruce Campbell, Program Director

Climate change Agriculture and Food Security, Consultative Group on International Agricultural Research (CGIAR), LIFE, University of Copenhagen

The agricultural sector is one of the major drivers of global environmental change, pushing global processes beyond safe operating boundaries. On the other hand, some researchers estimate that by 2050 agricultural production has to double in order to meet food demand as populations grow and get wealthier. And that this has to occur as climatic conditions for agriculture get worse in many parts of the world. Given the upcoming Rio+20, now is the time to develop a new vision for agriculture and food systems, where food security is secured under a changing climate at significantly lower environmental cost. As a step towards that direction some key indicators of success and preliminary targets are proposed.

Sustainable Agriculture: Producing More, Conserving More and Improving Lives

Lars Ipsen, Sales Manager Seed and Traits in Nordic and Baltic countries

Monsanto

Monsanto is one of the world's leading companies focused on sustainable agriculture. We discover and deliver innovative products that support the farmers who feed, fuel and clothe our world.

People around the world depend on farmers for their most basic needs. With global population expected to grow by 40 percent in the next few decades, agriculture will need to become more productive and more sustainable in order to keep pace with rapidly increasing demands.2 Many experts agree that we will need to grow as much food in the next 50 years as we did in the past 10,000 years combined if we are to sustain our planet's population.3 Compounding this challenge is the fact that farmers will need to keep up with demand while dealing with limited resources like land, water and energy.

Demand is growing, but supplies of these basic resources are not. Farmers will need to get more from every acre of land, every drop of water and every unit of energy. Sustainable agriculture is at the core of Monsanto. We're committed to developing the technologies that enable farmers to produce more while conserving more of the natural resources that are essential to their success. We do this by bringing seeds, traits developed through biotechnology and crop protection products to the marketplace.

Our commitment to sustainable agriculture
In 2008, we set a series of goals for ourselves to work with farmers to make agriculture more sustainable. These goals state that by 2030, we will do our part in:

- **Producing More –** Developing improved seeds that help farmers double yields from 2000 levels for corn, soybeans, cotton, and springplanted canola, with a $10 million grant pledged to improve wheat and rice yields through Monsanto's Beachell Borlaug International Scholars Program.
- **Conserving More –** Conserving resources through developing seeds that use one-third fewer key resources per unit of output to grow crops while working to lessen habitat loss and improve water quality.
- **Improving Lives –** Helping improve the lives of farmers and the people who depend on them, including an additional 5 million people in resource-poor farm families by 2020.

We believe that our goals are appropriate targets and that they are attainable through a combination of advanced plant breeding, biotechnology and improved farm-management practices. Farmers can meet the needs of our growing planet; better seeds and agronomic practices will help them do it.

Many people around the world play a part in shaping agriculture and in helping meet global demand in a sustainable way. In 2008, Monsanto made a commitment to work hand-in-hand with farmers and deliver technologies that help them produce more crops, conserve more resources and improve lives.

Our commitment to sustainable agriculture

Niels Bjerre, Crop Manager Cereals, Nordic

Bayer CropScience

With limited arable land and a continuously growing world population, the available farmland per capita will decrease dramatically. At the same time food and feed crops are competing with crops for fiber and biofuel. Sustainable agriculture is our response, but what does that mean in practice. The presentation contains an example of how Bayer invest in a major crop like wheat and with whom we are partnering. Sustainable development needs innovation and partnering.

Joining Forces in Europe: The Joint Programming Initiative: Agriculture, Food Security and Climate Change (FACCE-JPI)

Heather McKhann, European Officer

Institut National de la Recherche Agronomique, France, FACCE – JPI Secretariat

The Joint Programming Initiative "Agriculture, Food Security and Climate Change" (FACCE-JPI) brings together 21 Member States/ Associated Countries to address the challenge of food security in the context of demographic growth, global environmental changes, globalization of the economy and dwindling natural resources such as fossil fuels, water and arable land.
The main objective of FACCE-JPI is then to align national programmes across participating Member/Associated Countries over the long term to address the research questions defined in the Research Agenda and to "share the burden".
Outcomes of FACCE-JPI will include:
(1) A large mobilisation of the research community across Europe to work together to meet a grand societal challenge, including innovative solutions.
(2) Knowledge to inform European decision-making
(3) Enhanced European visibility and impact in the international context
(4) Increased competitiveness of European research through enhanced linkages to industry and SMEs.

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Starch bioengineering in *Brachypodium distachyon*

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Brachypodium distachyon was recently introduced as a model plant for temperate cereals (Opanowicz et al., 2008; IBI 2010). In order to explore pre-domesticated and novel features of cereal starch metabolism we aim to establish *Brachypodium* as a model.

Bioinformatics analysis identified starch biosynthesis genes including seven soluble starch synthases (SS), two granule bound starch syntheses (GBSS), four starch branching enzymes (SBE), two glucan- and one phosphoglucan- water dikinases (GWD, PWD). Transit peptides and putative carbohydrate-binding modules (CBMs) of the families CBM20, CBM45, CBM48 and CBM53 were identified. The gene setup for starch biosynthesis is very similar to barley and a phylogenetic analysis based on the SS genes provided evidence for a close relation to barley and wheat.

To investigate the starch structural features, grain starch from two lines Bd21 and Bd21-3 were characterized. Microscopic, chemical and structural data including amylopectin chain length distribution, phosphate content, and amylose content of the starch granules provided evidence for a close structural relationship to temperate cereals. Close relationship can be observed even though kernel starch content and starch granule size were considerably lower and β-glucan content was much higher than that for barley (*Hordeum vulgare*). Small-angle and wide-angle X-ray scattering (SAXS and WAXS) show low crystallinity of *Brachypodium* starch granules as compared to barley. These data were confirmed by differential scanning calorimetry (DSC) data. Polarization microscopy indicated ordered chain arrangements only in the outer layer of the granules.

We are currently identifying mutants and biolistic *Agrobacterium*-mediated transformation directed towards silencing and performing overexpression of key starch biosynthesis genes aiming at providing evidence for differential or conserved actions of specific genes in this grass as compared to domesticated cereals.

Our data show that *Brachypodium distachyon* can provide a valuable and efficient model for starch bioengineering in temperate cereals.

Key words:
Brachypodium distachyon, model plant, grain starch, starch biosynthesis genes, starch bioengineering

References:

Opanowicz, M., Vain, P., Draper, J., Parker D. and Doonan, J.H. (2008) *Brachypodium distachyon*: making hay with a wild grass. *Trends Plant Sci.* 13, 172-177.

The International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon. Nature* 463, 763–768.

Cyanogenic Glucosides and Mating Compounds in the Burnet Moth (*Zygaena filipendulae*) Life Cycle

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Cyanogenic glucosides (CNglcs) are amino acid derived bio-active natural products, present in more than 2,500 different plant species. CNglcs are important for plant defense against herbivores, because of their bitter taste and ability to release toxic hydrogen cyanide (HCN) as well as ketones or aldehydes upon tissue disruption. Zygaena larvae sequester the CNglcs linamarin and lotaustralin from their polymorphic food plants (Lotus) and also have the ability to *de novo* biosynthesize these compounds, if they should find themselves on a plant with a suboptimal ratio and/or content of CNglcs. In Zygaena, CNglcs serve as defence compounds during the entire insect life cycle, and their content and ratio are tightly regulated. There are also indications that CNglcs have other roles than defence in Zygaena, e.g. as storage of reduced nitrogen, as nuptial gifts and in mating communication in adults.

We have studied the roles of CNglcs, 5-glucoxy-tryptofan and degradation products thereof during *Zygaena filipendulae* mate attraction and mating. LC-MS and cyanide emission assays showed that females prefer to mate with males with a higher content of CNglcs, probably because *Z. filipendulae* males transfer a nuptial gift of CNglcs to females during mating. Especially lotaustralin may be related to female assessment of males since painting of this compound on rejected males often rendered them acceptable to females. The degradation products of CNglcs are emitted from male corremata during courting, and are also present in high amounts in glands associated with mating in both males and females. This indicates important functions of CNglcs during mate attraction and mating. In addition to CNglcs, 5-glucoxy-tryptofan was found to be transferred from males to females during mating, after 5 hours of coupling. 5-glucoxy-tryptofan could be a precursor of serotonin which is a major neurotransmitter in insects and may be involved in egg-laying.

Bioactivation and Turnover of Cyanogenic Glucosides in Burnet Moths.

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Cyanogenic glucosides (CNglcs) are amino-acid derived hydroxynitrile glucosides present in many plant and insect species. They function as defense compounds due to release of toxic hydrogen cyanide and bitter taste, as well as serve for nitrogen and sugar storage.
We use the burnet moth *Zygaena filipendulae* (Lepidoptera: Zygaenidae) as a model system to understand CNglc turnover and functions in insects. Burnet moths biosynthesize CNglcs and the larvae sequester them when feeding on their host plant *Lotus corniculatus*.

Furthermore, at least in *Zygaena* moths CNglcs are involved in mating behaviour. Bioactivation of CNglcs requires a two-step enzymatic breakdown resulting in hydrogen cyanide and a ketone compound. However, involved enzymes like ß-glucosidases (BGDs) and α-hydroxynitrile lyases (HNLs) are poorly known in insects.

Based on a 454 pyrosequencing approach we identified candidate genes for these enzymes. We express ß-glucosidase and α-hydroxynitrile lyase candidates in insect cells, run cyanide assays on recombinant proteins to reveal the cyanogenic glucosides bioactivation pathway in *Z. filipendulae*. In addition, we are working on elucidating the mechanism how *Zygaena* larvae sequester cyanogenic glucosides from its *Lotus* food plant without getting intoxicated.

Finally, we do feeding experiments with *Zygaena* larvae and *Lotus* plant mutants which differ in the level of hydroxynitrile glucosides to pinpoint larval feeding preferences and the influence of these plant compounds on insect development.

References:
- Bjarnholt et al (2008) Diversification of an ancient theme: Hydroxynitrile glucosides. Phytochemistry 69:1507-1516.
- Jensen et al (2011) Convergent evolution in biosynthesis of cyanogenic defence compounds in plants and insects. Nature communiations 2: 273
- Takos et al (2011) Genetic Screening Identifies Cyanogenesis-Deficient Mutants of Lotus japonicus and Reveals Enzymatic Specificity in Hydroxynitrile Glucoside Metabolism. Plant Cell 22:1605-1619
- Zagrobelny & Møller (2011) Cyanogenic glucosides in the biological warfare between plants and insects: The Burnet moth-Birdsfoot trefoil model system Phytochemistry 72 : 1585–1592
- Zagrobelny et al (2009) 454 pyrosequencing based transcriptome analysis of Zygaena filipendulae with focus on genes involved in biosynthesis of cyanogenic glucosides. BMC Genomics 10: 574

A single amino acid difference determines substrate specificity of β-glucosidases in hydroxynitrile glucoside metabolism

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Cyanogenic glucosides (α-hydroxynitrile glucosides) are defense compounds found in a large number of plant species. The glucosylation is required for the stabilization and storage of these compounds. Upon tissue disruption, for example by chewing insects, the cyanogenic glucosides come in contact with β-glucosidase enzymes. These remove the glucose moiety resulting in degradation of the unstable α- hydroxynitrile and the release of hydrogen cyanide gas. The model legume *Lotus japonicus* produces the cyanogenic glucosides linamarin and lotaustralin derived from the amino acids Val and Ile. It also produces related non-cyanogenic γ- and β-hydroxynitrile glucosides called rhodiocyanosides.

Two closely related β-glucosidases, BGD2 and BGD4, play a role in hydroxynitrile metabolism in leaves. Mutant analysis showed that only BGD2 was involved in leaf cyanogenesis (Takos A. *et al*. 2010). Biochemical analysis confirmed that BGD2 is able to hydrolyze both cyanogenic glucosides and rhodiocyanosides, while BGD4 efficiently degrades rhodiocyanosides. Using sequence comparison and 3D-modelling we predicted key amino acid residues responsible for the difference in substrate specificity. The role of these amino acids was experimentally tested using site directed mutagenesis of the BGD4 sequence. Modeling predicted that Gly 211 in BGD2, a Val in BGD4, was likely to be important for specificity as it determined the shape of the aglycone binding pocket. Changing the Val in BGD4 to a Gly enabled the modified BGD4 enzyme to use lotaustralin as substrate. Therefore a single amino acid change can be responsible for altering β-glucosidase specificity.

While leaves of mutants in *BGD2* were acyanogenic, flowers retained their cyanogenic potential. A related flower expressed β-glucosidase was identified based on the sequence requirements to hydrolyze cyanogenic glucosides and is being characterized presently.

Reference

- Takos A. *et al*. (2010). Genetic screening identifies Cyanogenesis-Deficient Mutants of Lotus japonicus and reveals enzymatic specificity in hydroxynitrile glucoside metabolism. *The Plant Cell* ***22:*** *1605-1619*

Mutant study of cyanogenic glucoside and related compounds in barley

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The only known cyanogenic glucoside in barley (*Hordeum vulgare*) is epiheterodendrin. Upon enzymatic hydrolysis of the glucosidic bond, this α-hydroxynitrile glucoside can release hydrogen cyanide (HCN). In general, the release of HCN is known to function as a defence mechanism against plant-feeding pests. Barley lacks the enzymatic activity of a cyanogenic glucoside-cleaving β-glucosidase and does not release HCN. Therefore the biological role of epiheterodendrin, and its closely related non-cyanogenic β- and γ-hydroxynitriles, is unclear. These five barley hydroxynitrile glucosides are all derivatives of the amino acid leucine, and they all accumulate in the leaf epidermis.

We used a HCN-release assay applying additional almond-derived β-glucosidase, to identify low-cyanogenic barley lines. As screening material, we used the epidermis of the first leaf from a fast-neutron mutagenized barley population in the cultivar Golden Promise. We screened 3,600 individuals and identified 156 initial candidates, of which three candidate mutant lines have been confirmed. A low content of all five hydroxynitrile glucosides was confirmed by LC-MS. In order to search for the responsible mutations, the proposed biosynthesis genes will be sequenced. If these genes are intact, a map-based cloning approach will be initiated.

The role of hydroxynitrile glucosides in barley, which represent the major proportion of the sugar content in epidermis cells, will be studied. We will especially focus on the response of the mutant lines towards the epidermis restricted biotrophic fungus *Blumeria graminis*, which causes powdery mildew, a disease with high impact in agriculture. Furthermore, it will be of major interest to characterize the role of hydroxynitrile glucosides in plant development as carbohydrate and/or nitrogen source, using our iso-genic barley lines with high and low content of hydroxynitrile glucosides.

Gene discovery: The biosynthetic enzymes involved in the synthesis of type II arabinogalactan – a key component of ArabinoGalactan-Proteins

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ArabinoGalactan proteins (AGPs) are highly complex glycosylated macromolecules found *in planta*. They consist of a protein backbone (2-10%) that is *O*-glycosylated by type II arabinogalactan (AG) chains (90-98%) in which the galactan component is (1,3)- and (1,6)-linked. In addition to the implication in a vast number of biological processes such as plant growth and development, these abundant molecules also hold a huge potential for future commercial exploitation *e.g.* as a functional food ingredient. However, the biosynthetic machinery involved in the synthesis of the type II AG component still remains to be discovered.

Using the recent work by Egelund et al., (2011), in which the authors adopted a bioinformatic approach to identify and systematically characterize the putative *Arabidopsis thaliana* galactosyltransferases (GalTs), responsible for synthesizing the (1,3)-Gal linkage from CAZy GT-family-31 (www.cazy.org), and when possible attempted to predict the possible substrate specificity, we have selected a number of candidate GalTs for further analysis using T-DNA insertional mutants, heterologous expression, sub-cellular localization and expression studies – as a first step in unravelling their catalytic specificity.

Egelund J, Ellis M, Doblin M, Qu Y, Bacic A (2011) The genes and enzymes of CAZy GT-family-31: Towards unravelling the function(s) of the plant glycosyltransferase family members. Annual Plant Reviews series “Plant cell wall polysaccharides - biosynthesis and bioengineering” Vol 41. Blackwell Publishing, Oxford.

Cereal bioengineering: Amylopectin-free and hyper-phosphorylated barley starch

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Barley lines producing grains with either amylopectin-free or hyper-phosphorylated starches were engineered by transgenic methods.

Amylopectin-free barley was generated by simultaneously silencing the three genes encoding the starch branching enzymes (SBEIIa, SBEIIb and SBEI) using a chimeric hairpin. This construct was inherited as a single locus with a 3:1 segregation, which makes the method useful for breeding as compared to combing alleles of the three different SBE genes segregating independently.

Transgenic grains were wrinkled. This is a phenocopy of Mendel's wrinkled peas, which were also based on a non-functional allele of an SBE gene. Amylopectin content was below detection level (< 1%) measured by both size exclusion chromatography (SEC) and differential scanning calorimetry (DSC). Scanning electron microscopy (SEM) showed that amylopectin-free starch granules were irregulary, elongated and globose shaped. Plants were propagated for three generations. Yield was determined in grains grown under semi-field conditions. Loss of SBE activity was compensated by the cereal grain by up-regulated gene expression of starch synthases. As an effect, yield loss is limited to 20%. This demonstrates for the first time a way for production of pure amylose in plants with limited yield loss.

In order to increase barley starch phosphorylation, endosperm specific overexpression of glucan water dikinase from potato (StGWD) was conducted. The content of phosphate esters in starch from consecutive generations (T_0 and T_1) of transgenic grains was tenfold higher than from vector control and wild type grains. Amylose content was not affected in hyper-phosphorylated grains. Hyper-phosphorylated starch granules had several pores on the surfaces, similar to pores seen on enzymatically semi-degraded granules. This provides support for the presence of a general mechanism in starch degradation in the plant kingdom that phosphorylation carried out by ectopic expression of StGWD tags barley starch granules for degradation executed by endogenous enzymes.

Together this work shows two new strategies for in planta starch bioengineering of cereals. It demonstrates that bioengineering may be used to obtain novel and technologically interesting cereal starches, and further that it facilitates the elucidation of the complex pathways of starch biosynthesis and the roles of individual starch biosynthetic enzymes.

Specialized roles for the two UDP-glucosyltransferases UGT85K2 and UGT85K3 in hydroxynitrile glucoside metabolism in *Lotus japonicus*.

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Cyanogenic glucosides are amino-acid derived plant chemical defense compounds against generalist herbivores. They are α-hydroxynitrile glucosides that are activated by specific β-glucosidases upon tissue disruption. The unstable α-hydroxynitrile will dissociate with the release of hydrogen cyanide. The legume model *Lotus japonicus* contains the cyanogenic glucosides linamarin and lotaustralin, and the non-cyanogenic γ- and β-hydroxynitrile glucosides rhodiocyanoside A and D, which are also thought to function as defense compounds. Glucosylation is a key-step in the biosynthesis of hydroxynitrile glucosides as it stabilizes and detoxifies these compounds, and allows for their storage. Both the UDP-glucosyltransferases UGT85K2 and UGT85K3 are able to catalyze the synthesis of linamarin and lotaustralin, but only UGT85K2 showed significant glucosylation activity for the synthesis of rhodiocyanosides *in vitro*. Mutants in the *UGT85K2* gene, obtained by TILLING, almost lacked rhodiocyanosides and showed severe growth defects. This suggested the toxicity of the rhodiocyanoside aglycones and supports their proposed defense role. The observed specificity of these UGTs further highlights the metabolic flexibility of the hydroxynitrile glucoside based defense pathway in *Lotus japonicus*.

Linking chlorophyll biosynthesis to photosynthesis

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The production of chlorophyll in higher plants is closely regulated in accordance with the requirements for the pigment, to ensure both a sufficient supply of the light-absorbing pigments for a fully functional photosynthetic apparatus but also to avoid the accumulation of free chlorophyll intermediates potentially leading to photo-oxidative damage.
Most steps in the biosynthesis of chlorophyll have been elucidated at the genetic and biochemical level, but the localization and the composition of subunit of some of the biosynthetic enzymes in the chloroplast is yet unclear. In particular the step leading to the formation of the fifth ring in the chlorophyll molecule is not fully characterized. This step is catalyzed by the so called aerobic cyclase. We have previously identified one catalytic subunit of this enzyme: CHL27 (Tottey et al., 2003). CHL27 contains two irons in its active site and in order to complete its catalytic cycle these irons need to be reduced from Fe^{3+} to Fe^{2+}. So far no enzyme involved in this has been identified despite huge efforts from many researchers. This prompted us to search for alternative ways to reduce the two irons in CHL27. We have used specific inhibitors of electron transport and mutants affected in regulation of electron flow to show that this particular step in chlorophyll biosynthesis indeed is directly regulated by photosynthetic electron transport. This has interesting consequences since there now is a direct connection between photosynthesis and the biosynthesis of photosynthetic pigments.

Tottey S, Block MA, Allen M, Westergren T, Albrieux C, Scheller HV, Merchant S & Jensen PE (2003) Arabidopsis CHL27, located in both envelope and thylakoid membranes, is required for the synthesis of protochlorophyllide. Proc. Natl. Acad. Sci. USA. 100: 16119-16124.

Silicon deposition in plant cell walls: transporters involved and implications for bioenergy production

Emiko Murozuka, Kristian Holst Laursen, Pia Haugaard Nord-Larsen, Claus Felby, Thomas Paul Jahn, Inge Skrumsager Møller and Jan Kofod Schjoerring

Incorporation of silicon (Si) in plant cell walls enhances their mechanical strength and contributes to alleviation of both biotic and abiotic stresses (Epstein, 1994). However, when plant residues are used for bioenergy puposes, Si is a problematic element which has negative impact on the quality of the biomass for biocatalytic and thermal conversion processes. Reduced Si content is therefore an important quality parameter and there is a strong interest in reducing the Si content in plant biomass (Gressel, 2008).
We have screened a panel of 20 wheat genotypes for natural variation in straw Si concentration. A 2-fold range in Si concentration was found, reflecting differences among both genotypes, nitrogen regime and growth locations. A micro-scaled enzymatic saccharification assay is carried out to investigate the effect of Si on straw sugar yield. The results obtained so far do not indicate that Si is a significant inhibitor of saccharification but the data analysis is still ongoing.

Si transport in plants is mediated by two aquaporins, Lsi1 and Lsi6. In addition, a secondary active transporter Lsi2 is involved. Lsi1 and Lsi2 are involved in Si uptake and transport through the roots while Lsi6 plays a role in the distribution of Si within the shoots (Ma et al., 2011). In order to find mutant plants defective in Si transport, we have screened a mutant population of the grass species *Brachypodium distachyon*. This species combines many desirable attributes such as a small plant size, short generation time, transformability and small genome (Garvin et al., 2008) and is increasingly used as a model for dedicated bioenergy crops. Phenotypic screening using germanium (Ge), which is a toxic analogue of Si, enabled us to identify mutants in Si transport since Ge is taken up via same pathway as Si but causes necrosis in the leaves. Based on selection of Ge tolerant plants, single mutants for each Si transporter were isolated together with a triple mutant which has mutations in all the Si transporters. After backcrossing, the mutant plants will be characterized with respect to Si accumulation and distribution in the cell walls. Further investigations of how Si interacts with and is integrated into the cell walls are in the pipeline.

References:

Epstein E (1994) The anomaly of silicon in plant biology. Proc Natl Acad Sci U S A. 91:11-7.

Garvin D.F, Gu Y-Q, Hasterok R, Hazen S.P, Jenkins G, Mockler T.C, Mur L.A.J, Vogel J.P (2008) Development of genetic and genomic research resources for Brachypodium distachyon, a new model system for grass crop research. Crop Sci 48(Supplement_1):69-84

Gressel J (2008) Transgenics are imperative for biofuel crops. Plant Sci 174: 246–263
Ma J.F, Yamaji N, Mitani-Ueno N (2011) Transport of silicon from roots to panicles in plants. Proc Jpn Acad Ser B Phys Biol Sci.87:377-85.

Van Soest P.J (2006) Rice straw, the role of silica and treatments to improve quality. Anim. Feed Sci. Tech. 130: 137–171

Triterpenoid saponins as plant defense compounds

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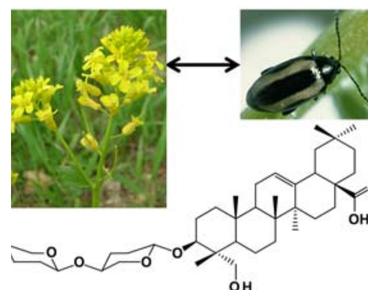
Background

Winter cress (*Barbarea vulgaris*) is a wild plant closely related to oil seed rape, radish, rucola, mustard and the reference plant *Arabidopsis thaliana*. It is resistant to a range of insect species because it produces triterpenoid saponins. We study natural plant resistance to insects with focus on saponin involvement in the resistance.



We used unbiased LC-MS ecometabolomic approach based on a segregating population of a cross between resistant and susceptible types of winter cress and identified four triterpenoid saponins as the main anti-insecticidal compounds against flea beetles: oleanolic acid cellobioside, hederagenin cellobioside, gypsogenin cellobioside, and 4-epihederagenin cellobioside. Transcriptomic datasets of the resistant and susceptible winter cress types were used for molecular marker design, as well as they were mined for putative key candidate enzymes for saponin biosynthesis: oxidosqualene synthases, cytochromes P450 and family 1 glycosyltransferases. We created a genome map of winter cress based on the molecular markers designed and localized genome regions containing genes for saponin and glucosinolate production, hairiness, and flea beetle resistance.

Conclusions

As indicated by the untargeted ecometabolomic approach as well as genome mapping studies, triterpenoid saponins act as defense compounds against flea beetle herbivory in winter cress. Genome regions containing genes for the resistance and saponin biosynthesis co-localized, and we are currently studying the genes located within these regions by mining the recently obtained genome sequencing data of the resistant and susceptible winter cress types. For instance we are characterizing oxidosqualene cyclases situated within these genome regions for their involvement in the saponin biosynthesis pathway. Read more about the project on www.ecogenomics.life.ku.dk.

Identification of sapogenin glycosyltransferases in *Barbarea vulgaris*

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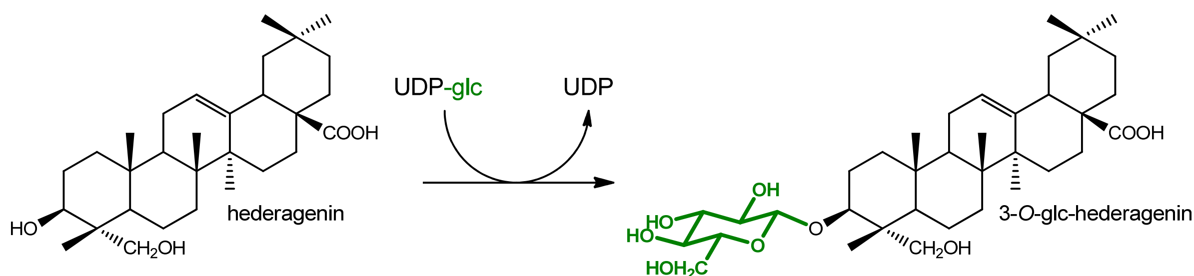
Saponins are bioactive compounds that are produced by a wide range of plants to counteract herbivorous and pathogenic attacks. They consist of a triterpenoidal derived aglycone attached to usually one or two saccharide side chains. The resulting amphiphilic structure enables most saponins to integrate into membranes, where there can cause severe damage due to membrane reorganization as a consequence of complex formation with membrane sterols.
The wild crucifer *Barbarea vulgaris* (winter-cress) produces several triterpenoid saponins. Some of which as, e.g., hederagenin cellobioside have been found to confer resistance towards phytophagous pests such as *Phyllotreta nemorum* (flea beetle) and *Plutella xylostella* (diamondback moth). The molecular mechanism of this deterrent effect still remains elusive, but glycosylation of the saponin aglycone is found essential for the insecticidal activity.



Activity screening of a *B. vulgaris* var. *variegata* cDNA expression library resulted in identification of an UDP-glycosyltransferase (UGT) capable of catalyzing sapogenin glucosylation. Subsequently, a total of five homologous UGT genes were cloned from two *B. vulgaris* spp. *arcuata* types that are either resistant or susceptible towards *P. nemorum.* Two enzymes of each *B. vulgaris* type were shown *in vitro* to efficiently catalyze glucosylation of all tested oleanane and lupane sapogenins as well as of sapogenin mixtures extracted from *B. vulgaris*. In contrast, glucosylation activity of the four UGTs towards flavonoids is significantly lower and no activity was observed towards any of the sterols tested, demonstrating substrate specificity towards sapogenins. NMR analysis confirmed β-configuration of the glucosidic bond at C3 of the oleanane sapogenins, as it also occurs *in planta*. One UGT of each *B. vulgaris* type was in addition found to additionally glucosylate lupane sapogenins in position C28 via ester linkage. Correlation of saponin distribution and expression of the identified UGTs in different *B. vulgaris* organs further corroborates a role in saponin biosynthesis.

Glycosyltransferases involved in the biosynthesis of arabinogalactan protein

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Arabinogalactan proteins (AGPs) are a group of glycoproteins analogous to animal proteoglycans, which are important throughout the plant development and are thought to also be involved in signalling. AGPs are synthesized by post-translational modification of a peptide backbone in the secretory pathway[1]. The arabinogalactan moiety of AGP is heterogeneous, but commonly composed of -1,3-galactan backbone substituted with complex carbohydrate side chains at the *O*6 position. At least 11 different glycosyltransferases (GTs) are involved in the biosynthesis of arabinogalactan. Recently, we identified a GT from CAZy family 31 as a galactosyltransferase involved in arabinogalactan biosynthesis by heterologous expression and *in vitro* enzymatic assays. In addition, *in silico* database analysis using GeneCAT[2] suggested that there are three putative GTs co-expressed with the GT31 described above. Currently, we are characterizing those putative GTs as well as the GT31 with respect to their functional roles and the biological significances of GTs-interaction in biosynthesis of AGP.

References:
1. Ellis, M., Egelund, J., Schultz, C.J., Bacic, A. (2010). Arabinogalactan-proteins: key regulators at the cell surface? *Plant Physiology* 153: 403-419.
2. Mutwil, M., Obro, J., Willats, W.G., Persson, S. (2008). GeneCAT̃novel webtools that combine BLAST and co-expression analyses. Nucleic Acids Research 2008; 36 (Web Server issue): W320-W326.

Possible evolution of alliarinoside biosynthesis from the glucosinolate pathway in *Alliaria petiolata*:

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Nitrile formation in plants involves the activity of cytochrome P450s. Hydroxynitrile glucosides are widespread in the plant kingdom but generally do not occur in glucosinolate-producing species. However, the biosynthetic pathways for hydroxynitrile glucosides and glucosinolates share common features and the ability to produce glucosinolates have been suggested to have evolved from the pathway of cyanogenic α-hydroxynitrile glucosides. *Alliaria petiolata* (garlic mustard, Brassicaceae) is the only species known to produce glucosinolates as well as a γ-hydroxynitrile glucoside. In addition, *A. petiolata* has been described to release diffusible cyanide, which indicates the presence of unidentified cyanogenic glucoside(s). Recently, *A. petiolata* has gained attention as an invasive species in North America. Our research on *A. petiolata* addresses the molecular evolution of P450s. By integrating current knowledge about glucosinolate and hydroxynitrile glucoside biosynthesis in other species and new visions on recurrent evolution of hydroxynitrile glucoside biosynthesis, we propose a pathway for biosynthesis of the γ-hydroxynitrile glucoside, alliarinoside (Frisch, T. and Møller, B.L., 2011). Homomethionine and the corresponding oxime are suggested as shared intermediates in the biosynthetic pathways of alliarinoside and 2-propenyl glucosinolate. The first committed step in the hydroxynitrile glucoside pathway is envisioned to be catalyzed by an oxime-metabolizing P450, which has been recruited from outside the glucosinolate pathway. Furthermore, hydroxynitrile glucoside biosynthesis is suggested to involve enzyme activities common to secondary modification of glucosinolates. Our results support that alliarinoside is the first known case of a methionine-derived hydroxynitrile glucoside. Furthermore, we report the presence of novel hydroxynitrile glucoside(s) structurally related to alliarinoside. We argue that the biosynthesis of hydroxynitrile glucosides in *A. petiolata* is the first known case of a hydroxynitrile glucoside pathway having evolved from the Brassicales-specific glucosinolate pathway. Elucidation of the pathway for biosynthesis of alliarinoside and other hydroxynitrile glucosides in *A. petiolata* is envisioned to offer significant new knowledge on the emerging picture of P450 functional dynamics as the basis for recurrent evolution of pathways for synthesis of bioactive natural products

References:

Frisch, T., Møller, B.L. (2011). Possible evolution of alliarinoside biosynthesis from the glucosinolate pathway in *Alliaria petiolata*. *FEBS Journal*, Accepted Article, doi: 10.1111/j.1742-4658.2011.08469.x

Vanillin biosynthesis pathway in *Vanilla planifolia*

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This research project is aimed at elucidating the biosynthetic pathway for vanillin glucoside in the vanilla orchid (*Vanilla planifolia*). Vanilla is the world's most popular flavor and therefore one of the most popular plant natural products with an estimated annual worldwide consumption of over 2000 tons. Vanillin (3-methoxy-4-hydroxybenzaldehyde) is the main flavor compound in vanilla and is one of the key additives to food products, beverages, perfumery, and an intermediate in the pharmaceutical industry. Production of natural vanillin from pods of *V. planifolia* is a laborious and slow process. Nowadays, it is only 1% of the global production of vanillin that is derived from vanilla pods. Vanillin can also be produced synthetically using different fossil hydrocarbons. Biotechnologically produced vanillin is an alternative to the synthetic vanillin and can be obtained using microorganisms and cell culture processes (Hansen et al., 2009). As vanillin is toxic in high concentrations to living cells, the glucoside derivative of vanillin is formed in plant cells. Vanillin glucoside is only found in the inner part of the vanilla pod. It is speculated that the vanillin glucoside is a product of the phenylpropanoid pathway from L-phenylalanine (Havkin-Frenkel and Belanger 2011).
This study has taken genomic, proteomic and biochemical approaches to elucidate the vanillin biosynthesis pathway. We have sequenced the transcriptome of a 5 month-old pod of *V. planifolia* using Roche 454 sequencing technology. Several candidate genes encoding putative enzymes of the vanillin biosynthetic pathway are being identified. The candidate genes identified belong to enzyme families of 4-hydroxybenzaldehyde synthase (4-HBS), cytochrome P450s (CYPs), *O*-methyl transferases (OMTs) and UDP-glycosyltransferases (UGTs). Currently substrate specificity is analyzed for 4-hydroxybenzaldehyde synthase (4-HBS), CYP98A3 and 5 putative UGTs, and 15 putative OMTs from *V. planifolia*. Moreover, intermediate compounds that are involved in vanillin biosynthesis were investigated by C14 and C13 isotope labeled feeding assays with both L-phenylalanine and *p*-hydroxybenzaldehyde. C14 isotope labeled L-phenylalanine feedings indicate that vanillin biosynthesis pathway only takes place in the inner part of the pod. Furthermore, *in situ* PCR confirms that *4-HBS* from *V. planifolia* is mainly expressed in the inner part of the pod where the vanillin glucoside biosynthesis possibly taking place. Soluble proteins from the inner part of the vanilla pod were extracted and analyzed by LC-MS and MALDI-TOF to obtain a profile of the proteins. In near future, putative genes that are involved in natural vanillin biosynthesis pathway will be assembled in expressible Yeast Artificial Chromosomes (eYACs from Evolva A/S,) to confirm if the suitable substrates can be converted to vanillin glucoside in yeast.

References:
Havkin-Frenkel D. and Belanger C. F., 2011. Handbook of Vanilla science and technology, Wiley-Blackwell, 300-327.
Hansen H. E., Møller L. B., Kock R. G., Bunner M. C., Kristensen C., Jensen R. O., Okkels t. F., Olsen E. C., Motawia S. M., Hansen J., 2009. De novo biosynthesis of Vanillin in Fission yeast (*Schizosaccharomyces pombe*) and Baker's yeast (*Saccharomyces cerevisiae*). Applied and Environmental Microbiology, 75., 2765-2774.

Thapsigargin – an elusive but important drug to be

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Thapsigargin is a guaianolide only found in two members of the genus *Thapsia* (Apiaceae) [1]. Covalently linked to a peptide, thapsigargin has been converted into a prodrug targeted for the treatment of solid tumors. After preclinical experiments have been proven highly promising, the drug is currently undergoing clinical trials for the treatment of prostate cancer [2].

Since neither natural sources nor chemical synthesis can ensure future market demands, biotechnological production of thapsigargin in a host organism like the moss *Physcomitrella patens* needs to be established [3]. A prerequisite for the latter is knowledge about the enzymes catalyzing fundamental steps of the biosynthesis.

Although terpenoids are one of the biggest groups of specialized products found in the plant kingdom, little is known about their biosynthesis. This counts especially for species belonging to the Apiaceae family, since investigations have been focused on artemisinin or structurally related compounds like costunolid that are produced by plants belonging to the Asteraceae family [4,5]. Guaianolides isolated from Asteraceae plants differ fundamentally in their stereochemistry from those found in Apiaceae, and this stereochemistry is vital for the pharmacological activity. Thus, enzymes involved in thapsigargin biosynthesis can be expected to differ from enzymes catalyzing similar reactions in Asteraceae.

New generation sequencing data enabled us to obtain two sesquiterpene synthases, *Tg*STS1 and *Tg*STS2, of which *Tg*STS1 catalyses δ-cadinene synthesis and *Tg*STS2 catalyses formation of an unstable germacrenol derivative that is thought to be an intermediate in thapsigargin biosynthesis. Candidate P450 genes thought to be responsible for the further steps in thapsigargin biosynthesis are currently under investigation.

Results obtained by our studies will contribute to basic understand of guaianolide biosynthesis in Apiaceae and facilitate biotechnological production of thapsigargin.

References:

1. Christensen, S.B. et al. (1997) Fortschr Chem Org Naturst 71:129-167
2. GenSpera Inc. US Patent 6,545,131, US Patent application 20070160536
3. Simonsen, H.T. et al. (2009) Perspect Medicin Chem 3:1-6
4. Covello, P.S. (2008) Phytochemistry 69:2881-2885
5 Nguyen, D.T. et al. (2010) J Biol Chem 285:16588-16598

In planta characterization of novel diterpene synthases using *Physcomitrella patens*

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Diterpenoids represent with 11 percent a substantial fraction of the currently over 44.000 terpenoid structures identified in the plant kingdom. Plant species that accumulate specialized diterpenoid metabolites have been exploited in modern and traditional medicine because of pharmacological activities that include anti-cancer, anti-malaria, analgesic, antimicrobial and antifungal, contraceptive as well as psychoactive properties. *Physcomitrella patens* is known to produce substantial amounts of the diterpene, *ent*-kaurene, a common gibberellic acid (GA) precursor essential for growth and development in vascular plants. Despite the importance of GAs in vascular plants viable *P. patens* lines lacking *ent*-kaurene (and GAs), have been generated through targeted knock out by homologous recombination. Due to their unused pools of GGPP, the universal precursor for diterpenoid biosynthesis, these *ent*-kaurene free lines constitute a great platform for *in planta* characterization of diterpene synthases. Using a biolistic transformation approach heterologous genes are introduced with a minimal number of handling steps. Furthermore, this platform will facilitate expression of candidate enzymes of the cytochrome P450 family possibly involved in further oxygenation of the diterpenes and hereby promote the elucidation of high-value diterpenoid biosynthetic pathways.

De novo Biosynthesis of Cyanogenic Glucosides in *Zygaena filipendulae*

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Cyanogenic glucosides (CNglcs) are amino acid derived bio-active natural products, present in more than 2,500 different plant species. CNglcs are mainly used as defence compounds against generalist herbivores, due to their bitter taste and through the release of toxic hydrogen cyanide (HCN) and ketones or aldehydes upon tissue disruption. The larvae of the specialist insects belonging to the Zygaena family are able to both sequester the CNglcs linamarin and lotaustralin from their polymorphic food plants (Lotus) and biosynthesise these compounds *de novo* when encountering a plant with suboptimal ratio and/or content of CNglcs. In Zygaena, CNglcs are used mainly as defence compounds, but also for storage of reduced nitrogen, in mating communication and as nuptial gifts in imagines. The biosynthesis of CNglcs in the burnet moth *Zygaena filipendulae* follows the same pathway as the food plant *Lotus corniculatus*, with two multifunctional P450 enzymes and a glucosyl-transferase acting in sequence, and contains the same intermediates. Remarkably, the pathways are not the result of horizontal gene transfer but have evolved convergently in plants and insects.

We are studying *Z. filipendulae* to identify the site of *de novo* biosynthesis of CNglcs in the larvae and the imagines as well as the roles of CNglcs during the life cycle. The transcripts of the three biosynthesis genes are heavily up regulated in larvae feeding on acyanogenic Lotus plants as indicated by RT-qPCR. *In situ* PCR and RT-qPCR showed transcripts of the three biosynthesis genes primarily in the larval integument and fat body. Western blots and imprints with whole larvae on nitrocellulose membranes indicate the corresponding enzymes to be localised mainly to the integument. We have further seen that the *Z. filipendulae* imagines are able to *de novo* biosynthesise CNglcs, a feature previously described only in the postman butterfly *Heliconius melpomone*. In the future we will conduct time series analysis of the biosynthesis during the entire life cycle, comparison between female and male larvae and tissue localisation in the imagines.

Selected references:
Jensen, N.B., Zagrobelny, M., Hjernø, K., Olsen, C. E., Houghton-Larsen, J., Borch, J., Møller, B.L. and Bak, S. (2011), Convergent evolution in biosynthesis of cyanogenic defence compounds in plants and insects, *Nature Communications*, **2**(273)
Zagrobelny, M. and Møller, B.L. (2011), Cyanogenic glucosides in the biological warfare between plants and insects: The Burnet moth-Birdsfoot trefoil model system, *Phytochemistry* **72**(13): 1585–1592
Zagrobelny, M., Scheibye-Alsing, K., Jensen, N.B., Møller, B.L., Gorodkin, J. and Bak, S. (2009). 454 pyrosequencing based transcriptome analysis of Zygaena filipendulae with focus on genes involved in biosynthesis of cyanogenic glucosides, *BMC Genomics*, **10**(574)
Takos, A. M., Knudsen, C., Lai, D., Kannangara, R., Mikkelsen, L., Motawia, M.S.
Olsen, C.E., Sato, S., Tabata, S., Jørgensen, K., Møller, B.L. and Rook, F. (2011), Genomic clustering of cyanogenic glucoside biosynthetic genes aids their identification in Lotus japonicus and suggests the repeated evolution of this chemical defence pathway,The Plant Journal, **68**(2): 273–28
Nahrstedt, A. and Davis , R.H. (1983), Occurrence, variation and biosynthesis of the cyanogenic glucosides linamarin and lotaustralin in species of the Heliconiini (Insecta: Lepidoptera), Comparative *Biochemistry and Physiology Part B: Comparative Biochemistry*, **75**(1): 65–73.

Heterologous expression, purification and characterization of barley (*Hordeum vulgare* L.) endoprotease B2

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During germination of the barley grain, cysteine proteases accounts for more than 90 % of the total proteolytic activity. Cysteine proteases are synthesized as pro-enzymes and are activated through reduction of the active site cysteines and by removal of the pro-domain. The complement of cysteine proteases is comprehensive and for detailed studies of the individual components of this complement, a fast and efficient eukaryotic expression platform is highly desirable.

In the current study, the barley key cysteine endoprotease B2 (HvEPB2) was expressed in *Pichia pastoris.* In order to achieve this, a c-terminal truncated version of HvEPB2 (HvEPB2ΔC) was inserted into the *P. pastoris* expression vector pPICZαA. The cosntruct was electrotransformed into *Pichia pastoris* strain KM71H and heterologous protein production was induced with 2% MeOH. A maximum yield were obtained when harvesting the supernatant after 4 days. Purification of the recombinant HvEPB2ΔC (rHvEPB2ΔC) from the supernatant were performed with IMAC by FPLC. The purified fractions were analyzed via SDS-PAGE, western blotting and via activity assaying. Kinetic parameters, effect of protease inhibitors, thermal stability, temperature and pH optimum was obtained by activity assays.

From the IMAC purification a yield of 4.26 mg purified rHvEPB2ΔC per l supernatant was obtained. rHvEPB2ΔC follows first order kinetics (K_m = 8,6 μM) for the chromogenic substrate Z-Phe-Arg-pNA and shows significant inhibition in the activity by the cysteine protease specific inhibitors E64 and leupeptin. The temperature optimum for rHvEPB2ΔC was determined to be 55˚C and the thermal stability T_{50} value to 44˚C. Activity of rHvEPB2ΔC at different pH values revealed a pH optimum at 4.5. Incubation of purified rHvEPB2ΔC with Osborne fractionated barley seed storage proteins for 12 hrs revealed after SDS-PAGE a significant degradation of the storage proteins. The degradation did not occur in the presence of E64.

Characterisation of a glycosyltransferase family 31 mutant with a xylan phenotype

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The Glycosyltransferase family 31 (GT31) has been shown to contain functionally proven galactosyltransferases, and plant GTs classified in GT31 are believed to be involved in the addition of galactose on ArabinoGalactan Proteins (AGPs), a group of highly complex glycoproteins in the cell wall. A specific GT31 GT, containing a lectin domain and a domain of unknown function, is characterised in the present study. Unexpectedly, a knockout mutant of the GT displayed a reduced xylan content in the lower part of the stem. Bioinformatics and modelling data have disclosed new conserved motifs and sub families of these domains. Putative functions are discussed in relation the presented data.

Unraveling genes involved in posttranslational modification of cell wall extensins

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Extensins are a group of ancient cell wall hydroxyproline rich glycoproteins found in some chlorophyte algae (such as Chlamydomonas), where they constitute the main wall building block, as well as in higher plant cell walls, where they constitute a relatively minor component of particular importance to cell wall assembly. Prolines of the proline rich extensin backbone are hydroxylated by 4-prolyl-hydroxylases, yielding hydroxyprolines, which are subsequently arabinosylated by arabinosyltransferases. Recently, the Arabidopsis arabinosyltransferase mutants (*r*educed *r*esidual *a*rabinose) *rra-1-3* and *xeg113* were shown to have aberrant arabinosylations of extensin and a truncated root hair phenotype – a phenotype they shared with three 4-prolyl-hydroxylase mutants (*p4h2*, *p4h5* and *p4h13*) implicated in hydroxylation of prolines in the extensin backbone (Velasquez et al. 2011, Science, 332: 1401-1403). We have now identified an additional arabinosyltransferase, where the corresponding T-DNA mutant displays a clear biochemical phenotype pointing to a particular function also in extensin arabinosylation. Suggested sites of action of the backbone 4-prolyl-hydroxylases and arabinosyltransferases in extensin glycan synthesis are presented.

Retrograde signaling from mitochondria by oxidized peptides

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Plant cells produce reactive oxygen species (ROS) continuously as a by-product of aerobic metabolism. Stress of any kind, abiotic or biotic, generally leads to an increased rate of ROS production. ROS can react with and irreversibly damage cellular components. Each cell compartment contains a number of enzymes designed to limit ROS accumulation, but under stress these enzymes might be overwhelmed and ROS accumulates leading to cell damage (and eventually even cell death) (Møller et al., 2007). To prevent this, as yet unknown signal transduction mechanisms trigger a programme of gene expression that is aimed at maintenance of homeostasis of the ROS concentration through antioxidant activity and metabolic adjustment. Meta-analysis has shown that the transcriptomic response is specific for oxidative stress signals originating from different subcellelular locations (Foyer & Noctor, 2003).

We have proposed that there is a special signaling system in eukaryotic cells in which oxidized peptides deriving from proteolytic breakdown of oxidatively damaged proteins, e.g., in mitochondria, act as specific messengers to regulate source-specific genes. In this way oxidatively modified peptides contribute to retrograde ROS signaling during oxidative stress (Møller & Sweetlove, 2010).

To test the hypothesis, we will tag oxidized peptides released from stressed mitochondria with desthiobiotin-hydrazide, affinity-enrich them using streptavidin and identify them by liquid chromatography mass spectrometry (LC-MS).The most promising peptides will be selected and synthesized. Antibodies raised against the synthetic peptides will be used to pull down the transcription complexes comprising peptide, transcription factor and DNA (Chromatin Immuno precipitation-Sequencing (ChIP-Seq). The protein fraction from each peptide isolate will be analyzed by LC-MS and the DNA tagged and analyzed by pyrosequencing. The proteomic analyses will identify the transcription factors binding the peptides whereas the sequencing will give us 600-800 bases, which will identify the gene regulated by the peptide.

References

Foyer, C. H., & Noctor, G. (2003). Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum, 119*(3), 355-364.
Møller, I. M., Jensen, P. E., & Hansson, A. (2007). Oxidative modifications to cellular components in plants. *Annual Review of Plant Biology, 58*, 459-481.
Møller, I. M., & Sweetlove, L. J. (2010). ROS signalling - specificity is required. *Trends in Plant Science, 15*(7), 370-374.

Rhizobia and the role of its lipopolysaccharide in the establishment of symbiosis

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Rhizobia are symbiotic Gram-negative soil bacteria that fix nitrogen into ammonia after becoming established inside root or stem nodules of legumes. A key step for the correct establishment of the symbiotic process is recognition between the host plant and the microbe at the early stage of the association. The "classic" mechanism of recognition relies on the detection of flavonoids by bacteria. The flavonoids are secreted by the host plant triggering the expression of bacterial *nod* genes, the gene products (Nod factors) are in turn recognized by the host plant. This recognition leads to the early processes of symbiosis such as root hair deformation, cell wall degradation, and infection thread formation. Recently, it has been demonstrated that not all symbiotic processes rely on this mechanism; for example, the nitrogen-fixing symbiosis between *Aeschynomene indica* and B*radyrhizobium* sp. BTAi1 (*Brad*) does not require Nod factors to establish their symbiotic relationship, and thus relies on a still unknown mechanism (Giraud *et al.,* 2007).
As an important role for lipopolysaccharides (LPS) in the recognition between bacteria and host plants already has been established (reviewed by Erbs and Newman, 2012), we decided to investigate if LPS could be a key factor in the early stage of the symbiotic process in the *A. indica* and *Brad* Nod-factor-independent system.We determined the complete structure of *Brad* LPS, and found that both its lipid A and the O-antigen have new and unique structures with no analogues so far indentified in nature. Furthermore we prepared, by mild acid hydrolysis, the lipid A and core oligosaccharides derived from *Brad* LPS, and examined the activity of these (structurally-defined) components for their ability to activate the innate immune system in two model plants, *L. japonicus* and *A. thaliana,* as well as in its symbiotic host plant *A. indica*. *L. japonicus* was chosen as a model legume representing the symbiotic host plant. As a control, we used the flagellin peptide flg22. Our results show that the LPS from the symbiotic *Brad* do not trigger the innate immune response in the different plant families. We also demonstrate that the putative LPS receptor(s) in *A. thaliana* does not recognize *Brad* LPS. On the other hand, flg22 induced the oxidative burst in all of the three plants, indicating an ability of both legume and non-legume plants to respond to elicitation by MAMPs in general.
To our knowledge, this is the first clear and unequivocal example of an LPS molecule to which plants do not react with a defence response. Like other legumes, *A. indica* relies heavily on nitrogen fixation by symbiotic bacteria and could either have evolved an ability to suppress its induced innate immune responses or an ability to lose the receptors for important immune elicitors from symbiotic bacteria. Both of these models could play a role in a successful symbiosis between *Brad* and its host *A. indica*.

References:
Giraud, E., Moulin, L., Vallenet, D., Barbe, V., Cytryn, E. et al. (2007). Legumes symbioses: absence of nod genes in photosynthetic bradyrhizobia.Science 316:1307-1312
Erbs, G. and Newman M-A. (2012).The role of lipopolysaccharide and peptidoglycan, two glycosylated bacterial microbe-associated molecular patterns (MAMPs), in plant innate immunity. Mol. Plant Pathol. 13: 95-104

Investigation of an Rsb-like signaling cascade involved in carbon metabolism in the cyanobacterium *Synechocystis* SP. PCC6803.

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As the world demands for growth of fuel and food productions increase, sustainable production of biomass for biorefineries is of great scientific, technological and societal interest, and there is a pressing need to identify novel molecular targets that can be manipulated to increase biomass production. Cyanobacteria are the main primary producers on Earth and because of their versatility in genetic manipulation and fast growth, cyanobacteria can be ideal organisms for production of green, sustainable biomass for biorefineries. Among the prerequisites for commercial cultivation of microalgae are robust growth of the organism at ambient CO_2 and efficient carbon allocation towards biomass.

In *Synechocystis*, the *icfG* gene cluster is known to be involved in carbon metabolism possibly through a phosphorylation-mediated signaling process[1]. The *icfG* gene (*slr1860*) encodes a protein phosphatase and is indispensable for growth under low CO_2 conditions when glucose is present in the media[2]. The *icfG* gene cluster resembles the Rsb signal transduction cascade in *Bacillus subtilis* which is a phosphorelay-dependent cascade that controls the alternative sigma factor B and enables the bacterium to respond to a range of stress conditions through transcriptional reprogramming[4,5]. A glucose-sensitive phenotype has also been described for a *Synechocystis* mutant of the *pmgA* gene, encoding a putative histidine kinase that is homologous to RsbW in *B. subtilis*[3]. This mutant accumulated higher amount of total sugar as compared to the wild type[3]. We hypothesize that an Rsb-like pathway involving *icfG*, and possibly *pmgA*, plays a role in sensing and transduction of an environmental or physiological cue(s) that govern the central carbon metabolism in a hitherto unknown manner.

We have identified homologs of the known Rsb kinases, phosphatases and switch protein components of the *B. subtilis* cascade in the *Synechosystis* genome and thus created deletion mutant strains in the following genes: *slr2099*, *slr1912*, *slr1983*, *icfG*, and *pmgA*. Results from physiological characterization of these mutants with respect to growth and biomass accumulating capacity in terms of total sugar at low and high CO_2 will be presented.

References:
1. Shi, L, Bischoff, K. M, Kennely, P. J. (1999). The *icfG* gene cluster of *Synechocystis* sp. Strain PCC 6803 encodes an Rsb/Spo-like protein kinase, protein phosphatase, and two phosphoproteins. J. Bact. 181(16): 4761-4767
2. Beuf, L., Bédu., S, Durand., M-C, Joset., F. (1994). A protein involved in co-ordinated regulation of inorganic carbon and glucose metabolism in the facultative photoautotrophic cyanobacterium *Synechocystis* PCC6803. Plant. Mol. Biol. 25: 855-864
3. Sakuragi, Y., Maeda, H., DellaPenna, D., Bryant, DA. (2006). α-Tocopherol plays a role in photosynthesis and macronutrient homeostasis of the cyanobacterium *Synechocystis* sp. PCC 6803 that is independent of its antioxidant function. Plant Physiol. 141: 508-52
4. Mittenhuber (2002). A phylogenomic study of the general stress response sigma factor σ^B of *Bacillus subtilis* and its regulatory proteins. J. Mol. Microbiol. Biotechnol. 4(4): 427-452
5. Völker, U., Maul, B., Hecker, M. (1999). Expression of the σ^B-dependent general stress regulon confers multiple stress resistance in Bacillus subtilis. J Bacteriol. 181(13):3942-3948

Protein-protein interactions in the biosynthesis of arabinogalactan protein

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Arabinogalactan proteins (AGPs) are a group of glycoproteins analogous to animal proteoglycans, which are important throughout the plant development and are thought to be involved in signalling. AGPs are synthesized by post-translational modification of a peptide backbone in the secretory pathway. The arabinogalactan moiety consists of a beta-1,3-galactan backbone substituted at the *O*6 position with complex side chains containing arabinose residues and arabinogalactan synthesis requires at least 11 different glycosyltransferases (GTs) as well as NDP-sugar interconversion enzymes and NDP-sugar transporters. Recently, we identified a GT from CAZy family 31 as a galactosyltransferase involved in arabinogalactan biosynthesis by heterologously expressing the enzyme and conducting *in vitro* enzyme assays. Further detailed biochemical characterization of the enzyme is in progress.

In silico database analysis suggested that there are a number of proteins co-expressed with the galactosyltransferase described above. We are investigating three putative NDP-sugar transporters and three putative GTs for functional relationships to the the galactosyltransferase and roles in AGP biosynthesis. Specifically, we are interested in whether some of the proteins form protein complexes and whether the complex formation reflects to their biochemical function. Using fluorescence resonance energy transfer (FRET), we observed that one transporter apparently forms homo- and heterodimers with two other transporters. Similarly, we observed that some of the GTs interact with each other as well as forming homodimers. Additionally, one transporter seems to interact with a GT. We are currently investigating the biological significance of these protein complexes in the biosynthesis of AGPs by heterologous expression.

Functions of individual cytosolic glutamine synthetase isogenes in nitrogen metabolism of *Arabidopsis*

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Plants have a fundamental dependence on nitrogen (N), usually acquired in the form of nitrate and ammonium. However, a substantial portion of the applied N is lost from the soil and leached into the environment. Several approaches have been followed to improve plant N use efficiency (NUE) by manipulating N uptake and N metabolism. A more thorough understanding of N metabolism is necessary to further explore the genetic strategies of N management in plants. Cytosolic glutamine synthetase (GS1) is central for plant N metabolism. It assimilates ammonium into glutamine following uptake of inorganic N into roots and re-assimilates ammonium released during senescence. In our project, a reverse genetics approach was taken to characterize two individual GS1 isoforms (Gln1;1 and Gln1;2) in *Arabidopsis*. Single and double knockout mutants were used to study how the *Gln1;1* and *Gln1;2* genes contribute to processes determining NUE. ^{15}N was used to monitor the N remobilization in single and double mutants. In addition, the response of single and double mutants to high ammonium supply was studied. The results show that the functions of individual GS1 isogenes are not redundant, as their phenotypes regarding growth, ammonium and total nitrogen content are different. Both *Gln1;1* and *Gln1;2* contribute substantially to the total GS activity in roots. However, how individual GS1 isogenes interact during normal plant growth still needs to be investigated.

Branching-enzyme treatment of starch slurries

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In this study enzymatic modification of starch is investigated as an alternative to the traditional chemical and physical modification techniques used in the food industry. Such processes can be considered as more environmentally friendly and also the products can be used in *clean-label* foods since they, as opposed to traditionally modified starches, do not require an E-number. It is further expected that new and interesting properties of enzymatically treated starches can be discovered.

A thermostable branching enzyme (E.C. 2.4.1.18) from *Rhodothermus obamensis* has been used in various concentrations to modify starch from potato tubers and maize. The water content was kept low during modification (60-70%) to suppress gelatinization during modification which was performed at 70°C and the. Keeping the water content low saves costs for energy in terms of heating and subsequent drying and is of interest for production on industrial scale. However the lack of water may affect the action of the enzyme.

The enzymatically treated samples were evaluated based on apparent amylose content assessed by iodine staining. It was found that a highly branched product was obtained by the enzymatic modification and that the botanical origin of the starch was determining for the molecular composition of the product. To investigate the observed differences between the botanical origins the samples are further being characterized by amylopectin chain-length distribution using Dionex, size exclusion chromatography and NMR.

Fast, In-field Technologies to Diagnose Plant Nutritional Disorders

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Plant and soil analyses are at present the best tools to assess the nutritional status of crops. However, these methods have several drawbacks, such as laborious sampling and slow response times of several weeks before results are obtained. Here we present two novel analytical methods for diagnosing plant nutritional disorders in a fast, cheap and accurate way.

Laser-Induced Breakdown Spectroscopy (LIBS): LIBS is an analytical method to determine the elemental composition of a sample, using the unique spectral lines emitted by each element when samples are heated intensely by a very short laser pulse. A tremendous development during the last decade has facilitated implementation of LIBS in areas as diverse as Mars exploration (Wiens *et al.* 2010) and within plant analysis (Galiová *et al.* 2011). A LIBS measurement takes no more than a second to perform and it requires no sample pre-treatment.

Near Infrared Absorbance (NIR): NIR uses the interaction of light with the molecules of a sample to obtain information about the chemical composition of the sample. Information is extracted and analyzed using multivariate mathematics, known as chemometrics. NIR is a commonly used method for quality assurance in the food and pharmaceutical industries (*e.g.* FOSS 2011), but work is now being done to use it on fresh plant samples (Calderon *et al.* 2009). This enables measurements of the bioactive concentration of several nutrients by detecting efficiency of essential plant functionalities. Each measurement only takes 18 seconds or less and requires no sample pre-treatment.

The key point for both methods is that the actual plant analysis can be moved into the field – thereby reducing the time consumption for sampling and providing instantaneous results. By doing so, both methods allow for unprecedented continuous monitoring of the plant nutritional status during growth, meaning that actions can be taken instantly if fertilization needs to be optimized.

References:
Calderon, F.J., Vigil, M.F., Reeves, J.B., Poss, D.J. (2009). Mid-Infrared and Near-Infrared Calibrations for Nutritional Parameters of Triticale (Triticosecale) and Pea (Pisum sativum). *Journal of Agricultural and Food Chemistry* 57:5136-5142

FOSS Analytical (2011): http://www.foss.dk/industry-solution/meat

Galiová M., Kaiser, J., Novotný K., Hartl, M., Kizek R. and Babula P. (2011), Utilization of Laser-Assisted Analytical Methods for Monitoring of Lead and Nutrition Elements Distribution in Fresh and Dried Capsicum annuum L. Leaves, *Microscopy Research and Technique*

Wiens, R.C., Clegg, S.M., Bender, S., Lanza, N., Barraclough, B., Perew, R., Forni, O., Maurice, S., Chemcam Team, Dyar, M. D. And Newsom, H. (2010), Progress on Calibration of the ChemCam LIBS Instrument for the Mars Science Laboratory (MSL) Rover, *41st Lunar and Planetary Science Conference*

Use of recombinant cereal hydrolytic enzymes for liquid feed application: screening, cloning and *in vitro* expression of triticale, barley, rye, and wheat enzymes for improving phosphorus and nitrogen bioavailability

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The grains from a collection of spring and winter triticale cultivars were biochemically screened for phytase, protease and xylanase activities. These enzymatic activities represented quite a variable and complex traits that were associated to known candidate enzymes using a comparative transcriptomics and genomics approach. For instance, the QTL for high phytase activity has been assigned to the purple acid phosphatases with phytase activity, PAPhy (Dai F., et al. 2011). We have demonstrated by a cisgenic approach that PAPhy isoforms "a" are the main phytases responsible of this trait (Holme I.B., et al. 2011). Other known cereals were chosen for their known transcriptomics and genetic backbone, i.e. barley (Hordeum vulgare cv. Golden promise) and tender wheat (Triticum aestivum cv. Bob white). While as less known at genetic and transcriptomic level, were chosen as control durum wheat (Triticum durum, different cultivars) and rye (Secale cereal, cv. Picasso and Rorik). Triticale cultivars possessing the highest and the lowest protease, xylanase or phytase activity were selected. Since the triticale genome has not been sequenced and the rye genome is only partly known, candidate gene sequences for the enzymes were selected mainly based on comparative transcriptomics. Primers for cloning of candidate genes and for semi-quantitative RT-PCR were designed. The main proteases expressed during grain development were found to belong to the aspartic proteases (phytepsins) and to the carboxypeptidase family. The cysteine endoproteases (gliadains) were also produced at low level during grain filling but their expression level varied among the genotypes. These latter were greater expressed during the early germination stages and were designated as the main hydrolyzing activities for the storage proteins. For xylanases, only type I (with CBM present) was found to be expressed during grain development, and only to a very limited level. Meanwhile xylanases with larger domains, type II, have been found expressed during germination. For the triticale phytases, their expression resembled what has already been seen in wheat and barley, with one set of isoforms (“a” type) expressed mainly during grain development and one set of isoforms (“b” type) expressed mainly during grain germination (Dionisio G. et al., 2011). The phytases and proteases have been expressed in *Pichia pastoris* for biochemical characterization and evaluation in liquid feed. From preliminary results a concerted action makes selected isoforms eligible as candidates for increasing bioavailable Pi, minerals and FAN (Free Amino Nitrogen) in liquid feeds.

References:
Dai F, Qiu L, Ye L, Wu D, Zhou M, Zhang G.(2011) Identification of a phytase gene in barley (Hordeum vulgare L.). PLoS One. Apr 21;6(4):e18829.
Dionisio G, Madsen CK, Holm PB, Welinder KG, Jørgensen M, Stoger E, Arcalis E, Brinch-Pedersen H. (2011) Cloning and characterization of purple acid phosphatase phytases from wheat, barley, maize, and rice. Plant Physiol. 156(3):1087-100.
Holme IB, Dionisio G, Brinch-Pedersen H, Wendt T, Madsen CK, Vincze E, Holm PB.(2011) Cisgenic barley with improved phytase activity.Plant Biotechnol J. Sep 29. doi: 10.1111/j.1467-7652.2011.00660.x. [Epub ahead of print]

Zinc Transport and Deposition in Barley Grain

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More than 25% of the world's population, primarily women and children, are suffering from zinc (Zn) deficiency. Zinc deficiency is prevalent in developing countries where cereals are the staple food. Cereals contain low amount of trace elements, e.g. Zn, but are rich in anti-nutritional compounds such as phytate. To meet the problem, different strategies have been used and among those, transgenic approach seems to be a promising one.

During grain filling, the maternal tissues supports the growing grain through the phloem strand and in the seed the transfer region mediates the transport into the endosperm, the edible part of the grain. The aleurone cells, the surrounding layer of the endosperm, contain a considerable proportion of total seed minerals such as Zn.and is excluded from the flour by milling Therefore, our aim is to direct more minerals into the endosperm via genetic modulation of Zn transporting genes in the grain tissues. In the present study we are using *H. vulgar* as a model plant.

Zinc is an essential micronutrient that is required as a cofactor in over 300 human enzymes but can also be toxic in excess, therefore, a tightly controlled Zn homeostasis is unavoidable. Zinc transporters have recently been identified in barley, functional in different tissues and subcellular compartments of the grain (Tauris et al. 2009).

The HMA2 transporter is member of the Zn transporting family of P1B type heavy metal ATPases and is in the grain, localized to the plasma membrane of transfer cells. HMA2 exports Zn from transfer cells into the apoplastic endosperm cavity, from where Zn again is taken up by modified aleurone cells before entering the endosperm.

MTP1 belongs to CDF (cation diffusion facilitator) family and is in the barley grain localized to the aleurone layer where it is involved in Zn influx into the protein storage vacuoles.
To increase Zn content in the endosperm of barley grain, our strategy covers

1) Overexpression of HvHMA2 in the transfer region and
2) The down regulation of HvMTP1 in the aleurone layer.

Our hypothesis is that overexpression of HvHMA2 gene in the transfer region will increase the loading capacity of zinc into the endosperm. The hypothesis for the down regulation of MTP1 in the aleurone layer is the redirection of zinc from the storage vacuoles

To have transfer cells specific overexpression, JekyllI promoter has been used to promote HvHMA2 gene as Ltp promoter for HvMTP1 to be driven in the aleurone layer.
To identify new candidate genes, laser capture based micro dissected tissues from zinc deficient and sufficient barley grains will be used for RNA sequencing.

References:
Tauris, B., Borg, S., Gregersen, L. P. and Holm, P.B. (2009). A road map for zinc trafficking in the developing barley grain based on laser capture microdissection and gene expression profiling. *J. Exp. Botany*. 4: 1333-1347.
Brinch-Pedersen, H., Borg, S., Tauris, B. and Holm, P.B. (2007). Molecular genetic approaches to increasing mineral availability and vitamin content in cereals. *J. Cereal Sci.* 46: 308-326.

Integrated transcriptomics and proteomics analysis of storage protein composition in developing barley grain to improve nutritional profile

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Barley (*Hordeum vulgare*), is extensively cultivated in Denmark for fodder and malt. Nitrogen (N) is a key plant macronutrient and a major type of fertiliser used to achieve higher yield. A high N dose increases protein content but promotes a very unfavourable class of the barley storage proteins (C-hordeins). Like other cereals, barley utilises only about one-third of the available nitrogen, the rest is leached to groundwater, detrimental to the environment. Additionally, animals do not utilise glutamine and proline, causing further nitrate/ammonia contamination problems. Therefore, solving this problem, between high yield and good grain quality is a challenge. Understanding the molecular and biochemical mechanisms underpinning the effect of N on storage protein composition during grain filling could be a first step to respond to this challenge.

Hordeins form four big gene families; we found different expression patterns among them during grain development (Hansen et al., 2009). Extending this study, we analysed the developmental expression of hordein homologues in grains of field grown barley cv. Barke at N levels of 50 kg/ha, 120kg/ha and 150kg/ha.

Using a combination of advanced biochemistry methods, we could comprehensively describe changes in transcripts (using DNA microarray and qRT-PCR), amino acids (Ultra Performance Liquid Chromatography) and protein abundance (2D-DIGE combined with MS/MS). A gene chip assay and a novel high throughput qRT-PCR system with allele-specific primers, developed in our laboratory, allowed us to separate the expression of the different alleles within hordein gene families. Examining all known hordein homologues, we revealed markedly different temporal profiles in their expression patterns. Furthermore, different N-regimes caused significant differences in both quantity and quality of the storage proteins transcripts. Increasing availability of N caused an increase in total proteins, but the relative share of B and D hordeins decreased, while that of C increased. We characterised the allelic contribution to these changes. Principal Component Analysis of the amino acid (AA) profiles also indicated dissimilarity in individual AA percentages, correlated to hordein content. The individual hordein homologues were quantified by a combination of two-dimensional difference gel electrophoresis (2D-DIGE) and by mass spectroscopy (MS/MS). The abundance values of proteins of interest confirmed the various hordein patterns during grain development and the differential response to environmental stimuli.

This combined transcriptomics and proteomics approach helps to select alleles for breeding that will decrease non-essential and increase essential AA content in barley grain.

Reference:

Hansen,M., Friis, C., Bowra, S., Bach Holm, P., Vincze, E. (2009). A pathway-specific microarray analysis highlights the complex and co-ordinated transcriptional networks of the developing grain of field-grown barley. *Journal of Experimental Botany* 60:153-167

Grain Zn content and protein quality/quantity of barley

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Zn plays a crucial role in biological systems. In silico studies of the human proteome reveal that 10% of proteins (i.e. 2800) potentially bind Zn. A total of 2367 proteins in 181 gene familes were identified as Zn-related in Arabidopsis (Broadley et al. 2007). Atmospheric CO_2 enrichment during 21st century could increase the yields among the majority of plant species (eg. C_3 plants) but there will be a "dilution" of other nutrients like minerals and proteins in the grain. The nutritional short-comings can not be met solely by using higher amounts of nitrogen and mineral fertilizer since it would increase nitrogen and mineral load in the environment. Therefore, alternative approaches are needed to increase grain Zn content like creation of transgenic lines that accumulate more Zn or exploitation of the natural variation in zinc content.

From bioinformatic studies we have identified protein families which potentially might bind more zinc. The expression levels of the selected gene/protein families are currently being assessed by quantitative real time PCR. In addition, we are also working on establishing colorimetric assays and radioactive methods in our laboratory to identify and characterize Zn binding proteins in barley. We have a collection of barley cultivars (<300) with high grain protein content and substantial genetic variation in grain Zn concentration. We isolated proteins showing Zn binding abilities by the Immobilized metal ion affinity chromatography (IMAC) together with dithizone (DTZ) staining and the analysis by mass spectrometry is in progress.

We will use the promising cultivars with higher Zn content to further explore the potential link between protein quality/quantity and zinc content under elevated atmospheric CO_2 levels.

References:

Broadley, M.R., White, P. J., Hammond, J. P., Zelko, I., Lux, A. (2007). Zinc in plants. New Phytologist. 173 (4): 677-702.

Persson, D.P., Hansen, T.H., Laursen, K.H. Schjoerring, J.K. and Husted, S. (2009). Simultaneous iron, zinc, sulphur and phosphorus speciation analysis of barley grain tissues using SEC-ICP-MS and IP-ICP-MS. Metallomics 1: 418-426.

Recent advances in compartmentation and speciation analysis of iron and zinc in the cereal grain

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Iron (Fe) and zinc (Zn) deficiency in humans is a serious problem in major parts of the world, not least in regions where people live on a cereal based diet. Iron and Zn deficiency leads to a number of diseases including anemia, mental retardment, stunted growth, immune dysfunction and various skin diseases. Consequently, there is an urgent need to increase the density and bioavailability of Fe and Zn in the edible parts of cereals. In this presentation we will provide a state-of-the-art overview on the current methods to study compartmentation of Fe and Zn between different tissue types of the cereal grain and present the most advanced methods to study their chemical speciation. This information is essential in order to study and improve the bioavailability of Fe and Zn.

Recent developments in compartmentation analysis using micro X-ray fluorescence Spectroscopy (µXRF), nano-Secondary Ion Mass Spectrometry (nano-SIMS) and LA-ICP-MS (Laser Ablation - Inductively Coupled Mass Spectrometry) have significantly expanded our knowledge on how Fe and Zn are distributed between the key-tissue types of e.g. wheat, rice and barley grains. Moreover, novel information about the chemical binding forms of Fe and Zn are emerging, using a combination of multi-dimensional chromatography coupled to ICP-MS. This has recently shown that Zn predominately is bound to thiol-rich water soluble proteins, whereas Fe is bound to phytate oligomers. In addition, it has been shown that the bioavailability of Fe and Zn in grains can be markedly improved by increasing the biosynthesis of low molecular weight ligands such as nicotianamine (NA). The increased amount of these ligands *in planta* also increased the amount of Fe and Zn in the grain and it was found that the speciation also had changed toward a binding between these small ligands and Fe and Zn.

References

An, G., Lee, S., Persson, D.P., Hansen, T.H., Husted, S., Schjoerring, J.K., Kim, Y.S., Jeon, U.S., Kim, Y.K., Kakei, Y., Masuda, H., Nishizawa, N.K., 2011. Bio-available zinc in rice seeds is increased by activation tagging of nicotianamine synthase. Plant Biotechnology Journal 9, 865-873.
Hansen, T.H., Laursen, K.H., Persson, D.P., Pedas, P., Husted, S., Schjoerring, J.K., 2009. Micro-scaled high-throughput digestion of plant tissue samples for multi-elemental analysis. Plant Methods 5, -.
Husted, S., Persson, D.P., Laursen, K.H., Hansen, T.H., Pedas, P., Schiller, M., Hegelund, J.N., Schjoerring, J.K., 2011. Review: The role of atomic spectrometry in plant science. Journal of Analytical Atomic Spectrometry 26, 52-79.
Lombi, E., Smith, E., Hansen, T.H., Paterson, D., de Jonge, M.D., Howard, D.L., Persson, D.P., Husted, S., Ryan, C., Schjoerring, J.K., 2011. Megapixel imaging of (micro)nutrients in mature barley grains. Journal of Experimental Botany 62, 273-282.
Persson, D.P., Hansen, T.H., Laursen, K.H., Schjoerring, J.K., Husted, S., 2009. Simultaneous iron, zinc, sulfur and phosphorus speciation analysis of barley grain tissues using SEC-ICP-MS and IP-ICP-MS. Metallomics 1, 418-426.

How are plant diseases affected by climate change?

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It is well established that burning of fossil fuels leads to climate change because of the increasing concentration of CO_2 in the atmosphere. This will give us higher temperature and changed precipitation patterns. Furthermore, ozone concentrations will increase near big cities and industrial areas. The effects of climate change on plant diseases have gained lots of attention in the previous years. Many reviews, theoretical predictions and studies, where only one or two climatic factors are changed, have been made. However, we lack knowledge on how plants react on pathogen infection, when they are exposed to several abiotic stresses simultaneously.

Here we present data from experiments conducted in an advanced phytotron with different levels of CO_2, temperature and ozone in combination and as single factor experiments, ranging from ambient (2010) to predicted levels in 2075. We are using barley (*Hordeum vulgare*) as a model crop together with three fungal pathogens: the biotrophic *Blumeris graminis* f.sp. *hordei* causing powdery mildew, the hemibiotrophic *Bipolaris sorokiniana* causing spot blotch disease and the hemibiotrophic and mycotoxin producing *Fusarium graminearum* causing head blight.

The disease development of *B. graminis* and *B. sorokiniana* is differently affected in the altered climatic conditions. For *B. graminis* we found a significant penetration resistance in plants exposed to single factor treatments with either high ozone or temperature compared to ambient conditions. This was in contrast to *B. sorokiniana* where especially temperature was the main increasing factor for disease development – both as visual symptoms and when measuring fungal biomass. However when ozone and temperature were combined with CO_2 in a multifactorial experiment, penetration resistance (*Bgh*) and symptoms and biomass (*B. sorokiniana*) was compromised. Interestingly, for *B. sorokiniana* we found that disease development observed visually as symptoms (both number and size) does not always correlate to the actual amount of fungal biomass in the leaf.

Fusarium head blight was also predominantly most severe in high temperature. Toxin production was not correlated directly to climatic conditions, but instead to the amount of fungus present in the plant.

The cellular background for these observations is investigated using microscopy, non-targeted-metabolomic analysis, gene expression analysis and measurements of photosynthesis in order to explain the differences in the barley - fungal interactions.

Plant-fungal intimacy: the cellular origin and molecular basis of extrahaustorial membrane formation.

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Biotrophic powdery mildews are fully dependent of their host to fulfil their life cycle. During its infection, the pathogen has an intimate interaction with its host. After germination, the fungus forms an appresorium that attaches to the leaf surface. Using a combination of turgor pressure, enzymatic digestion of the cell wall and a specialized structure, the penetration peg, the fungus breaches the cell wall and attempts to enter the plant cell. Successful entry triggers the formation of a specialized intracellular feeding structure, the haustorium. The membrane surrounding the haustorium, the extrahaustorial membrane (EHM), is of plant origin. The molecular mechanisms leading to the formation of the EHM and the subcellular origin of this membrane are unknown. Furthermore, the fungal effectors that force the plant molecular machinery into generating the EHM and allowing the haustorium to be formed still need to be isolated.

The model system used is the interaction between barley (*Hordeum vulgare*) and the powdery mildew fungus, *Blumeria graminis* f.sp. *hordei* (*Bgh*). By particle bombardment, marker constructs or sequences for transient induced gene silencing (TIGS) are expressed in barley epidermal cells (Douchkov *et al*., 2004, Böhlenius *et al*., 2010). Subsequently, pathogen ingress and marker localisation can be followed at the single cell level. We use confocal laser scanning microscopy (CLSM) studies with fluorescent markers for cellular organelles combined with interference with plant vesicular trafficking to find the cellular origin of the EHM. In addition, by expressing selected fungus-derived effector proteins in barley and an unbiased screen using a library of fungal proteins stably expressed in Arabidopsis cells, we attempt to identify fungal components that modulate vesicle trafficking and secretion.

References:

Douchkov D.,Nowara D., Zierhold U.,Schweizer P. (2004) A high-throughput gene-silencing system fort he functional assessment of defense-related genes in barley epidermal cells. Molecular Plant-Microbe Interactions 18 (8):755-761

Böhlenius H., Mørch S.M., Godfrey D., Nielsen M.E., Thordal-Christensen H. (2010) The multivesicular body-localized GTPases ARFA1b/1c is important for callose deposition and ROR2 syntaxin-dependent preinvasive basal defense in barley. Plant Cell 22(11): 3831-3844

Deep profiling of the transcriptome of potato to identify late blight resistance gene networks in potato

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Phytophthorainfestans is an oomycete causing the devastating disease late blight in solanaceae species such as potato**.** Different cultivars of potatoes are displaying differential degrees of resistance towards the disease, in general thought to be mediated by the presence of specific R-genes in the different cultivars.In this study we have analyzed 14 late blight infected potato cultivars, using a sequence tag-based Transcriptome platform.Analysing the abundance of sequence tags derived from the *tef1* gene (EF1α) of *Phytopthorainfestans*, and thus charting disease progression at the molecular level, generally a good correlation between recent but not older phenotypical resistance score data was found probably reflecting changing in the genetic makeup of current *P. infestans* populations to overcome existing resistance genes.. Results of this analysis showed Bintje, Matador, Jutlandia, Spunta, Signum all were low resistant cultivars, where disease expression could be observed 65 hours post infection, Ditta, Karnico, Desiree, Dianella, Kuras, Kardal, 97-HGP-07 were medium resistant cultivars, and infection was observed at 120 hours, and finally Toluca and Sarpomira showed no sign of infection during the period of observation (258 hours).Using Principal Component Analysis and multiple pair wise comparisons a set of candidate genes with gene regulation associated with infection was identified. Following hierarchical clustering of the genes a specific early up-regulation of several genes was identified in the highly resistant Sarpomira variety. Several MAP kinases and a WRKY transcription factor, important candidates for an R-gene mediated signaling pathway was identified; as well as a LRR-NBS-TIR, a typical R-gene of solanaceae, which was partly cloned and sequenced from Sarpomira. We propose this gene is the candidate resistance gene of Sarpomira.

Unraveling plant regulatory networks by studying a NAC transcription factor's role towards biotic and abiotic stress

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Pathogens induce the expression of genes encoding plant transcription factors, by which plants are capable of extensive reprogramming of their transcriptome in a highly dynamic and temporal manner. NAC transcription factors are a large family of regulatory proteins forming such a network. They have key roles gene in cross talk between different pathways, and are involved in processes as diverse as flower development and reproduction, hormones, biotic and abiotic stress responses, light responses and senescence. This study aims not only to provide novel insights to the plant-pathogen interactions between the biotrophic powdery mildew fungus, *Blumeria graminis* f.sp. *hordei* (*Bgh*) and barley, but also to unravel plant regulatory networks through NAC transcription factors' diverse functions in barley.

Jensen *et al.* (2007) have shown that *HvNAC6* is a positive regulator of penetration resistance in barley towards *Bgh* by transient transformation assay, which led us to generate stable *HvNAC6* transformation lines to further investigate its function in biotic and abiotic stress. Interestingly, transgenic barley plants harbouring an *HvNAC6* RNA interference (RNAi) construct displayed lower levels of *HvNAC6* transcripts and were more susceptible to powdery mildew than wild-type plants. Moreover, *HvNAC6* RNAi plants exhibit dosage-dependent ABA hyposensitivity during seedling development, which implies *HvNAC6* modulates ABA-associated phenotypes in seedling developmental processes.

The other perspective of this study is to investigate the transcriptional regulation of the *HvNAC6* gene. *In silico* analysis of this promoter and comparison with the orthologues in Arabidopsis *ATAF1* and rice *OsNAC6* demonstrate the presence of similar putative regulatory elements including W box, GCC box, MYC, MYB and ABRE. Stably transformed barley plants with *HvNAC6* promoter linked to the reporter genes *GUS* and GFP are generated to analyze temporal and spatial gene expression patterns occur on a tissue and organ level during biotic and abiotic stress.

Reference:
Jensen, M. K., J. H. Rung, P. L. Gregersen, T. Gjetting, A. T. Fuglsang, M. Hansen, N. Joehnk, M. F. Lyngkjaer, and D. B. Collinge. (2007). The *HvNAC6* transcription factor: a positive regulator of penetration resistance in barley and Arabidopsis. *Plant Molecular Biology* 65(1):137-150

Hydroxy nitrile glucosides (HNGs) in *Hordeum vulgare* and the importance of HNGs for colonization by powdery mildew

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Barley (*Hordeum vulgare*) is producing five known leucine derived hydroxy nitrile glucosides; Sutherlandin, Osmaronin, Dihydroosmaronin, Epidermin and Epiheterodendrin. The latter is an α-hydroxy nitrile glucoside, hence it is cyanogenic upon cleavage of the β-glucosidic bond. However, no β-glucosidase activity is found in the leaves of barley.

The other HNGs are either γ-HNGs (Osmaronin) or β-HNGs (Sutherlandin, Epidermin and Dihydroosmaronin), which are acyanogenic. Furthermore, an unknown compound is encountered with same m/z as Sutherlandin, but with a completely different retention time. Based on the fragmentation pattern seen in LCMS analysis, it is not a glucosidic compound. The identity of the compound is currently being investigated. The compound is also speculated to be part of a quenching mechanism of the ROS production taking place in the cell upon pathogen attack (Møller, 2010).

The presence of HNGs is restricted to the epidermal layer, where >99% of the HNGs are found (Nielsen *et al*., 2002). Our analyses indicate that this may be subject to a further subdivision, as we encounter HNGs in wax extractions from the barley plants as well. The route of transport for the HNGs will be investigated, as it has been shown previously that the compounds are not synthesized in the epidermal layer.

Even though α-HNGs are generally known as defence compounds, it has been reported that barley powdery mildew (*Blumeria graminis*) prefers plants with high levels of HNGs as host plants. We are exploring the relationship between the HNG-levels and the ability of the fungus to colonise the plant. It is hypothesised that the powdery mildew is using the HNGs as a nitrogen source, as well as a host recognition factor. To look further into this, CYP79 RNAi lines have been generated, and are currently subjected to metabolite profiling.

References:
Møller, B.L. (2010). Functional diversifications of cyanogenic glucosides. Current Opinion in Plant Biology, Vol. 13, No. 3, pp. 337-346.

Nielsen, K.A., Olsen, C.E., Pontoppidan, K., Møller, B.L.(2002). Leucine-derived cyano glucosides in barley. Plant Physiology, vol. 129, pp.1066-1075

Nielsen, K.A., Hrmova, M., Nielsen, J.N., Forslund, K., Ebert, S., Olsen, C.E., Fincher, G.B., Møller, B.L. (2006). Reconstitution of cyanogenesis in barley (*Hordeum vulgare*) and its implications for resistance against the barley powdery mildew fungus. Planta Vol. 223, pp. 1010-1023.

Yeast two-hybrid identification of peptide aptamers for the barley powdery mildew effector motif, YxC

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Biotrophic fungi, such as *Blumeria graminis* f. sp. *hordei*, have developed structures called haustoria, which enable them to penetrate living plant cells. In order to control the host plant, fungi secrete a number of effector proteins. More than 300 barley powdery mildew effectors has been identified, sharing a YxC-motif in the N-terminal of the mature protein. To identify the role of the motif in the plant-fungal interaction, yeast two-hybrid screens of the peptide aptamer libraries were made. Peptide aptamers are artificial proteins that consist of randomized peptide sequences inserted into a structurally stable scaffold protein. Peptide aptamers are a very useful tool in protein-protein interactions assay because of their restricted conformational range, which results in high affinity to the target and increased binding specificity. Selected peptide aptamers are able to inhibit the function of target proteins e.g. by disrupting their interactions with other proteins. To make the yeast two-hybrid screening more efficient, the ‘interaction mating’ has been used, which enables screening of more baits and preys simultaneously. We expect that YxC-motif-binding peptide aptamers will become useful tools in our studies of barley powdery mildew effectors and that they can reveal new ways of plant protection.

Determination of R gene specificity in wheat

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Plants defend themselves against pathogens through a range of resistance genes (R genes), many of which belong to the nucleotide binding (NB) and leucine rich repeat (LRR) class of R genes. It has been found that NB-LRR genes with a mutation in the conserved p-loop domain act as a dominant negative conferring susceptibility when expressed in plants having the equivalent wild type R gene. Based on this discovery, a strategy to match R genes to specific pathogens has been designed in wheat.

The wheat-yellow rust system is used for proof-of-concept. The wheat cultivar 'Bobwhite S-26' transformed with the *Yr10* gene will be resistant to a yellow rust isolate harboring the corresponding *AvrYr10*. Transgenic plants with both wild type and mutated *Yr10* will remain susceptible to the isolate.

We will identify an inventory of wheat R genes via deep transcriptome sequencing. Publicly available DNA sequence information will work as scaffolds for establishing a 'Bobwhite S-26'-specific collection of expressed NB-LRR type R genes. Transgenic 'Bobwhite S-26' lines with mutated forms of R genes will be inoculated with selected avirulent isolates of yellow rust. Susceptibility will indicate that a mutated form of the R gene that confers resistance to a particular isolate is present in the transgenic plant, and in this way the R gene can be identified and exploited in breeding for disease resistance.

Role of the novel Arabidopsis bHLH transcription factor HNY in innate immunity

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A key aspect of plant innate immunity is the recognition of potential pathogens via the perception of pathogen-associated molecular patterns (PAMPs). This is mediated by ligand binding surface-localized transmembrane pattern-recognition receptors. The perception of PAMPs leads to a series of early and late responses, collectively termed PAMP-triggered immunity (PTI), which result in restriction of pathogen growth. Despite recent advances, the signaling events that occur downstream of recognition remain elusive. The basic helix-loop-helix (bHLH) transcription factor HNY was identified in a reverse genetic screen for novel components of plant immune signaling. *HNY* over-expression causes diminished defense responses, suggesting that HNY acts as a key negative regulator of plant innate immunity by transcriptionally regulating direct or indirect targets involved in defense signaling.

A novel cysteine-rich receptor like kinase (*HvCRKl*) functions as a disease susceptibility gene for powdery mildew in barley

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The receptor-like protein kinases constitute a large and diverse group of proteins controlling numerous plant physiological processes, including development, hormone perception and stress responses. The transmembrane-anchored cysteine rich receptor-like protein kinases (CRKs) represent a prominent sub-family of RLKs in Arabidopsis (Wrzaczek *et al.,* 2010) and rice (Shui *et al.,* 2004). Barley genome encompasses several members of the CRK family that are transcriptionally active in response to various biotic and abiotic stresses. *HvCRK1*, a putative barley (*Hordeum vulgare*) *CRK* gene family member, was functionally characterized for its role in barley-powdery mildew interactions. The mature putative protein comprises 645 amino acids, and at the N terminal part includes a putative receptor domain containing two characteristic duf26 domains followed by a transmembrane domain. The C terminal part comprises of a highly conserved, characteristic putative protein kinase domain. A transient increased accumulation of *HvCRK1* transcripts following inoculation of susceptible barley with the biotrophic fungus *Blumeria graminis* f.sp. *hordei* (*Bgh*) was observed. Silencing of *HvCRK1* expression by transient gene silencing led to an enhanced resistance to *Bgh*, but did not affect R-gene mediated resistance. Interestingly, silencing of *HvCRK1* phenocopies the effective penetration resistance found in *mlo* resistant barley plants (Rayapuram *et al.,* 2011) and the possible link between *Hv*CRK1 and MLO is substantiated by the fact that *HvCRK1* induction upon *Bgh* inoculation is *Mlo*-dependent. An *in silico* analysis of the transmembrane revealed a short 17 amino acid domain with a 'AAA' conserved motif; two features characteristic of endoplasmic reticulum (ER) targeted proteins (Szczesna-Skorupa and Kemper, 2000). In agreement with our *in silico* analysis, we demonstrate that *Hv*CRK1 indeed localizes to the ER of barley cells, unlike most of the known CRKs that are plasmamembrane localized. In this study, we provide evidence supporting the claim that a powdery mildew causing fungus (*Bgh*) exploits an ER localized CRK, as a host susceptibility factor in barley by a mechanism which either intercepts/involves MLO.

References:
Rayapuram, C *et al.* (2011). *Molecular plant pathology,*(DOI: 10.1111/J.1364-3703.2011.00736.X)
Shiu S. H., Karlowski W. M., *et al.* (2004). *Plant Cell*, **16**, 1220–1234.
Szczesna-Skorupa, E. and Kemper, B. (2000). *J. Biol. Chem.,* **275**, 19409-19415.
Wrzaczek, M., Brosche *et al.* (2010). *BMC Plant Biol.,* **10**.

Silencing of a barley glutamate receptor-like gene gives resistance against powdery mildew

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Research conducted by the use of the model system of barley (*Hordeum vulgare*) and the powdery mildew fungus (*Blumeria graminis* f.sp. *hordei*, *Bgh*) has led to the discovery of many important components involved in the establishment of plant/pathogen interactions. The first plant glutamate receptor-like proteins (GLRs) to be discovered were in the model plant *Arabidopsis*. Plant GLRs are homologous to ionotropic glutamate receptors (iGluRs) from animals, where they are well-documented receptors in the animal brain, mediating nerve transmitter perception and Ca^{2+}-influx during cell-to-cell signalling. Animal iGluRs and plant GLRs share the same conserved domains important for ligand-binding and pore formation. Most reports on plant GLRs revolve around various roles in the physiology of the plant, whereas reports of GLR function in relation to pathogens are more limited. Here, we present the identification of barley *HvGLR2.1*, which is the first barley GLR to be described. Silencing of this ion-channel mediates resistance towards *Bgh* in single, epidermal cells of wild-type barley. However, this effect was not seen in the *ror2* mutant, suggesting a functional link between GLR2.1 and ROR2.

Two Effectors From *Albugo laibachii* Nc14

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A striking attribute of *Albugo laibachii* (*Al*) is its capacity to suppress host plant defences, conferring susceptibility not only to *Al*, but also to parasites normally resisted by the host (Cooper *et al.*, 2008). The recent completion of the *Al* Nc14 genome enabled identification of candidate effectors, which were screened for contribution to virulence of *Pseudomonas syringae* (*Pst*) DC3000 infection of *Arabidopsis* (Kemen *et al.*, 2011).

Here, we present the characterization of two candidate effectors from different classes of *Al* effectors: a short secreted protein SSP6, and the CHXC type effector CHXC1. CHXC1 is a secreted HECT type E3 ubiquitin ligase, which localizes to the plant nucleus after transient expression in *Nicotiana benthamiana*. We find that CHXC1 confers enhanced virulence of *Pst* DC3000 lux on Nd-0, but not Col-0, when delivered by T3SS. Interestingly, CHXC1 is present in the related species *Albugo candida* 20DD5 (*Ac* 20DD5), suggesting it to be a core effector. In contrast, SSP6 is absent from *Ac* 20DD5 but exists in multiple polymorphic copies in *Al* haplotypes. Transient heterologous expression of SSP6-2c shows that it localizes to the plasma membrane and suppresses FLS2 dependent ROS production. Based on these findings we propose two evolutionary classes of effectors: Fast evolving effectors without conserved functions, and core effectors with conserved functional domains.

References:

Cooper *AJ, Latunde-Dada AO, Woods-Tör A, Lynn J, Lucas JA, Crute IR & Holub EB,* 2008. Basic compatibility of Albugo candida in Arabidopsis thaliana and Brassica juncea causes broad-spectrum suppression of innate immunity. MPMI 21:745-56.

Kemen E, Gardiner A, Schultz-Larsen T, Kemen AC, Balmuth AL, Robert-Seilaniantz A, Bailey K, Holub E, Studholme DJ, Maclean D & Jones JD, 2011. Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of Arabidopsis thaliana. PLoS Biology 9(7):e1001094

Impact of Climate change on emerging plant diseases and their threat to food security

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Climate change is influencing our agro-eco system and the consequences related to plant diseases are expected to include:

- changed crop physiology, growth and quality
- new crops/ cropping systems and new emerging diseases
- changed severity of already established diseases.

Increased temperature and elevated CO_2 and ozone gases are predicted to have a progressively negative effect on yield and biomass production, but possible effects on plant disease are less clear. We are investigating the interaction of the climate change factors temperature, CO_2 and ozone with cereals and their diseases.

Spot blotch caused by the fungus *Bipolaris sorokiniana* is used as model disease. In some areas *B. sorokiniana* has become one of the most devastating pathogens in barley and wheat production. Spot blotch disease outbreaks have increased severely in areas that have become more humid and warmer within the last years due to climate change. After a symptomless early biotrophic stage this hemi-biotrophic fungus kills the mesophyll cells with the help of phytotoxins which leads to necrotic leaf spots. These phytotoxins are demonstrated to be responsible for the development of symptoms on the leaf of infected plants (Berestetskiy, 2007).

We are investigating how environmental conditions are linked to the aggressiveness of the fungus and if production of phytotoxins is directly related with disease severity. Normally symptom development and the number of necrotic lesions is an indicator of disease severity and plants with fewer spots are expected to have mechanisms that restrict fungal growth in the leaf. However, our experiments indicate that there is more fungal biomass in plants when there is no characteristic symptom development which is in contrast to the expected necrotrophic growth strategy of *B. sorokiniana.*

Barley genotypes were grown under different treatments of climate change factors and the leaves were exposed to the toxic compounds that are produced by *B. sorokiniana*. Some genotypes developed the clearest necroses under elevated CO_2, while others were more sensitive under elevated temperature or did not show differences in symptom occurrence at all.

Our results show that symptom development and disease severity are driven by several factors like environmental conditions and the genetic predisposition of the plant. Another important factor might be the aggressiveness of the fungus. The conditions that stimulate disease outbreaks will be studied. Moreover, the phytotoxins and the mechanism behind symptom development will be investigated further to understand the variability in lesion occurrence.

Berestetskiy, A.O. (2008): A review of fungal phytotoxins: from basic studies to practical use. *Applied Biochemistry and Microbiology.* Vol. 44:5, pp. 453-465.

***A*nnotation of *de novo* transcriptome in perennial ryegrass**

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Perennial ryegrass (*Lolium perenne* L.) is an important grass species for both forage and amenity purposes. Denmark is the world's largest exporter of grass seed and therefore it plays an important role in the country's economy. It is envisaged that breeding efforts may be enhanced with the assistance of new breeding technologies such as genomic selection. A major step towards genomic selection will be the availability of a reference genome, and efforts are underway within our group to deliver this. An important step in *de novo* assembly will be defining the gene set, and the availability of transcriptome sequencing data will greatly aid gene prediction and validation, and the development of functional markers for improved ryegrass breeding. In this study we have isolated RNA from the following tissues: leaf, stem, inflorescence, leaf sheath and meristem. The samples were sequenced on an Illumina Genome Analyzer system. The goals of this study are to annotate the gene set of two different genotypes, find SNPs for marker-assisted selection (MAS) and to study tissue and developmental specific patterns of gene expression. With the use of the Trinity *de novo* assembly software (Grabherr et al. 2011), we are also studying transcript diversity by looking at alternative splicing.

References:

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. D. Zeng, Z. H. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman & A. Regev (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology,* 29**,** 644-U130.

Genotyping by Sequencing to resolve allele frequencies in perennial ryegrass breeding populations

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Lolium perenne (perennial ryegrass) breeding programs are primarily based on recurrent selection to develop improved populations. For accurate phenotyping of many target traits in these programs, measurements need to be performed on populations planted in swards, as opposed to single plants. Resolving the allele frequencies within these populations at multiple SNP locations may allow us to associate the allele SNP frequencies with the phenotype. In order to test this approach, a strategy is required to accurately determine allele frequencies in perennial ryegrass populations. We have used a genotyping by sequencing (GBS) approach (Elshire et al., 2011) that involved genome complexity reduction with restriction enzymes, barcoding fragments to allow multiplexing, and pooling samples for sequencing on the Illumina GAII and HiSeq 2000. Different enzymes were tested to generate a range in the extent of genome complexity reduction. We have applied this approach to eight perennial ryegrass cultivars to get an insight into expected SNP harvest and the coverage required to accurately determine allele frequencies. The developed strategies will ultimately be used to characterise genetic variation in a training population being used to develop models for genomic selection.

References

ELSHIRE, R. J., GLAUBITZ, J. C., SUN, Q., POLAND, J. A., KAWAMOTO, K., BUCKLER, E. S. & MITCHELL, S. E. 2011. A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species. *Plos One,* 6.

The Lolium genome zipper – targeted use of comparative grass genomics for ryegrass breeding

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In the past decades, intense research efforts in model grass species has led to the establishment of whole genome sequences that constitute a major resource for genetic and genomic applications. In outbreeding forage and turf grass species such as ryegrasses (*Lolium* spp.), such resources are not available yet. Thus, targeted use of grass genome sequence resources provides a major opportunity to efficiently exploit genomic information for genetic and breeding applications in non-model species.

Here, we present a linear gene order model of the Lolium genome (the Lolium genome zipper) on the basis of conserved synteny to barley (Mayer et al., 2011) and the model grass genome Brachypodium as well as rice and sorghum. A Lolium transcriptome map constructed using SNPs developed from next generation transcriptome sequencing and Illumina GoldenGate genotyping, served as scaffold for chromosomal arrangement of syntenic genes to the model species which were anchored based on a collection of Lolium reference sequences. As a result, 3,747 Lolium genes were unambiguously associated to single Lolium chromosomes. In total, the Lolium genome zipper incorporates 4,139 gene loci. Genome-wide, we were able to anchor 2,760 syntenic genes of Brachypodium, 2,280 of rice, and 2,331 of sorghum, respectively, which were matched by 3,005 Lolium reference sequences as evaluated by stringent best bidirectional hit sequence comparisons.

The transcriptome map, the SNP markers and the Lolium zipper presented here constitute an important tool for the assignment of candidate genes to QTL, for map-based cloning, functional genomics and the genome assembly in ryegrass. Moreover, the Lolium genome zipper as an ordered, information rich scaffold of the ryegrass genome represent a milestone in describing synteny between sequenced model grasses and the most important forage and turf grass species.

References:

Mayer K.F.X., Martis M., Hedley P.E., Šimková H., Liu H., Morris J.A., Steuernagel B., Taudien S., Roessner S., Gundlach H., Kubaláková M., Suchánková P., Murat F., Felder M., Nussbaumer T., Graner A., Salse J., Endo T., Sakai H., Tanaka T., Itoh T., Sato K., Platzer M., Matsumoto T., Scholz U., Doležel J., Waugh R., Stein N. (2011) Unlocking the barley genome by chromosomal and comparative genomics. *The Plant Cell* 23:1249-1263.

Changes in the *Lolium perenne* transcriptome during induction of flowering

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Perennial ryegrass (*Lolium perenne L.*) is the principal turf and forage grass utilized in temperate regions. Due to the high fodder quality provided during its vegetative growth phase, perennial ryegrass is considered the most important forage grass in agriculture. However, the fodder quality decreases significantly during the reproductive phase due to stem and inflorescence production. The controlled inhibition or delay of flowering would result in an extended period of vegetative growth, leading to a significant increase of forage quality. Our goal is to develop and validate functional molecular markers suitable for marker-assisted breeding for delayed flowering time in perennial ryegrass. We use an RNA-Seq approach to investigate the transcriptome of perennial ryegrass during initiation of flower induction in two genotypes with contrasting vernalization requirements. We observed a significant change in the transcriptome related to the different time points during vernalization and long day induction. Clustering analysis revealed groups of genes with patterns of expression that are indicative of them playing a role in both vernalization induction and long day induction. Candidate genes will be selected based on gene expression profiling in both genotypes, Single Nucleotide Polymorphism (SNP) molecular markers will be developed and will be mapped in a population segregating for vernalization response. The candidate genes and the SNP markers will be integrated in the perennial ryegrass physical map.

References:

Amasino, R.M., Michaels S.D. (2010). The Timing of Flowering. *Plant Physiology* 154: 516–520

Distelfeld, A.,Li, C., Dubcovsky J. (2009). Regulation of flowering in temperate cereals. *Current Opinion in Plant Biology* 12:178–184

Greenup, A., W. Peacock, W.J., Dennis E.S., Trevaskis, B. (2009). The molecular biology of seasonal flowering-responses in *Arabidopsis* and the cereals. *Annals of Botany* 103: 1165–1172

Jensen, L.B., Andersen, J.R., Frei, U., Xing, C., Taylor, P.B.,Luberstedt, T.,L. (2005). QTL mapping of vernalization response in perennial ryegrass *(Lolium perenne L.)* reveals co-location with an orthologue of wheat VRN1. *Theoretical and Applied Genetics* 110: 527-536.

Jung, C., Müller, A.E. (2009). Flowering time control and applications in plant breeding. *Trends in Plant Science* 14 /10: 563-573

Andersen, J.L., Jensen, L.B., Asp, T., Lübberstedt, T. (2006). Vernalization response in perennial ryegrass (*Lolium perenne L.*) involves orthologues of diploid wheat (*Triticum monococcum*) VRN1 and rice (*Oryza sativa*) Hd1. *Plant Molecular Biology* 60: 481-494

Evaluating the propensity for targeted mutagenesis using TAL effector nucleases in *in vitro* cultured barley ovules

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Targeted mutagenesis at specific genomic loci is a desirable tool to study gene function in several organisms. These mutations are facilitated by double stranded breaks (DSBs) which can induce incorrect DNA repair, resulting in random insertions or deletions which can cause the disruption of the gene function. Introduction of these DSBs at specific loci has been successfully demonstrated with Zinc Finger Nucleases and Meganucleases. However, synthesizing these custom nucleases requires a significant amount of time, resources and expertise. Recently a new molecule, an engineered TAL effector nuclease (TALEN), showed the ability to cleave genomic DNA in a target specific manner (Christian et al., 2010). The primary advantage of those TALENs is the relative ease with which custom molecules can be assembled (Cermak et al., 2011).

We have successfully implemented a technique for the assembly of custom TALENs (Cermak et al., 2011). Four TALENs were generated using this approach. These nucleases were designed to target a gene cassette consisting of the ubiquitin promoter and the green fluorescence protein. Those TALENs are currently tested for their DNA cleavage specificity *in vivo*, using a yeast-2-hybrid assay.

The next part of the project is to test those TALENs *in planta*, using genetically engineered barley plants that contain the targeted GFP cassette. Plants homozygous for a single copy of the GFP cassette are used as pollinators for non-transgenic barley plants resulting in zygotes heterozygous for the transgene. Thus a TALEN-induced mutation in the GFP cassette may result in the loss of GFP expression. We are currently transforming these *in vitro* cultured ovules with the custom TALENs using the Agrobacterium-mediated ovule transformation method developed by Holme et al. (2006). Regenerated plants from embryos without GFP expression will be selected for further characterizations of potential mutation events.

References:

Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, Voytas DF (2010) Targeting DNA Double-Strand Breaks with TAL Effector Nucleases. Genetics 186 (2):757-761.
Cermak T, Doyle EL, Christian M, Wang L, Zhang Y, Schmidt C, Baller JA, Somia NV, Bogdanove AJ, Voytas DF (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. Nucleic Acids Research. doi: 10.1093/nar/gkr218
Holme I, Brinch-Pedersen H, Lange M, Holm P (2006) Transformation of barley (Hordeum vulgare L.) by Agrobacterium tumefaciens infection of in vitro cultured ovules. Plant Cell Reports 25 (12):1325-1335.

Pre-anthesis high-temperature acclimation alleviates damage to the flag leaf caused by post-anthesis heat stress in wheat.

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The objective of this study was to investigate the effect of pre-anthesis high-temperature acclimation on leaf physiology of winter wheat in response to post-anthesis heat stress. The results showed that both pre- and post-anthesis heat stresses significantly depressed flag leaf photosynthesis and enhanced cell membrane peroxidation, as exemplified by increased $O_2^{\cdot-}$ production rate and reduction in activities of antioxiditave enzymes. However, under post-anthesis heat stress, plants with pre-anthesis high-temperature acclimation (HH) showed much higher photosynthetic rates than those without pre-anthesis high-temperature acclimation (CH). Leaves of HH plants exhibited a higher Chl a/b ratio and lower chlorophyll/carotenoid ratio and superoxide anion radical release rate compared with those of the CH plants. In addition, antioxidant enzyme activities in HH plants were significantly higher than in CH. Coincidently, expressions of photosythesis-responsive gene encoding Rubisco activase B (*RcaB*) and antioxidant enzyme-related genes encoding mitochondrial manganese superoxide dismutase (*Mn-SOD*), chloroplastic Cu/Zn superoxide dismutase (*Cu/Zn-SOD*), catalase (*CAT*) and cytosolic glutathione reductase (*GR*) were all up-regulated under HH, whereas a gene encoding a major chlorophyll a/b-binding protein (*Cab*) was up-regulated by post-anthesis heat stress at 10 DAA, but was down-regulated at 13 DAA. The changes in the expression levels of the HH plants were more pronounced than those for the CH. Collectively, the results indicated that pre-anthesis high-temperature acclimation could effectively alleviate the photosynthetic and oxidative damage caused by post-anthesis heat stress in wheat flag leaves, which was partially attributable to modifications in the expression of the photosythesis-responsive and antioxidant enzymes-related genes.

References:

Adams K.L. (2010) Dandelions ‘remember’ stress: heritable stress-induced methylation patterns
in asexual dandelions. *New Phytologist* 185:867–868.
Burke J. (2001). Identification of genetic diversity and mutations in higher plant acquired thermotolerance. *Physiologia Plantarum* 112:167–170.
Zhang X, Wollenweber B, Jiang D, Liu F, Zhao J. (2008) Water deficits and heat shock effects
on photosynthesis of a transgenic Arabidopsis thaliana constitutively expressing ABP9, a bZIP transcription factor. *Journal of Experimental Botany* 59:839–848.

Improvement of the amino acid composition of cereals using RNAi gene-silencing technology

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The main storage proteins in barley and wheat are the prolamines, accounting for up to ~50% of the total grain protein content. However in a feed context, the amino acid compositions is unbalanced due to abundance of glutamine and proline and low content of essential amino acids, especially lysine in the prolamines. Over the last five decades very large efforts have been devoted to improve the amino acid composition of cereals. In barley large-scale mutagenesis programs have resulted in the identification of a range of primarily recessive mutants that confer a high lysine phenotype (Munck, 1992). However, the high lysine trait is invariably associated with lower yield. Earlier we reported the improvement of storage protein composition of the barley grain by suppression of storage protein called C-hordein (Lange et al. 2007; Hansen et al. 2007).

Our present project comprises the generation of new lines by genetic modification using RNAi gene-silencing technology: we altered the C-hordein level in barley and suppressed its close homologue ω-gliadin in wheat. RNAi constructs of a C-hordein gene was transformed into barley by *Agrobacterium* mediated transformation. The generated transgenic lines were screened by PCR, followed by Southern hybridization and 20 lines were proven positive. Among the lines the insertion patterns were as follows: 55 % of the lines showed single, 15% double and 30% more than 2 insertions. We cloned the wheat ω-gliadin gene from *Triticum aestivum* cv. Bobwhite and made the appropriate RNAi construct. The construct was introduced by particle bombardment into wheat together with a vector carrying the Bialaphos resistance gene for transgenic line selection. The construct was also inserted into barley by *Agrobacterium* mediated transformation. We got transgenic plants from both wheat and barley. From wheat 12 lines was proven transgenic by PCR (Southern hybridization is in progress), while 24 barley lines were proven transgenic by PCR followed by Southern hybridization. The analysis of the nutritional values of the genetically modified barley and wheat lines are in progress.

We conclude that RNAi mediated suppression of C-hordein synthesis in barley and ω-gliadin synthesis in wheat could be a promising approach for improving the nutritional value of barley and wheat as a feed crop while at the same time reducing the environmental nitrogen load.

References:
Munck L. (1992). The case of high-lysine breeding. In "Barley Genetics, Biochemistry, Molecular Biology and Biotechnology" (P.R. Shewry, ed.) CAB International (1992) 573 -601
Hansen, M., Lange, M., Friis, C., Dionisio, G., Holm, P.B., Vincze, E. (2007). Antisense-mediated suppression of C-hordein biosynthesis in the barley grain results in correlated changes in the transcriptome, protein profile, and amino acid composition. Journal of Experimental Botany 58:3987-3995
Lange, M., Vincze, E., Wieser, H., Schjoerring, J. and Holm, P.B. (2007). Suppression of C-hordein synthesis in barley by anitsense constructs results in a more balanced amino acid composition. J. Agr. Food Chem. 55: 6074-6081

Redox regulation of transferases involved in starch biosynthesis in *Arabidopsis thaliana*

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Starch is an abundant storage carbohydrate in plants, and essential for human nutrition. The properties of starch are also exploited in industrial applications such as paper and bioethanol production. Starch consists of two glucose polymers; amylose, a linear chain of residues connected by α-1.4-glucosidic bonds, and amylopectin a highly branched polymer, residues are linked with both α-1.4-glucosidic bonds and α-1.6-glucosidic bonds (branch points). Starch is present in leaves, stem, tubers and grains of the plant. Multiple enzymes are involved in the synthesis of cereal starch, starch synthases, branching and debranching enzymes among others. Due to the complex synthesis of starch grains, many important structural and catalytic functions of the enzymes involved in the grain synthesis, including the redox state of the cell, remain unknown. Redox regulated enzymes implicated in starch synthesis and degradation have been identified *in vitro* in *Arabidopsis thaliana.* These are ADPglucose pyrophosphorylase, beta-amylase (BAM1) and starch phosphorylator GWD1 among others. With the use of an activity screen based on manipulation of redox potentials in *A. thaliana* extracts, several enzymes including, starch synthase I (SSI), branching enzyme II (BEII), and starch synthase III (SSIII), where shown to be dependent on redox potential. The aim of the current study is to clone and express *A. thaliana* SSI, SSIII and BEII in *Escherichia coli*. The dependence on redox-potential of each individual enzyme will be determined in addition to kinetic characterisation.

Leaf senescence regulation by NAC transcription factors in barley

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Increasing plant productivity is vital to meet the growing world-wide demand for primary plant products for food, feed and fuel. New crop plant varieties with both enhanced and sustainable productivity are urgently needed. One approach to achieve this is to focus on leaf lifespan as a major determinant of plant productivity in order to develop new breeding strategies for prolonging leaf photosynthesis and delaying senescence processes.

NAC transcription factors are known to be involved in regulation of various developmental processes including important agronomic traits such as senescence, nutrient remobilisation, and grain protein content (Uauy *et al*., 2006; Gregersen *et al*., 2008). In cereal crops a large number of genes from the plant-specific NAC transcription factor family have been shown in expression studies to be associated with senescence processes. With the aim of identifying transcriptional regulators of leaf senescence in barley (*Hordeum vulgare* L.), we characterised 48 members of the barley NAC gene family (Christiansen *et al*. 2011). Based primarily on the transcript levels of these 48 *HvNAC*s, several genes stood out as good candidates for senescence regulators. Two genes, *HvNAC005* and *HvNAC013*, were selected for further in-depth characterisation.

These genes represent two distinct NAC subfamilies and display different induction patterns during the course of flag leaf senescence. Promoter analyses have revealed putative abscisic acid (ABA) responsive cis-regulatory elements in the promoter of *HvNAC005*. We confirmed by qRT-PCR that *HvNAC005* is indeed induced by ABA. Interestingly, the *HvNAC013* promoter contained putative NAC binding sites suggesting that *HvNAC013* could either be auto-regulated or part of a NAC gene cascade. Several lines of transgenic plants have been constructed for each of the two genes, including overexpression, knockdown and promoter:GUS lines. The analyses of these plants are still ongoing; however, there are indications that overexpression of *HvNAC013* results in a delayed senescence phenotype. Thus, *HvNAC013* may be a negative regulator of senescence.

Gregersen, P.L., Holm, P.B., and Krupinska, K. (2008) Leaf senescence and nutrient remobilisation in barley and wheat. *Plant Biology* 10:37-49.

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., and Dubcovsky, J. (2006) A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314:1298-1301.

Christiansen, M.W., Holm, P.B., Gregersen, P.L. (2011) Characterization of barley (*Hordeum vulgare* L.) NAC transcription factors suggests conserved functions compared to both monocots and dicots. *BMC Research Notes*, 4:302.

Wheat for Celiac disease patients

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Gliadins constitute a significant part of wheat gluten and are thus important for baking quality of wheat. However they have a nutritionally unbalanced amino acid profile and are reported to be responsible for a number of adverse reactions to wheat including celiac disease.

Although both α/β, γ, and ω-gliadins have T-cell stimulatory properties, the main problem seems to be caused by a specific 33-mer peptide derived from the α/β-gliadins. Content of alpha-gliadins is controlled by the *Gli-A2*, *Gli-B2* and *Gli-D2* loci located on the short arm of wheat group 6 chromosomes. Of these only the *Gli-D2* locus seems to encode proteins containing the 33-mer peptide described above and there are indications that relative expression from the three loci may vary between wheat lines.

We have a collection of wheat accessions that has been claimed to be either deficient in these epitopes or reduced in the content of alpha-gliadin in general. Three different antibodies, G12, HYB 314-01 and HYB 314-02, all of which are specific for different parts of the 33mer epitope, has been used to test some of these wheat accessions using Western blots. Further screening will be carried out using the most efficient antibody, G12. In addition we have started to screen the wheat accessions using A-PAGE, which is the classical method for classifying and scoring the different wheat storage proteins, including alpha-gliadins.

Segregating populations are available for some of these varieties / accessions, which eventually may support the development of a variety without these proteins or a significantly reduced content. This may be of interest since the only effective treatment, for celiac disease patients, is strict avoidance of gliadins in the diet.

GENOME WIDE ASSOCIATION STUDY FOR CONVERSION OF BARLEY STRAW INTO SECOND GENERATION BIOFUEL

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Inherent recalcitrance of plant cell wall structures to be accessible by hydrolytic enzymes is a key factor for second generation bioethanol production. P-coumaric and ferulic acid are major p-hydroxycinnamic acids in plant cell-wall. They are linked to polysaccharides and to lignin respectively by ester and ether bonds. They are considered negatively correlated to glucose released after enzymatic hydrolysis (Culhaoglu et al., 2011).

In this study a collection of 115 old and modern winter barley (*Hordeum vulgare L.*) varieties was grown in field trials in the north of Italy for 2 years as part of the ERA-PG EXBARDIV project. At the maturity stage plants were harvested and straw collected. Lines were genotyped with 9000 SNPs using the high throughput Illumina iSelect assay (BOPA 3, 4), a novel and highly flexible array technology capable of whole genome scanning for SNP polymorphism.

To characterize lignocellulosic biomass ferulic and p-coumaric acid content as well as sugars released after enzymatic hydrolysis are studied (Gomez et al., 2010). Classic wet chemistry for biomass analysis is time consuming and considered the bottleneck for GWAS where many samples has to be considered. To speed up the phenotypization process we applied modern partial least square (PLS) techniques to create provisional models for biomass composition using near infrared (NIR) spectroscopy and ad hoc software (Naik et al., 2010). Association study allowed identifying QTLs involved in P-coumaric and ferulic acid cell wall content and monosaccharides yield. Synteny between Barley and Rice, Sorghum and Brachypodium Distachyon was explored to identify candidate genes.

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References:
Culhaoglu T, Zheng D, Mechin V, Baumberger S. (2011). Adaptation of the Carrez procedure for the purification of ferulic and p-coumaric acids released from lignocellulosic biomass prior to LC/MS analysis. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences* 879: 3017-3022

Gomez LD, Whitehead C, Barakate A, Halpin C, McQueen-Mason SJ. (2010). Automated saccharification assay for determination of digestibility in plant materials. *Biotechnology for biofuels* 3

Naik S, Goud VV, Rout PK, Jacobson K, Dalai AK. (2010). Characterization of Canadian biomass for alternative renewable biofuel. *Renewable Energy* 35: 1624-1631

GENETIC MAPPING OF PA MUTATIONS IN PENTIUM MATERIAL

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Phytic acid (PA) is the major phosphorous storage compound in plant seed and can account for up to 80% of total phosphorous in seed. It has long been considered as an anti-nutrient because of its strong ability to complex metal ions, especially Fe, Zn and Ca. This can result in a deficit in the absorption of some dietary minerals in populations where cereals are the main staple. In monogastric animals, phosphate must be supplemented in their feeds or phytase must be added to the feed as a pre-treatment because the phosphate bound in PA cannot be digested. This could lead to eutrophication with phosphorous leaching into water bodies. One possible solution to this is to identify germplam with a low content of PA.

In a previous screen for high content of inorganic P (Pi) in a M2 population of EMS treated Pentium seeds we have identified a number of potential mutants with altered content of PA and Pi. These mutant plants have been crossed with the wheat cultivar Hereford to develop segregating F2 populations. One of these populations was selected for further studies since it was found to contain a missense mutation in the A genome copy of the Ta MIPS01 gene encoding *myo*-inositol-3-phosphate synthase (MIPS01) which catalyses the first committed step in the biosynthesis of PA.

Initial results indicate that there is a significant segregation for PA content in the selected F2 population. In addition there seems to be correlation between genotype of the MIPS01 mutation and content of PA. These results however need to be confirmed through phenotypic evaluation (PA and Pi) and genotyping of a larger number of F2 plants from the population.

Development of high throughput qRT-PCR system to study genetic variation in the temporal expression of barley storage protein genes

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Hordeins, the main storage proteins of barley (*Hordeum vulgare*) grain, are mostly responsible for unfavourable amino acid composition addressed for food and feed applications. Hordeins consist of gene families and we reported in our previous DNA microarray study that the different members of these families are expressed with different temporal specificities (Hansen et al., 2009). Analysis of temporal gene expression combined with genetic variation in big gene families with high homology among the alleles is very challenging. We created a high throughput qRT-PCR system with specific qPCR primers to qualitatively and quantitatively characterise the allelic variation of the expression of the different genes of the hordein families.

All the known hordein-coding sequences (172) were collected from commonly available databases (NCBI, TIGR) and classified into groups and subgroups (B1, B3, C, D and γ). Furthermore we cloned new alleles of hordein genes (20) from different developmental stages of six Nordic cultivars with high protein content and cv. Golden Promise and those new sequences were classified into the above mentioned groups and included in the analysis. We designed 26 B-, 11 C-, 6 D-and 2 γ- specific primers with Primique and AlleleID softwares and manually. These primers are covering DNA coding region and in two cases also 3'-UTR.

We analysed developing grains of spring barley cultivars. Six Nordic cultivars with high protein content (cv. Netto, Kontiki, Fairytale, PR3440, IC364, PR3528) and Golden Promise as a control were grown in a greenhouse. Material harvested 10, 20, 30 and 40 days after pollination (DAP) was used in the creation of pools of cDNAs. We performed qRT-PCR first on the control cultivar Golden Promise followed by the gene expression analysis of the collected material from the high protein cultivars. The analysis of obtained DNA dissociation curves provided information about homologue-specificity of the designed primers. According to the standard curve prepared for actin, the concentration of cDNA in all samples was evaluated and amount of individual transcripts was measured.

We obtained big differences in quantity and quality of storage proteins transcripts in consecutive developmental stages and among the studied cultivars grown under the same conditions. We confirmed that individual alleles are expressed in certain cultivars but not in the others. The results imply that our throughput qRT-PCR system is sensitive enough to screen a big number of different barley accessions for natural barley storage proteins variation. It can be used to check the temporal fluctuations in hordein expressions or to find differences in their response to environmental stimuli.

Reference:
Hansen,M., Friis, C., Bowra, S., Bach Holm, P., Vincze, E. (2009). A pathway-specific microarray analysis highlights the complex and co-ordinated transcriptional networks of the developing grain of field-grown barley. *Journal of Experimental Botany* 60:153-167

Improved cereal seeds through investigation of key enzymes in the phytic acid biosynthesis

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Phytic acid (PA) is a naturally occurring polyphosphorylated carbohydrate and has for a long time been known as a storage compound for phosphorus in seeds. The full biosynthesis and accumulation of PA in seeds involves many interacting activities and control points in the biosynthetic pathway, translocation, site of accumulation and storage.

Previous research shows that a barley 5/6-kinase, HvIpk seem pivotal to phytic acid synthesis (Josefsen et al., 2007). New, yet unpublished results of a genome wide association study (GWAS) on a collection of barley cultivars have consolidated the importance of the 5/6-kinase as a gene of high importance for the phytic acid level in the seeds.

The initial study of HvIpk revealed different activities (kinase, phosphatase, isomerase and 3-position specific kinase activity) of the 5/6-kinase, and these activities will be substantiated using different substrates either alone or in a challenge assay to see which substrate is preferred under the experimental conditions. It has been claimed that a single kinase was responsible for all phosphorylation steps (Chakrabarti and Biswas, 1981) which found further support from an *in vitro* assay using recombinant HvIpk (Josefsen et al., 2007).

The investigations of the substrate dependence, structure and function of the HvIpk family has been initiated by cloning a codon optimized version of HvIpk and expression as a His-tagged protein in *E. coli.* The purified enzyme will be used for detailed kinetic studies of HvIpk using HPIC (high-pressure ion-chromatography) with post-column detection, which allows a concomitant detection and quantification of both product and substrate over time.

References:

Chakrabarti, S., Biswas, B.B. (1981). Two Forms of Phosphoinositol Kinase from Germinating Mung Bean Seeds. *Phytochemistry* 20: 1815-1817

Josefsen, L., Bohn, L., Sørensen, M.B., Rasmussen, S.K. (2007). Characterization of a multifunctional inositol phosphate kinase from rice and barley belonging to the ATP-grasp superfamily. *Gene* 397: 114-125

Genome-wide association scan for genes controlling resistant starch in barley grains

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Resistant starch (RS) is “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals”, and it is favorable for the dietary management of metabolic disorders such as diabetes and hyperlipidemia, and for prevention of colonic diseases. The increased consumption of resistant starch is associated with improved cardio-vascular and gut health. Enhancing resistant starch content in cereal crops such as wheat and rice, the staple foods for humans, showed to be beneficial to health. Through induce mutation, high resistant starch rice (Shu et al.,2009) and wheat (Jia et al., 2007), respectively, have been developed and characterized. Consumption of high resistant starch rice and wheat food can stablelize the postprandial blood glucose and shows to be beneficial to the type II diabetes. Currently, a similar screening method is extended into barley and genome wide association studies on 240 spring barley varieties have been done to obtain the major contributors to the high resistant starch in cereals. The barley collection was genotyped with 9000 iSelect SNP markers in the ERA-PG EXBARDIV network.

References:
Jia L., Zhang Z., Shu X., Li C., Wu D., 2007. Starch properties and structure of a wheat mutant high in resistant starch. The Open Agric J. 1, 5-10.
Shu, X., Jia, L., Ye, H., Li, C., Wu, D., 2009. Slow Digestion Properties of Rice Different in Resistant Starch. J. Agric. Food Chem. 57 (16), 7552-7559.

The genetics of high mature grain phytase activity in Triticeae cereals

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High **m**ature **g**rain **p**hytase **a**ctivity (MGPA) in cereals benefits the sustainability of animal intensive agriculture and minimizes the need for phosphate supplements. Furthermore it may help counter some forms of human malnutrition such as zinc deficiency which affects millions.

Cereal species show great variation in MGPA from <100 FTU/kg for rice, maize and oat, to ~5000 FTU/kg for rye. All cereals with more than 100 FTU/kg belong to the Triticeae. Cultivars of the same species also show variation. In wheat we have found a 5-fold variation, in rye two fold but so far we have found little variation in barley. Understanding the molecular basis for this variation would be valuable for the effort to increase MGPA by breeding or cisgenic approaches.

It can be demonstrated biochemically that the Purple Acid Phosphatase Phytases (PAPhy's) are responsible for the high MGPA of the Triticeae. The PAPhy's are highly conserved at the mature protein level and variation in specific activity of enzymes characterized thus far is small and do not correlate with the variation of the phytase activity in grains when different species are compared.

Hence we propose that the variation in phytase activity between species and cultivars are mainly attributable to gene regulation. To investigate this hypothesis we have used genomic library screening, PCR and IPCR to isolate phytase genes from wheat, barley, rye and wild / relict relatives. The promoters were analyzed for known regulatory elements and compared. We have demonstrated that the PAPhy genes are regulated by two distinctly different types of promoters that are active primarily during grain filling and germination, respectively. Cross reference with the sequenced genomes of rice, maize and sorghum showed that the *PAPhy_a* gene which is active during grain filling is unique to the Triticeae. We can therefore ascribe the high MGPA of the *Triticeae* to this one gene. Furthermore, we have identified variants of the *PAPhy_a* gene that are either inactive or correlates with higher MGPA.

Marker assisted breeding and mass selection of wheat composite cross populations

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Utilizing diverse populations instead of single line varieties is expected to lead to a number of advantages in cereal production. These include reduced epidemics of plant diseases, improved weed competition and better exploitation of soil nutrients, resulting in improved yield stability. However, a number of challenges must be met before diverse wheat populations can be introduced into commercial wheat production: one of these is the development of breeding technologies based on mass selection which enable breeders and farmers to improve specific traits in populations and maintain diversity at the same time.

BIOBREED is a project started in Denmark in 2011 to meet these challenges for wheat population breeding. The project is focusing on the development of tools and methods for mass selection of traits relevant for organic and low input production, as it is expected that the highest benefits by utilizing diverse populations can be achieved there. These tools and methods include the development of genetic markers for common bunt (Tilletia caries) resistance and for traits affecting baking quality, such as gluten content and seed hardiness, as well as for the content of nutritive components like anthocyanin and phytate. The development of a composite cross population both with and without common bunt stress will be observed by means of molecular markers and disease readings.

The project is screening a selection of 300 wheat varieties for resistance to common bunt in the framework of an association analysis based on field data and DArT marker data. A specific study in a doubled haploid population from the cross between PI 554099 (carrying the common bunt resistance gene Bt 9) and the susceptible variety Cortez (Wiersum, Netherlands) segregating for the common bunt resistance gene Bt-9 aims at the localization of this gene.

A number of 218 crosses have been made between 30 varieties with a moderate to high degree of bunt resistance. These crosses are now in F_3 (169 crosses) and F_4 generation (49 crosses). The F_4 generations are grown as a bulk population both with and without bunt infection. The diversity of the composite cross population will be assessed by molecular markers, and changes in population structure when growing the populations with and without common bunt infection will be followed using these markers. Head rows of the crosses will also be grown separately, and so far 20 heads of 44 of the F_4 generation have been grown with bunt infection. The distribution of bunt resistance between the head rows will give some hints to the underlying genes determining common bunt resistance.

The seed of the populations will be sorted prior to sowing on a gravity separator and by single seed separation based on near infrared transmission (NIT) in order to remove lines with inferior quality traits like low seed hardiness and low gluten content. The genetic markers developed in the association analysis will be applied to the composite cross population.

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Detoxification of NO_x pollution by plants

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NO_x is a primary pollutant of the atmosphere causing: smog, ozone formation and acid rain. This has a negative effect on environment and health, and in urban areas with high NO_x pollution the incidence of respiratory and cardiovascular diseases and mortality is increased due to both the direct effects of NO_x and the effects of derived compounds such as ozone. The major anthropogenic sources for environmental NO_x are industry and transportation and there are several international political programs aiming to reduce NO_x emissions. In science and technology, there is a focus on developing technologies for the removal of NO_x. We here present data demonstrating for the first time, that plants are able to effectively remove NO_x from the surrounding atmosphere, suggesting a new method for NO removal in which plants can be used locally for NO_x removal in areas with health threatening NO_x concentration. We show that the efficiency of NO_x removal varies among plant species, and discuss how measurements of NO_x removal in different setup of plants can be used to design what plants can be used, and how they can be arranged to get optimal NO_x removal. We also demonstrate that the cellular mechanism for NO_x removal is based on the ability of plant hemoglobin to oxidize NO to NO_3^- by measuring NO removal in transgenic plants with over-expression or silencing of plant hemoglobin gene expression.

The composition and structure of photosystem I in the moss *Physcomitrella patens*

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Research on the structure and function of Photosystem I (PSI) so far has mainly focused on cyanobacteria, algae (diatoms, green and red algae) and higher plants. Only recently Bryophytes (mosses, liverworts and hornworts) which diverged from the ancestor of seed plants more than 400 million years ago (Falkowski, 2006), came into focus since they represent the transition from aquatic to terrestrial life. Analyzing the structure and function of the photosynthetic machinery in moss can provide valuable insights into the evolution of the system during the adaptation to a terrestrial environment.

We isolated PSI with its light-harvesting complex (LHCI) from the moss *Physcomitrella patens* and characterized its structure, polypeptide composition and light-harvesting function using electron microscopy, mass spectrometry, biochemical- and physiological methods.

The *P. patens* PSI shares the overall structure known from higher plants with two functional moieties: the PSI core and at its PsaF/PsaJ site the half moon-shaped LHCI (Ben-Shem et al, 2003). While sharing this higher order structure with vascular plants it became evident that *P. patens* possesses a strikingly high number of isoforms for the different PSI-core subunits as well as PSI associated light-harvesting proteins. We could demonstrate that all those different subunit isoforms are expressed on protein level and are incorporated into functional PSI-LHCI complexes. This variability of different PSI subunits might reflect *P. patens* remarkable resistance against various abiotic stresses such as salt, drought, osmotic stress and UV.

References:

Ben-Shem A, Frolow F, Nelson N (2003) Crystal structure of plant photosystem I. *Nature* **426:** 630-635
Falkowski PG (2006) Evolution. Tracing oxygen's imprint on earth's metabolic evolution. *Science* **311:** 1724-1725

Targeted and untargeted approaches to identify protein-protein interactions

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Physical associations between proteins, so-called protein-protein interactions, are an essential aspect of all biological processes. There is an increasing awareness that we have to think in terms of the dynamic interactome as opposed to the classical one-protein-at-a-time approach. A crucial prerequisite for studying this dynamic interactome is to identify the proteins involved and their physical associations. We work on protein-protein interactions within transport, regulation and biosynthesis of secondary metabolites using glucosinolates as a model compounds. Here, we present our recently established protein-protein interaction platform which is founded on a cytosolic split-ubiquitin-based yeast two hybrid system (CytoY2H) optimized for transcriptionally active bait proteins (Möckli et al., 2007).

We have screened our normalized cDNA library from mixed tissues of *Arabidopsis thaliana* to identify interacting partners in regulons centered around a key regulator of glucosinolate biosynthesis. As a complementary untargeted approach to isolate and indentify regulatory protein complexes *in vivo*, we are currently conducting tandem affinity purification-mass spectrometry experiments. By contrast, we chose a targeted CytoY2H approach to provide evidence for the proposed metabolon driving the core structure biosynthesis of glucosinolates. We have generated a “targeted prey library” consisting of more than 80 genes involved in or associated with glucosinolate biosynthesis which enable us to test any given bait for all possible binary interactions amongst all known players within a week using CytoY2H.

References:
(2007) Möckli, N., Deplazes, A., Hassa, P.A., Zhang, Z., Peter, M., Hottiger, M.O., Stagljar, I., and Auerbach, D. Yeast split-ubiquitin-based cytosolic screening system to detect interactions between transcriptionally active proteins, *BioTechniques* 42(6):725-730

EXPLORING XYLANOLYTIC AND PROTEINACEOUS XYLANASE INHIBITOR ACTIVITIES IN DIFFERENT BARLEY CULTIVARS

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Xylanolytic enzymes (e.g. xylanases, xylosidases, arabinofuranosidases, etc), widely produced by fungi, actinomycetes and some bacteria, have many biotechnological applications including the improvement of animal feed, the quality of baked products and bioconversion of lignocelluloses for bioethanol production [1,2]. Xylanases (EC 3.2.1.8) hydrolyze internal β-1,4-glycosidic linkages between xylose units in the arabinoxylan backbone of cell walls. Cereals like barley produce xylanases for remodeling of the cell walls during seed development and germination [3]. In addition, cereals also contain microbial xylanases produced by microbes residing on surface/outer kernel layers of the grains. However, microbial xylanase activity/efficiency is often significantly inhibited by the presence of proteinaceous xylanase inhibitors (XIs). Hitherto, three structurally distinct classes of XI have been identified i.e. *Triticum aestivum* xylanase inhibitor (TAXI), xylanase inhibitor protein (XIP), and thaumatin-like xylanase inhibitor (TLXI), respectively [4].
In the current study, the variability of xylanase (plant vs. microbial) and xylanase inhibition activities in different barley cultivars was investigated. This was done with application of different techniques including activity assays, zymogram analysis and proteomics analysis (2DE and MALDI-TOF MS, MS/MS) coupled with immunoblotting. Activity measurements suggest that there is considerable inter-cultivar variability in the level of both microbial and endogenous xylanase activities, as well as at xylanase inhibitor levels. Ongoing experiments will reveal whether the observed trends are a function of genetic, environmental factors and/or their interaction. 2D-immunoblots enabled detection of the occurrence, variability and different isoforms of the different XIs. Moreover, it is also of interest to unravel the indigenous microbial community and the associated proteins present on the surface/outer kernel layers of barley. This was carried out with application of 2DE and MS, together with microscopic evaluation and metabolite profiling of fungi isolated from grain surfaces. Preliminary data reveals presence of different field fungi on different cultivars.

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References

1. Q. K. Beg, M. Kapoor, L. Mahajan, G. S. Hoondal. *Appl. Microbiol. Biotechnol.*, 56, 326 (2001).
2. A. Moure, P. Gullón, H. Domínguez, J.C. Parajó. *Process Biochem.,* 41, 1913 (2006).
3. Bewley, J.D. (1997). *The Plant Cell, 9:* 1055-1066.
4. Dornez, E., Croes, E., Gebruers, K., De Coninck, B., Cammue, B.P.A., Delcour, J.A, Courtin, C.M. (2010). *Critical Reviews in Plant Science, 29*, 244-264.

Gene expression response to drought stress in potato plants

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Potato is a space efficient crop with more than twice the energy yield per area unit compared to cereals and is therefore an important crop to consider when trying to meet the increasing demand for food and renewable precursors for chemicals, pharmaceuticals and energy production in the future. According to existing climate models, the global mean temperature is predicted to increase and longer periods without rain is predicted in the season of growth in most of potato producing areas in the future. All increasing the risk of drought leading to decreased crop yield.

In this study two separate dehydration-experiments of potato plants were studied using tag-based gene expression profiling on the potato leaves. In the first experiment, the transcriptional response to short term drought of 14 field-grown potato cultivars (97-HGP-01, Bintje, Desirée, Dianella, Ditta, Jutlandia, Kardal, Karnico, Kuras, Matador, Toluca, Sarpo Mira, Signum and Spunta) was monitored during a drought period of 12 days. Four of these cultivars (Kuras, Desirée, Sarpo Mira and Jutlandia) were chosen for a second and faster progressing dehydration-experiment under more controlled conditions in pots in a greenhouse.

The gene expression data was analysed using Partial Least Squares Regression, Principal Component Analyses and hierarchical clustering. The analysis allowed the drought response of potato to be dissected into different phases and interestingly gene expression changes responses prior to the emergence of markers of root ABA, which is the classical marker for drought induced gene expression changes, were observed.

Molecular Dissection of Lipid Flippases: Towards Synthetic Biology

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Lipid flippases regulate the lipid arrangement across cellular membranes by pumping lipids from one side of the membrane to the other. This creates local changes in membrane curvature and thereby lipid flippases serve important roles in vesicular traffic. Elucidating the molecular function and dynamics of lipid flippases is an important step towards the development of artificial biological systems with new tailored characteristics, for example, for personalized medicine. We have established a purification procedure for a plant lipid flippase, ALA2, and its partner protein, ALIS5. Methods for the reconstitution of the lipid flippase complex into giant unilamellar vesicles have been established. The size of giant vesicles makes it possible to study active pumps live in a light microscope by shape changes. Giant vesicles created with complex lipid compositions form lipid domains, lipid rafts which can be visualized by fluorescent lipophilic probes, which are either excluded or included in the rafts. Lipid preference of fluorescently tagged pumps can thereby be identified. The purified pump complex displays an ATPase activity that is specifically stimulated by phosphatidylserine, in line with its in *vivo* transport activity.

References:

Papadopulos et al, (2007), Flippase Activity Detected with Unlabeled Lipids by Shape Changes of Giant Unilamellar Vesicles, *The Journal of Biological Chemistry* 282, 15559-68.

López-Marqués et al, (2010) Intracellular targeting signals and lipid specificity determinants of the ALA/ALIS P4-ATPase complex reside in the catalytic ALA alpha-subunit. *Mol Biol Cell.* 21, 791-801.

Genome-wide distribution of *cis* regulatory elements and reconstruction of transcriptional network governing the expression of carbon concentrating mechanisms in the cyanobacterium *Synechocystis* sp. PCC 6803

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Transcriptional regulatory networks play central roles in organismal physiology. Short stretches of DNA sequences known as *cis* regulatory elements (thereafter *cis*), lie in the vicinity of genes and serve as “barcodes” to recruit specific transcriptional factors. Understanding the genome-wide and gene-ontological (GO) distribution of a *cis* is an important step towards elucidating the function of its TF and future transcriptional network engineering for microbial bioenergy and biochemical production.
Efficient carbon acquisition through carbon concentrating mechanisms (CCM) is a hallmark of cyanobacteria. This ability enables to concentrate inorganic carbons around RuBisCO and supports efficient CO_2 fixation. LexA is a well-established transcriptional repressor of the SOS DNA damage repair response in *E.coli* but in the cyanobacterium *Synechocystis* sp. PCC 6803 LexA has been shown to be involved in regulation of CCM gene expressions[1]. Canonical LexA *cis* has been identified as CTA-N_9-CTA by *in vitro* gel shift experiments[2]. In this study, we determined the genome-wide distribution of the canonical LexA *cis*. Taking advantage of the publically available microarray results[3] we analyzed its bioactivity upon downshift of CO_2 concentration. No significant bioactivity was observed for the canonical element upon CO_2 downshift. *In silico* mutagenesis of the *cis* identified a variant, CGA-N_9-CTA, which confers significant bioactivity during CO_2 downshift. Interestingly genes co-occuring with this variant did not show significant enrichment in custom-made CCM GO category and instead were found to be enriched in multiple GO categories including nitrogen metabolism, NADH dehydrogenases and isoprenoid quinone biosynthesis. These results suggest that LexA functions in *Synechocystis* sp. PCC 6803 are global. The CGA-N_9-CTA motif was found upstream of *cmpR* that encodes a transcriptional activator involved in the regulation of low-CO_2 inducible bicarbonate ABC transporter[4]. Furthermore, *cis* of CmpR was found upstream of *ftsH2*, a protease implicated as a negative regulator of NdhR a transcriptional repressor also involved in the CCM regulation[5]. This led to the hypothesis of a novel CCM transcriptional regulatory network where LexA sits at the top of the hierarchy and regulates the expression of CmpR and FtsH2 and ultimately the activity of NdhR, meaning a global control over CCM gene expression. Our findings pave a path towards future transcriptional rewiring with a hope of enforcing the organism´s ability in carbon acquisition and so the robustness of the organism as photobiological cell factory.

[1]Domain, F., Houot, L., Chauvat, F., and Cassier-Chauvat, C. (2004) Function and regulation of the cyanobacterial genes *lexA*, *recA* and *ruvB*: LexA is critical to the survival of cells facing inorganic carbon starvation. *Molecular Microbiology* 53(1), 65-80.
[2]Patterson-Fortin, L.M., Owttrim, G.W. (2008) A *Synechocystis* LexA-orthologue binds direct repeats in target genes. *FEBS Letters* 582, 2424-2430.
[3]Whang, H.L., Postier, B.L., and Burnap, R.L. (2004) Alterations in Global Patterns of Gene Expression in *Synechocystis* sp. PCC 6803 in Response to Inorganic Carbon Limitation and the Inactivation of *ndhR*, a LysR Family Regulator. *J Biol Chem.* 279(7):5739-51.
[4]Omata, T., Price, G. D., Badger, M. R., Okamura, M., Gohta, S., and Ogawa, T. (1999) Identification of an ATP-binding cassette transporter involved in bicarbonate uptake in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *Proc. Natl. Acad. Sci. U. S. A.* 96, 13571–13576.
[5]Zhang, P., Sicora, C.I., Vorontsova, N., Allahverdiyeva, Y., Battchikova, N., Nixon, P.J., and Aro, E.M. (2007) FtsH protease is required for induction of inorganic carbon acquisition complexes in *Synechocystis* sp. PCC 6803. *Molecular Microbiology* 65(3), 728-740.

Mechanism and Regulation of a P-type H^+-ATPase: A Nanodisc Approach

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Regulation of the H^+-ATPase involves regulator proteins acting on the H^+-ATPase eg. by phosphorylation. The interactions between the H^+-ATPase and these regulator proteins, called the signalosome, are not easily studied due to the natural lipid environment of membrane proteins. A novel tool to facilitate such studies is the nanodisc. Best described as a solubilized patch of lipid bilayer, nanodiscs allows for the reconstitution of membrane proteins in a process similar to that used for vesicles. In addition to having access to both sides of the membrane simultaneously, the monodispersity of nanodiscs permits the use of techniques normally applied to soluble proteins, such as small angle X-ray scattering and electron microscopy. Reconstitution of the H^+-ATPase, including different parts of the signalosome, into nanodiscs, makes it possible to obtain structural information of this complex through the use of the above mentioned techniques.

SHI transcription factors in model plant species:

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Compact growth is a desirable trait in many crop plants and can be difficult to obtain by traditional breeding. As an alternative, use of biotechnology could provide plant varieties with optimized growth habits. Here we introduce the family of SHI transcription factors, which through overexpression have resulted in compact plants of very diverse species.

SHI proteins are transcription factors and this family of genes is found throughout the Plant Kingdom. We present here a phylogenetic analysis of entire *SHI* gene families in 11 model plant species. *SHI* gene sequences, and possibly also the functions of the proteins, are well conserved and widespread from the lycophyte *Selaginella moellendorffii* and the bryophyte *Physcomitrella patens* to higher plants. They clearly divide into two branches with all species represented in both subgroups.

The possible functions and regulations of the SHI proteins are discussed, and the potential of using overexpression as means to dwarf plants is assessed. In conclusion the breeding of some species, especially flowering ornamentals, could benefit from this strategy. Furthermore, detailed knowledge about the role of SHI proteins in plant growth and development could help shed more light on the interactions between plant hormone signaling pathways.

Expanding the biosynthetic diversity of chloroplasts

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The photosynthetic protein complexes use sunlight to oxidize water and transfer the released electrons to stable electron carriers and produce NADPH. Photosystem I (PSI) produces the most negative redox potential in nature and is perfectly suited to drive a number of redox reactions. In this work, we have introduced a new biosynthetic pathway consisting of cytochrome P450 enzymes (P450s) into the chloroplast. Plant P450s are naturally localized in the endoplasmatic reticulum where they catalyse hydroxylations of bioactive compounds. Normally, electrons necessary for their reactions are delivered by a cytochrome P450 reductase using NADPH.

We used the enzymes involved in the biosynthesis of the plant defence compound Dhurrin. In this pathway, the first enzyme is the membrane-bound CYP79A1 which converts the amino acid tyrosine into an oxime. This oxime is converted by another membrane bound P450, CYP71E1, into a nitrile and finally the soluble glucosyltransferase UGT85B1 completes the formation of Dhurrin by adding a sugar moiety.

The three genes encoding CYP79A1, CYP71E1 and UGT85B1 were cloned and transiently expressed, both individually and in combination, in leaves of *N. benthamiana*. A transit-peptide derived from another chloroplast localized protein was engineered in front of each protein ensuring targeting of the three enzymes to the chloroplast. Activity assays on isolated thylakoids and intact chloroplast showed that all three enzymes are active in the chloroplast. More interestingly, it was shown that the activities of CYP79A1 and CYP71E1 – individually and combined – can be supported by reducing power from PSI in a light-dependent manner. This demonstrates that it indeed is possible to express all the enzymes of the pathway in an active state in the chloroplast.

Future work will be directed towards establishing stable transformants with multi enzyme pathways expressed in the chloroplast and produce interesting bioactive compounds directly driven by light.

The biological relevance of the Conformational Changes of NADPH-dependent Cytochrome P450 Reductase studied by SAXS, Cryo-EM, SMFS and Neutron Reflectometry

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The NADPH-dependent cytochrome P450 reductase (CPR) is the key electron donor to eukaryotic cytochromes P450 (CYPs). CPR shuttles electrons from NADPH through the FAD and FMN-coenzymes to the iron of the prosthetic heme-group of the CYP. In the course of these electron transfer reactions, CPR undergoes large conformational changes. New evidence enables us to elucidate the molecular mechanisms controlling these conformational changes facilitating effective electron transfer to a huge diversity of different cytochromes P450. The effects of phosphorylation, lipid environment, lipid curvature and cofactors on the activity and regulation will be studied by a variety of Bio-Physical techniques, which includes Small Angle X-ray scattering (SAXS), Cryo Electron Microscopy (Cryo-EM), Single molecule Fluorescence Spectroscopy (SMFS) and Neutron Reflectometry.

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Engineering of light-driven biosynthetic pathways in cyanobacteria

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Plants can synthesize around 200,000 different bio-active natural compounds, many of which are of commercial interest since they can be utilized as drugs. Some of the enzymes that take part in the biosynthesis of these compounds are the cytochrome P450s (P450s). In this synthetic biology project we are aiming at coupling photosynthetic electron transport directly to a metabolon of P450s to develop a system in which the high energy electrons derived from water can be used directly in the biosynthetic reactions of the P450s. As a model system we are working with the enzymes producing the cyanogenic glucoside dhurrin, a defence compound in *Sorghum bicolor*. Three enzymes are involved in dhurrin biosynthesis, two P450s (CYP79A1 and CYP71E1) and a glycosyl transferase, UGT85B1.

Recent results from our laboratory has shown that the electron transfer from photosystem I to CYP79A1 works with high efficiency *in vitro* with the small soluble electron carrier ferredoxin (Fd) as electron mediator and that the reaction catalyzed by CYP79A1 indeed can be driven by light. We are now developing an *in vivo* system with the final goal to obtain light-driven dhurrin biosynthesis in the thylakoid membranes of a photosynthetic host organism. As host we use the naturally transformable and fast growing cyanobacterium *Synechococcus* sp. PCC 7002.

Different approaches for the *in vivo* coupling of photosystem I (PSI) and the P450s are being pursued. In one system the soluble enzymatic domains of the P450s are being fused directly to one of the small subunits of PSI, thereby integrating the P450s into the PSI complex. To facilitate highly efficient electron transfer, the fusion protein complex has been designed to keep the interacting proteins in close proximity to each other. All three enzymes of the dhurrin biosynthesis pathway will be introduced in the PSI complex. So far the fusion of the CYP79A1 to PSI has been completed. Preliminary results show that the PSI-CYP79A1 fusion construct is expressed and result in active CYP79A1 in purified thylakoid membranes. In a second approach the whole dhurrin pathway is introduced in *Synechococcus* sp. PCC 7002 as an artificial gene cluster. The three genes (CYP79A1, CYP71E1 and UGT85B1) that constitute the pathway are being codon optimized, designed and synthesised with relevant optimized transcription and translation elements. In order to ensure thylakoid localization of the expressed proteins a thylakoid targeting peptide are engineered into the N-terminus of CYP79A1 and CYP71E1.

For both approaches expression levels, metabolon formation and activity will be characterised and compared.

Engineering of plant metabolites in yeast facilitated by pathway optimization in *Nicotiana benthamiana*

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Glucosinolates (GLs) are sulphur containing amino acid derived secondary metabolites produced in plants of the Brassicaceae family. They are part of the plant's natural defense against herbivores and microorganisms.

In human nutrition, GLs are mostly known for their sharp taste characteristic of plants such as mustard, radish and wasabi. Epidemiological studies have shown that consumption of cruciferous vegetables is associated with a reduced risk of developing cancer. This phenomenon has been attributed to specific glucosinolates (or rather their hydrolysis products) among the ~30 GLs that are typically present as natural products characteristic for these plants. Therefore, there is an increasing interest to use GLs as possible dietary supplements or pharmaceuticals. To achieve this application, high amounts of GLs needs to be produced by a cheap reliable source. As Glucosinolates are highly complex molecules, chemical synthesis is difficult and expensive, and isolation from their natural source is typically low in yield.

Transient reconstitution of the biosynthetic production system of GLs derived from non-modified amino acids (Indole- & Benzyl-glucosinolate) in tobacco was employed as "proof of concept". Production of these simpler GLs has been successfully established by stable integration into yeast.

Accordingly,the production platform will be moved to the next level to produce the more complex GL glucoraphanine which is derived from chain-elongated methionine. Here, the major task is to implement the plastidal chain-elongation machinery to another compartment or the cytosol in the target organism yeast. Again, transient expression in tobacco serves as basis for establishing a stable production in yeast cell factories.

References:
Geu Flores, F, Olsen, CE, Halkier, BA (2009): Towards engineering glucosinolates into non-cruciferous plants. Planta, 229 (2): 261-270.
Mikkelsen, MD, Olsen, CE, Halkier, BA, (2010): Production of the Cancer-Preventive Glucoraphanin in Tobacco. Molecular plant, 3: 751-759.
Mikkelsen, MD, Buron, LD, Salomonson, B, Olsen, CE, Hansen, BG, Mortensen, UH, Halkier, BA, (submitted): Microbial production of indolylglucosinolate through engineering of a multi-gene pathway in a versatile yeast expression system.

Characterization of plant cytochromes P450 involved in diterpenoid metabolism

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Specialized metabolism in plants is the source of more than several hundred thousand natural products. The family of terpenoid compounds is one of the largest families of natural compounds with more than 44 000 known structures. Diterpenes (C-20) are a sub category of terpenoids, which often have ring a like structure with regio and stereo specific "decoration" of hydroxyl-groups. These features make the diterpenes a very complex and diverse family of molecules which are hard to synthesize and therefore have a high commercial value. Cytochrome P450s enzymes (P450) are the main contributor to the decoration of diterpenes, and therefore in the future production of a wide range of high value diterpenes, the understanding and characterization of the P450s are crucial.
In this study the goal is to structurally characterize the functionally established P450, CYP720B4 involved in resin formation in *Sitka spruce* [1]. From the plant *Tripterygium wilfordii* we have identified possible P450s candidates involved in the biosynthesis of the diterpene triptolide, which has an antitumor and immunosuppressant activity [2], and the goal is to characterize these.

P450's are interacting with the cytochrome P450 reductase (CPR) and are membrane bound enzymes, making them difficult to study. Therefore we want to incorporate the P450s, potentially together with CPR, into nanodiscs for stabilization and mimicking the native membrane environment of the P450.

Amounts in the 10-100mg range of purified and active P450 is needed for the incorporation of P450 into nanodiscs. Therefore we are using a high throughput screening method for optimization of P450 expression in *E. coli*. This is done by linking the target P450 to GFP which only will fold correctly if the P450 is properly folded, hence the GFP signal will be correlated to the amount of active P450 being produced in *E. coli* [3].

1. Hamberger, B., et al., *Sitka spruce CYP720B4 catalyses multiple oxidations in the biosynthesis of diterpene resin acids of conifer defense against insects.* Plant Physiology, 2011.
2. Titov, D.V., et al., *XPB, a subunit of TFIIH, is a target of the natural product triptolide.* Nat Chem Biol, 2011. **7**(3): p. 182-188.
3. Drew, D., et al., *Rapid topology mapping of Escherichia coli inner-membrane proteins by prediction and PhoA/GFP fusion analysis.* Proceedings of the National Academy of Sciences, 2002. **99**(5): p. 2690-2695.

Enrichment of glycopeptides from wheat albumin extracts by ZIC®-HILIC

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The study of plant proteomes is important to further the understanding of biological processes and enhance the agronomical and nutritional value of crops and food products. To gain deeper understanding on the proteome level, it is important to characterize post-translational modifications that influence the function, structure, localization, turnover and interaction with other proteins (1,2). For glycoprotein analysis, zwitterionic hydrophilic interaction liquid chromatography (ZIC®-HILIC) has been demonstrated as a useful tool for depletion of hydrophobic peptides and retention of hydrophilic glycopeptides, e.g. from in-solution tryptic digests of human plasma (3,4). The aim of this work is to apply this methodology to screen and identify glycosylated proteins from wheat albumin extracts.

Glycopeptides enriched by ZIC®-HILIC were analyzed by MALDI-TOF MS and the enriched peptides were further subjected to deglycosylation with PNGase A in the presence of ^{18}O labeled water followed by LC-MS/MS analysis. In preliminary experiments, 19 glycosylation sites from 18 matched proteins were detected in wheat albumin extracts.

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References

1. Hitchen P.G., Dell A. (2006). Bacterial glycoproteomics. Microbiology 152:1575-1580.
2. Mann M., Jensen O.N. (2003). Proteomic analysis of post-translational modifications. Nature Biotechnology 21:255-261.
3. Hägglund P., Bunkenborg J., Elortza F., Nørregaard O.J., Roepstorff P. (2003). A new strategy for identification of N-glycosylated protein and unambiguous assignment of their glycosylation sites using HILIC enrichment and partial deglycosylation. Journal of Proteome Research 3:556-566.
4. Hägglund, P., Matthiesen, R., Elortza, F., Højrup, P., Roepstorff, P. Jensen, O. N., Bunkenborg, J. (2007) An enzymatic deglycosylation scheme enabling identification of core fucosylated N-glycans and O-glycosylation site mapping of human plasma proteins. Journal of Proteome Research 6:3021-3031.

Engineering mammalian type O-Glycosylation in plants

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Mammalian mucins are large heavily O-glycosylated glycoproteins (>200 kDa) abundant in mucus layers, where they hydrate, lubricate and protect cells from proteases as well as from pathogens. O-glycosylation in plants and mammals is intrinsically different with respect to *i)* families of proteins subjected to O-glycosylation, *ii)* the particular amino acids modified and *iii)* the sugars constituting the O-glycans. Accordingly, plants lack all known gene families responsible for mammalian types of protein O-glycosylation. Plants, therefore offer a unique cell platform for engineering designed O-glycosylation in principle enabling de novo introduction of the entire mammalian O-glycan repertoire including the truncated cancer specific tumor-associated antigens. In the present study, we have produced the cancer specific mucin type glycopeptide epitope (Tn), which may be used as a potential cancer vaccine candidate, in plants thus providing proof of concept of the feasibility of engineering plants with respect to mucin type O-glycosylation.

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