Programme

TUESDAY, 29 January 2008

09.15 – 09.45	Coffee and registration
09.45 – 09.50	**Welcome**, by Preben Bach Holm, member of steering committee, Plant Biotech Denmark
	Session 1 – Products and Productivity (chair: Anna Haldrup)
09.50 – 10.30	*Keynote talk* **The dependence of yield on photosynthesis: Opportunities for improvement**, by Donald R. Ort, professor, University of Illinois, USA
10.30 – 10.50	**A R2R3 MYB gene subfamily regulates aliphatic glucosinolate accumulation in Arabidopsis**, by Ida Elken Sønderby, PhD student, Department of Plant Biology, LIFE, University of Copenhagen
10.50 – 11.10	**Arabinan have diverse function in the cell wall,** by Jesper Harholt, postdoc, Department of Plant Biology, LIFE, University of Copenhagen
	Session 2 – Nutrition and Diseases (chair: David Collinge)
11.10 – 11.50	*Keynote talk* **Improving nitrogen use efficiency in maize and wheat. How far are we from practical applications?** by Bertrand Hirel, professor, INRA (French National Institute for Agricultural Research), France
11.50 – 12.10	**Crystal structure of a plasma membrane proton pump**, by Morten J. Buch, associate professor, Department of Plant Biology, LIFE, University of Copenhagen
12.10 – 12.30	**The MPK4 Map kinase cascade in plant innate immunity in Arabidopsis,** by Maria Cristina Suarez-Rodriguez, postdoc, Department of Molecular Biology, NAT, University of Copenhagen
12.30 – 13.15	Lunch
	Session 3 – Breeding and Systems Biology (chair: Ahmed Jahoor)
13.15 – 13.55	*Keynote talk* **Systems-level analysis of plant low-temperature responses**, by Matthew Hannah, postdoc, Max-Planck Institute of Molecular Plant Physiology, Germany
13.55 – 14.15	**Fine mapping of sugarcane mosaic virus (SCMV) resistance gene Scmv2 in maize**, by Christina Rønn Ingvardsen, project scientist, Department of Genetics and Biotechnology, DJF, University of Århus
14.15 – 14.35	**Proteomics of Lotus japonicus seed development,** by Svend Dam, PhD student, Department of Molecular Biology, NAT, University of Århus
14.35 – 15.00	Coffee
	Session 4 – Technologies (chair: Bill Willats)
15.00 – 15.20	**The model plant Physcomitrella patens – a unique resource for gene discovery and analysis**, by Christina Lunde, assistant professor, Department of Plant Biology, LIFE, University of Copenhagen

15.20 – 15.40 **Bioinformatics driven design of anchor markers bridging model and crop plants**, by Birgit K. Hougaard, postdoc, Department of Molecular Biology, NAT, University of Århus

15.40 – 16.00 **Photoactivation and other fluorescence techniques for graft compatibility studies**, by Helle Juel Martens, associate professor, Department of Plant Biology, LIFE, University of Copenhagen

16.00 – 16.20 **New technologies for high-throughput DNA sequencing**, by Pernille Kræmer Andersen, research assistant, Department of Genetics and Biotechnology, DJF, University of Århus

16.20 – 16.40 **Metabolic engineering of carbohydrate partitioning and turnover**, by Jens Kossmann, professor, Institute of Plant Biotechnology, Stellenbosch University, South Africa

16.40 – 18.30 Poster session in the Marble Hall – wine and snacks are served

18.30 – 22.00 Dinner at Gimle, Dyrlægevej 9, Frederiksberg

WEDNESDAY, 30 January 2008

	Session 1 – Opening session (chair: Jens Stougaard)
09.00 – 09.30	**Plants for the future**, by Torben Bo Toft Christensen, head of office, Project and Innovation Office, Landbrugsrådet
09.30 – 10.00	**AgroTech – How can we support plant research?,** by René Logie Damkjer, director, AgroTech
10.00 – 10.30	**Expert communication with the public: Modernist ideals vs. post-modernist attitudes,** by Lise-Lotte Holmgreen, assistant professor, Department of Language and Culture, Ålborg University
10.30 – 10.55	Coffee
	Session 2 – Plants for Health (chair: Cathie Martin)
10.55 – 11.15	**Towards regulating isolavonoid biosynthesis**, by Fred Rook, associate professor, Department of Plant Biology, LIFE, University of Copenhagen
11.15 – 11.35	**Rational screening for health promoting bioactive compounds in plants**, by Karsten Kristiansen, professor, Department of Biochemistry and Molecular Biology, University of Southern Denmark
11.35 – 11.55	**Do food bioactive compounds have disease reduction potentials?**, by Peter Olesen, CSO, Chr. Hansen A/S
11.55 – 12.15	**Modern NMR techniques for characterisation of plant products and bioactive constituents,** by Jerzy W. Jaroszewski, professor, Department of Medical Chemistry, Faculty of Pharmaceutical Sciences, University of Copenhagen
12.15 – 12.35	**Cyanogenic glucosides in primary and secondary metabolism,** by Raquel Sánchez-Pérez, postdoc, Department of Plant Biology, LIFE, University of Copenhagen
12.35 – 12.55	**Breeding for better oil quality in winter oilseed rape**, by Morten H. Poulsen, field station manager, Dow AgroSciences, Field Station Abed
12.55 – 13.40	Lunch
	Session 3 – Molecular Plant Microbe Interactions (chair: John Mundy)
13.40 – 14.00	**Presentation of the CARB Centre**, by Jens Stougaard, professor, Department of Molecular Biology, NAT, University of Århus
14.00 – 14.20	**Global spread of new wheat rusts**, by Mogens Hovmøller, senior scientist, Department of Integrated Pest Management, DJF, University of Århus
14.20 – 14.40	**Colonisation of barley roots with DsRed expressing Fusarium avenaceum and GFP expressing Fusarium culmorum-competition and effect on toxin production**, by Thomas Johansen, PhD student, Department of Ecology, LIFE, University of Copenhagen
14.40 – 15.00	**Breeding for disease resistance at SW Seed**, by Annette Olesen, director, Product Development, SW Seed, Svalöf Weibull AB, Sweeden
15.00 – 15.25	Coffee

15.25 – 15.45 **Mycorrhiza – for improving phosphate uptake**, by Iver Jakobsen, head of programme, Biosystems Department, RISØ, Technical University of Denmark

15.45 – 16.05 **The power of genetics in signalling studies**, by Hans Thordal-Christensen, senior scientist, Department of Agricultural Sciences, LIFE, University of Copenhagen

Final session (chair: Preben Bach Holm)

16.05 – 16.30 **Globalization, biofuels and bioeconomics – implications for Danish agriculture**, by Henning Otte Hansen, senior advisor, Institute of Food and Resource Economics, LIFE, University of Copenhagen

Abstracts selected for presentation

Key Note Talk

The dependence of yield on photosynthesis: Opportunities for improvement

Donald Ort[1,2], Xin-Guang Zhu[1], Steve Long[1]

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The yield potential (Yp) of a grain crop is the seed mass per unit ground area obtained under optimum growing conditions without weeds, pests and diseases. It is determined by the product of the available light energy and by the genetically determined properties: efficiency of light capture (Ei), the efficiency of conversion of the intercepted light into biomass (Ec) and the proportion of biomass partitioned into grain (η). Plant breeding brings η and Ei for some crops close to their theoretical maxima, leaving Ec, primarily determined by photosynthesis, as the only remaining major prospect for improving Yp. Numerous potential routes of increasing Ec by improving photosynthetic efficiency are explored, ranging from altered canopy architecture to improved regeneration of the acceptor molecule for CO_2. Collectively, these changes could improve Ec and, therefore, Yp by c. 50%. Because some changes could be achieved by transgenic technology, the time of the development of commercial cultivars could be considerably less than by conventional breeding and potentially, within 10-15 years.

Products

A R2R3 MYB Gene Subfamily regulates aliphatic glucosinolate accumulation in Arabidopsis

Ida Elken Sønderby[1], Bjarne Gram Hansen[1], Nanna Bjarnholt[1], Daniel J. Kliebenstein[2], and Barbara Ann Halkier[1]

[1]Plant Biochemistry Laboratory, Faculty of Life Sciences, Copenhagen University, Denmark
[2]Department of Plant Sciences, University of California, Davis, USA

Glucosinolates are natural metabolites in the order Brassicales that defend plants against both herbivores and pathogens and can attract specialized insects. We identified three R2R3 MYB transcription factors regulating aliphatic glucosinolate biosynthesis in *Arabidopsis* by combining several systems biology tools. *MYB28* was identified based on its co-expression with several aliphatic glucosinolate biosynthetic genes. Furthermore, it co-localized within a genomic region controlling variation both in aliphatic glucosinolate content (metabolite QTL) and in transcript level for genes involved in the biosynthesis of aliphatic glucosinolates (expression QTL). A phylogenetic analysis with the R2R3 motif of *MYB28* showed that it and two homologues, *MYB29* and *MYB76*, were members of an *Arabidopsis*-specific clade that included three characterized regulators of indole glucosinolates.

Over-expression of the individual *MYB* genes showed that they all had the capacity to increase the production of aliphatic glucosinolates in leaves and seeds and induce gene expression of aliphatic biosynthetic genes within leaves. Analysis of leaves and seeds of single knockout mutants showed that mutants of *MYB29* and *MYB76* have reductions in only short-chained aliphatic glucosinolates whereas a mutant in *MYB28* has reductions in both short- and long-chained aliphatic glucosinolates. Furthermore, a double knockout in *MYB28* and *MYB29* was completely devoid of aliphatic glucosinolates, suggesting a complex regulatory mechanism since the absence could not have been predicted by the chemotypes of the single knockouts. This provides a unique system within which to study the evolution of *MYB* regulatory factors and their downstream targets.

Productivity

Arabinan have diverse function in the cell wall

Jesper Harholt[1], Yumiko Sakuragi[1], Jacob Krüger Jensen[1], Casper Søgaard[1], Jens Øbro[3], Peter Ulvskov[2], Peter Ryden[4], and Henrik Vibe Scheller[1]

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[2]Biotechnology group, Faculty of Agricultural Sciences, University of Aarhus, 40 Thorvaldsensvej, DK-1871 Copenhagen
[3]Copenhagen Biocentre, Department of Molecular Biology, Ole Maaløes Vej 5, DK-2200 Copenhagen
[4]Institute of Food Research, Norwich Research Park, Colney Lane, Norwich, NR4 7UA, UK

Arabinan deficient 1 (arad1) have reduced level of pectic arabinan but biochemical characterisation of arad2 (At5g44930) have so far not given any clue to a putative function. ARAD1 and ARAD2 do not appear to be functionally redundant as complementation of the arad1 phenotype is not achievable using a 35S::ARAD2 construct. As arad1 is not completely arabinan deficient an arad1xarad2 double knock out was generated in order to investigate possible increased severity of arabinan deficiency. Cell wall composition analysis did not reveal further reduction in arabinose content compared to arad1 signifying that other genes then ARAD2 could be responsible for the residual arabinan in arad1.

Arabinan have previously been shown to influence the mechanical properties of the cell wall (Jones et al. 2003, Ulvskov et al. 2005). The mechanical properties of arad1 were therefore investigated. In arad1 a decrease in both modulus and failure stress could be observed. Initial cell wall composition analysis has shown that arad1 had a reduction in arabinose content in the hypocotyls used for the mechanical tests. Additionally it could be concluded that the qrt background, a pectinmethylesterase deficient line used for the SAIL T-DNA lines, has a decreased modulus and increased failure stress. When subjected to infection by the fungal pathogen Botrytis cenerea, the arad1 mutant showed increased lesion size (ca. 30%, depending on experiments) as compared to WT by 2dpi. This result indicates that the arabinan deficiency results in increased susceptibility, which in turn suggests that arabinan plays a role in resistance against Botrytis infection. Using arabinanase deficient Arabidopsis generated by expression of arabinanase it can be suggested that arabinan is a new elicitor for pathogen defence initiation. Aside from arad1, cell wall composition analysis has not revealed any biochemical phenotype in T-DNA knock out lines in five of the remaining seven members and no TDNA knock out are available for the last two.

Key Note Talk

Improving nitrogen use efficiency in maize and wheat. How far are we from practical applications?

Bertrand Hirel[1], Jacques Le Gouis[2], Thomas Kichey[3], Frédéric Dubois[3], and André Gallais[4]

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[4] Laboratoire de Biologie des Plantes et Contrôle des Insectes Ravageurs. Faculté des Sciences33, rue St Leu, F-80039 Amiens cedex 1. France.

In this presentation, the recent developments and future prospects of obtaining a better understanding of the regulation of nitrogen use efficiency in maize and wheat, two of the main crops cultivated in the world, will be presented. In these crops, an increased knowledge of the regulatory mechanisms controlling the plant nitrogen economy is vital for improving nitrogen use efficiency and for reducing excessive input of fertilisers, while maintaining an acceptable yield. Using plants grown under agronomic conditions at low and high nitrogen fertilisation regimes, it is now possible to develop whole plant physiological studies combined with gene, protein and metabolite profiling to build up a comprehensive picture depicting the different steps of nitrogen uptake, assimilation and recycling to the final deposition in the seed. We will provide a critical overview on how our understanding of the physiological and molecular controls of nitrogen assimilation under varying environmental conditions in crops, has been improved through the use of combined approaches, mainly based on whole plant physiology, quantitative genetics, forward and reverse genetics approaches. Current knowledge and prospects for future agronomic development and application for breeding crops adapted to lower fertiliser input are explored, taking into account the world economic and environmental constraints in the next century.

Nutrition

Crystal Structure of a Plasma Membrane Proton Pump

Morten J. Buch-Pedersen[1,2,], Bjørn P. Pedersen[1,*], Jens P. Morth[1], Michael G. Palmgren[2], and Poul Nissen[1]*
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A prerequisite of life is the ability to maintain electrochemical imbalance across biomembranes. In plants and fungi, proton pumps from the family of P-type ATPases establish electrochemical gradients of protons across the plasma membrane which, in turn, are essential for energisation of secondary active transport systems. Here we present the first structure of a P-type proton pump determined by X-ray crystallography. Our results provide insight into the mechanism of proton export and how it is coupled to ATP hydrolysis in P-type H+-ATPases. We present a model to explain how proton transport, against high potential, is achieved by means of positively charged residues acting in concert with the conserved Asp 684 residue and the solvent-filled cavity in the membrane.

Diseases

The MPK4 Map kinase cascade in plant innate immunity in *Arabidopsis*

Maria Cristina Suarez-Rodriguez[*†], Shuqun Zhang[‡], Andrew F. Bent*, Patrick J. Krysan*, Jin-Long Qiu[†], Berthe Katrine Fiil[†], Klaus Petersen[†], Henrik Bjørn Nielsen[£], Stephan Thorgrimsen[†], Peter Morris[ξ], John Mundy[†], and Morten Petersen[†]*

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MAP kinases are evolutionary conserved signaling components that play essential roles in plant innate immunity. In *Arabidopsis* the bacterial ellicitor flagellin (flg22) activates a MAPK cascade proposed to include the triple kinase MEKK1 and the two single kinases MPK3 and MPK6. However, recent genetic and biochemical studies showed that MEKK1 is only required for flg22-induced activation of MPK4 but not for MPK3 or MPK6. Similarly, defense genes are de-repressed in *mekk1* and *mpk4* loss-of-function mutants and both are extreme dwarfs. The defense activation turned on in both mutants depends on the phyto-hormone salicylic acid (SA) indicating that both MEKK1 and MPK4 function as negative regulators of salicylic acid-dependent responses. MEKK1 directly interacts with MKK1 and MKK2 and more recent work has supported the requirement of MKK1 and MKK2 for the flagellin-mediated activation of MPK4. Recently, we established a direct link among MPK4, MKS1, WRKY33. Ongoing research aim at identifying targets directly controlled by WRKY33 through the MPK4 kinase cascade but also identify other transcription factors below MPK4 and their targets.

Key Note Talk

Systems-level analysis of plant low-temperature responses

Matthew Hannah, Max-Planck Institute of Molecular Plant Physiology, Germany

Low temperature is a major challenge to plant growth and survival. Many plants are able to counter this by increasing their freezing tolerance in response to low but non-freezing temperatures in a process known as cold acclimation. Our aim is to dissect the molecular basis of freezing tolerance and to understand the underlying regulatory networks. Initially, via meta-analysis of available expression data we identified pathways and functional groups of genes that respond to cold in the accession Columbia. We then used accessions of Arabidopsis originating from Scandinavia to the Cape Verde Islands to relate molecular changes to adaptive variation.

Importantly, we identified low temperature as a significant selective pressure for Arabidopsis – freezing tolerance was correlated with minimum habitat temperature. Underlying this we found that during cold exposure, global changes of transcripts, but not of metabolites, correlate with the ability of Arabidopsis to cold acclimate. We also identified specific metabolites and transcripts associated with acclimated and constitutive freezing tolerance. Further analysis of public data revealed, despite using paired diurnal controls, diurnal and circadian-regulated genes are responsible for the majority of variation between experiments to identify cold-responsive genes. Targeted expression analyses showed that this is because cold dampens or disrupts the cycles of many clock components and output genes while on the other hand there is extensive diurnal gating of cold signalling.

The mechanisms and physiological significance of cold-diurnal interactions are being investigated. As light is the key diurnal input and affects cold responses, we are investigating signalling pathways underlying the integrated response of plants to temperature and light. This approach will also extend our recent work to identify candidate small molecule signals based on their in vivo co-response with transcripts across a diverse range of treatments. Incorporating temporal information will assist in assigning causality and through this we hope to identify known and novel environmentally-activated small molecule signalling pathways.

Breeding

Fine Mapping of Sugarcane Mosaic Virus (SCMV) Resistance gene Scmv2 in Maize

Christina Roenn Ingvardsen[1], Yongzhong Xing[2], Uschi Frei[3], and Thomas Lübberstedt[3]

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The potyvirus sugarcane mosaic virus (SCMV) is an important pathogen of maize (*Zea mays* L.), causing chlorosis, stunting and serious yield loss in susceptible cultivars. Three major resistance genes confer resistance to SCMV, one gene located on chromosomes 3 and two genes closely linked on chromosome 6. However, the molecular mechanisms underlying the establishment and progression of SCMV infection in maize are poorly understood.

A near-isogenic line, F7R, carrying the resistance regions from chromosome 3 (*Scmv2*) and 6 (*Scmv1a and 1b*) in a susceptible background was developed. Using SSR markers linked to the two loci, the near isogenic line F7R was further developed into 9 genotypes differing in the two gene regions but having the background of the susceptible parent. Testing the nine genotypes for response to SCMV infection showed that one resistance region alone was not sufficient for complete resistance against SCMV. When the resistant allele is fixed at either the *Scmv1* or *Scmv2* locus, the susceptible homozygote at the other locus is easily distinguishable from the genotypes carrying one or two resistance alleles. The oligogenic inherited SCMV resistance has thus been broken down into two “monogenic” cases, giving a very reliable association between phenotype and genotype classes, which is an important prerequisite for successful map-based gene isolation. Large F_2 mapping populations for each of both genome regions have been developed.

We are currently in the process of map-based gene isolation of Scmv2 using a BAC library, constructed from the resistant inbred line FAP1360A.

Systems Biology

Proteomics of *Lotus japonicus* Seed Development

Svend Dam[1], Brian S. Laursen[1], Astrid L. Siegumfeldt[1], Jane H. Ørnfelt[1], Bjarne Jochimsen[1], Ida Thørgersen[1], Jan J. Enghild[1], Andrea M. Lorentzen[2], Peter Roepstorff[2], and Jens Stougaard[1]*

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Legumes are important for the general food and feed supply because the seeds are very protein-rich (up to 50 %). However, the storage proteins in the seeds only contain a small amount of the sulphur containing amino acids (Cys and Met). Earlier studies (mostly histological) on legumes have concentrated on bean and pea. Legume seed development can be divided into three phases (cell division / development, maturation / seed filling and desiccation phase).

Bean and pea are not ideal for proteomics studies because of lack of genome sequence. *Lotus japonicus* have all the features to be a good model plant for proteomic (for example a small and sequenced genome).

Proteins were extracted from 14 developmental stages ranging from very small to mature seeds and pod walls. The extracted proteins were separated on SDS-PAGE. The major storage proteins bands were identified. Thin sections of the seeds were made from all 14 developmental stages and stained with toluidine blue. The development of seed coat, embryo and endosperm were followed. In the light of these results, 5 stages were chosen for detailed analyses by 2-D gels. From the 2-D gels, we have identified proteins from approximately 1050 spots giving rise to approximately 550 unique protein accessions. Identification and quantification of protein content is a work in progress.

Furthermore, a MudPIT like analysis from 2 developmental stages was made. From green seeds approximately 520 unique protein accessions were identified and from mature seeds approximately 150 unique protein accessions were identified.

A comparison of the unique protein accessions from 2-D gels and MudPIT like analysis signifies that about 50 % of the unique protein accessions were identified in both methods and about 50 % of unique protein accessions were specific for 2-D gels or MudPIT like analysis.

The aim of this project is to identify the timing of pathway regulation during seed development. Our results may be transferred to the crop legumes.

Technologies

The model plant *Physcomitrella patens* – a unique resource for gene discovery and analysis

Christina Lunde, Department of Plant Biology, Faculty of Life Sciences, University of Copenhagen

In recent year a new model plant species, *Physcomitrella patens*, has emerged. Unlike any vascular plants known to date *Physcomitrella* is able to perform homologous recombination with high efficiency. In addition, the vegetative state of *Physcomitrella* is haploid and each cell maintains in ability to generate new gametophytes. These features render *Physcomitrella* a highly efficient model plant in molecular studies and allow the generation of predictable and specific gene knock-outs in one step without the need for whole plant regeneration and crossing. The full genome sequence of *Physcomitrella* is now available and one of the conclusions which can be drawn is that multi-gene families generally contain fewer members in *Physcomitrella* compared to angiosperms (Nishiyama et al., 2003). Doing comparative genomics across this ancient non-vascular plant and angiosperms will therefore allow the discrimination between highly conserved genes which have been retained during the evolution of plants and contemporary genes evolved in vascular plants to deal with their specific environmental challenges.

Another important aspect is that stress protective mechanisms have not only become more complex during plant evolution (Nishiyama et al., 2003). *Physcomitrella* diverged from higher plants more than 450 million years ago ad is highly tolerant to a range of abiotic stresses and identifying the underlying mechanisms giving this tolerance could supply valuable information on how to improve stress tolerance of vascular plants. In algae and moss several novel genes involved in abiotic stress protection have been identified (Benito and Rodriguez-Navarro, 2003; Lunde et al., 2007; Zurbriggen et al., 2007). These genes have apparently been lost during evolution in time periods where they had no selective advantage. These ancient genes could however hold solutions for some of problems faced by modern agriculture.

Bioinformatics Driven Design of Anchor Markers Bridging Model and Crop Plants

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Comparative genetic mapping allows transfer of genetic information between species. In the legume family the genomes of a few species (e.g. *Lotus japonicus*) are well characterised, and genome information from these can contribute important information to breeding programs in crop legumes with less well-characterised genomes.

In a general effort to bridge the maps of crop legumes with those of model legume plants we have established an automated bioinformatic pipeline suggesting intron-spanning primers for use throughout the legume family (1,2). The pipeline take advantage of sequence data from related species and identifies evolutionarily conserved sequences followed by design of intron-flanking PCR primers. To ensure that the markers represent true anchor points between the individual maps, the regions amplified correspond to single copy genes. The bioinformatic pipeline has so far yielded a total of 459 legume based primer sets (http://cgi-www.daimi.au.dk/cgi-chili/GeneticMarkers/table) and 1335 primer sets for the grasses (http://cgi-www.daimi.au.dk/cgi-chili/GeneticMarkers/grass). A number of the legume based primer sets have been tested in common bean (*Phaseolus vulgaris*) and the distantly related legume groundnut (*Arachis*) and about 50% of the primers tested yielded a marker. One hundred and twenty Leg markers have been made in bean and we see large regions of conserved macrosynteny between bean, *Lotus* and *Medicago*.

The bioinformatic pipeline was made as a dynamic setup, so when new EST collections become available they can be submitted to the web program GeMprospector (http://cgi-www.daimi.au.dk/cgi-chili/GeMprospector/main). Here the new information will be combined with the information in the existing database and new cross-species anchor marker candidates will be produced for personal use (3). Likewise, the primer-finding unit (PriFi) of the bioinformatic pipeline is web accessible (http://cgi-www.daimi.au.dk/cgi-chili/PriFi/main) and can be used separately for design of primer pairs for PCR amplification of genomic DNA in species where prior sequence information is not available. PriFi works with an alignment of DNA sequences from phylogenetically related species and outputs a list of possibly degenerate primer pairs (1).

References:
(1) Jakob Fredslund, Leif Schauser, Lene H. Madsen, Niels Sandal and Jens Stougaard. PriFi: using a multiple alignment of related sequences to find primers for amplification of homologs. Nucleic Acids Res. 2005 Jul 1;33 (Web Server issue):W516-20.
(2) Jakob Fredslund, Lene H. Madsen, Birgit K. Hougaard, Anna Marie Nielsen, David Bertioli, Niels Sandal, Jens Stougaard and Leif Schauser. A general pipeline for the development of anchor markers for comparative genomics in plants. BMC Genomics. 2006 Aug 14;7:207.
(3) Jakob Fredslund, Lene H. Madsen, Birgit K. Hougaard, Niels Sandal, Jens Stougaard, David Bertioli and Leif Schauser. GeMprospector-online design of cross-species genetic

marker candidates in legumes and grasses. Nucleic Acids Res. 2006 Jul 1;34(Web Server issue):W670-5.

Photoactivation and other fluorescence techniques for studies on graft compatibility

Helle Martens, Plant Physiology and Anatomy Laboratory, Department of Plant Biology, Faculty of Life Sciences, University of Copenhagen

Uniting two plant species together by grafting is as well an ancient cultivation technique and a modern tool for cell biology studies. Many economically important woody plant species, such as pear, almond and apricot, are grown as grafted plants in order to improve fruit quality, disease-resistance and growth. And only by grafting is it possible to test whether transcription factors or other signalling compounds make use of the phloem for whole plant coordination events as for instance flowering.

The formation of direct vascular (phloem and xylem) connections is crucial for the transport of water and nutrients between graft partners. Within the same plant family intergeneric grafting is possible, however, the number of successful combinations is limited. Different species belonging to the same genus are compatible in some cases but incompatible in others. The complexity of interspecific grafting is further demonstrated by reciprocal grafts; a plum cultivar may be compatible as a scion on peach, but the reverse graft, peach on plum is not. A major problem in tree fruit production is late incompatibility, called “cellular incompatibility”. In this case vascular connections are well established but the seemingly successful grafts break at the union many months after grafting.

Our aim is to test whether cellular incompatibility in apricot is caused by reduced cell communication between the graft partners. As an approach to early detection of incompatibility we are developing bioimaging techniques such as fluorescence recovery after photobleaching and photolysis of caged probes. This allows us to visualise and quantify the symplasmic transport within plant tissue. For development of the techniques we used herbaceous grafts (cucumber/pumpkin). The experiments were performed with apricot/plum combinations on in-vitro grown plant material, both on callus unions and on small plants. The results achieved by comparing compatible with incompatible graft combinations of apricot and plum cultivars are discussed.

New technologies for high-throughput DNA sequencing

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Over the last years, genome sequencing has started a revolution in biological sciences by allowing determination of the complete DNA sequence from any organism, thus obtaining a full description of the whole set of genes. The demand for cost-effective methods for high-throughput DNA sequencing has prompted the development of powerful platforms including the GS20/GSFLX (454 Life Sciences) as well as the Illumina Genome Analyzer System (Solexa). Both technologies are based on a robust sequencing-by-synthesis method producing on average 100 megabases and 1 gigabase of raw sequences, respectively, in a single run. In general, the systems allow for massively parallel sequencing of the DNA fragments without a cloning step during sample preparation. The 454 system utilizes the principles of pyrosequencing whereas solexa sequencing is build on reversible terminator chemistry, resulting in different parameters between the two systems such as accuracy and average length of the generated reads.

The platforms are competitive in a broad range of applications varying from genome sequencing, transcriptome analysis and small RNA discovery. However the choice of instrument depends on the respective study and the desired post-sequencing treatment of data. As an example, sampling of the Arabidopsis transcriptome using the 454 technology (Weber et al. 2007) demonstrated an unbiased representation of transcripts which enabled analysis of gene expression, discovery of genes and annotation of the genome. Furthermore this technology has proved useful in the discovery of high quality single nucleotide polymorphisms as well as novel alternative splicing events. All in all the new research technologies in the context of large-scale DNA sequencing create a foundation for deeper understanding of how genes and their predicted proteins give rise to biological form and function.

Metabolic engineering of carbohydrate partitioning and turnover

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Sugarcane is one of the most productive crops world-wide. In high-sucrose varieties more than 60% of its dry weight is accumulated as sucrose in the culm. As commercial sugarcane varieties represent polyploid and highly heterozygous genotypes, sugarcane breeding is to a certain extent difficult. Genetic engineering might therefore enable to further increase the sugar content by manipulating targeted enzymes influencing sucrose accumulation. To understand the regulation of carbohydrate partitioning, transgenic sugarcane clones were generated where key steps of carbohydrate metabolism were down-regulated using antisense RNA or RNAi approaches:

- to repress the partitioning of carbon towards respiration
- to minimize futile carbon cycling between sucrose, hexoses, and intermediates of respiration
- to optimize the partitioning of carbon between cell wall polysaccharides and sucrose
- to enhance the flux through the sucrose biosynthetic pathway

Several examples will be discussed emphasising differences in the regulation of carbohydrate partitioning between sugarcane and other more widely studied species such as Arabidopsis, potato and tobacco and describe approaches where it was possible to almost double the sucrose contents in transgenic sugarcane.

The regulation of starch turnover seems to be influenced by the amount of phosphate that is attached to the starch molecules as well as by both, the processes of starch phosphorylation and dephosphorylation. Examples will be given to illustrate that this seems to be a general principle in the regulation of storage polysaccharide turnover.

Opening session

Plants for the Future

Torben Bo Toft Christensen, Project and Innovation Office, Landbrugsrådet

Plants for the Future is a European Technology Platform that aims to strengthen the European Plant Research and Innovation Area, mobilizing support – both public and private – at the European and regional level. It aims to involve and motivate a wide cross-section of stakeholders to cooperate in common research projects in FP 7 (Seventh Framework Programme in EU). The platform involves companies, research institutions, farmers organizations, regulatory bodies, education and communication, financial, consumer and environmental groups.

The Danish Plants for the Future platform is now being launched with support from the Danish Agency for Science Technology and Innovation. The consortium is formed by The Danish Agricultural Council, Plant Biotech Denmark, Copenhagen University Faculty of Life Sciences, Udviklingscenter Årslev, Øresund Food Network and The Danish Agricultural Advisory Service.

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Expert communication with the public: Modernist ideals vs. post-modernist attitudes

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Research indicates that, in general, Europeans are sceptical towards biotechnology. With the Danish population at large, this scepticism follows a 'Frankensteinian' argument, involving notions such as uncertainty, danger and risk (Hviid Nielsen et al. 2002). Previous studies suggest that in dealing with biotechnology the Danish press uses this argument as a framework for its metaphorical and discursive constructions either in supporting or rejecting the technology (Holmgreen (2008); Holmgreen & Vestergaard (submitted)).

Despite the identification of risk as one of the major sources of scepticism, and hence the possibility for the biotech community of discursively and metaphorically addressing this point, the community continues nonetheless to experience a lack of popular support (Interviews conducted with Danish biotech experts in January 2005).

The paper approaches this problem by investigating whether the lack derives from the community's own inability to metaphorically construct a culturally and socially acceptable image of the technology and whether this inability is rooted in underlying paradigmatic differences between the scientific community and the public (Cook et al. 2004; Wynne 2001).

Theoretically, the analysis is based upon Conceptual Metaphor Theory (Lakoff and Johnson 1980/2003), including the concept of 'motivation', propounded by among others Radden and Panther (2004), to establish the conceptual as well as the culturally and socially contingent aspects of metaphor use. Furthermore, the focus on pairs of values has proven valuable for analysing whether the use of metaphors reflects commonly accepted value pairs in Danish society that would potentially make a readership accept the arguments presented (Holmgreen & Vestergaard (submitted)).

The data for the analysis are a number of interviews given by prominent experts in the biotech field and published in 2002 by Biotekcenter, a co-operative information unit set up by among others Aventis and Monsanto, two major players in the biotech industry (Christiansen 2002).

References:

Christiansen, J. L. (ed.) (2002), *11 Attitudes to the Crops of the Future*. Biotekcenter
Cook, G. et al. (2004), "The scientists think and the public feels": expert perceptions of the discourse of GM food. *Discourse and Society* 15: 433-449
Holmgreen, L. (2008) Biotech as 'biothreat'? - metaphorical constructions in discourse. *Discourse and Society* 19
Holmgreen, L. & Vestergaard, T. (ms), Evaluation and audience acceptance in biotech news texts. Submitted to *The Journal of International Applied Linguistics*
Hviid Nielsen, T. et al. (2002), Traditional blue and modern green resistance. In Bauer, M. W. et al. (eds), *Biotechnology – The Making of a Global Controversy*, 179-202. Cambridge: Cambridge University Press
Lakoff, G. & Johnson, M. (1980/2003), *Metaphors We Live By*. Chicago: Chicago University Press
Radden, G. and Panther, K. (eds) (2004), *Studies in Linguistic Motivation*. Berlin and NY: Mouton de Gruyter
Wynne, B. (2001), Creating public alienation: expert cultures of risk and ethics on GMOs. *Science as Culture* 10: 445-481

Plants for Health

Towards regulating isoflavonoid biosynthesis

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Isoflavonoids are natural products produced almost exclusively by plant species belonging to the legume family and play an important role in the interaction with environmental micro-organisms. Isoflavonoids are antioxidants and have additional phytoestrogenic activity. Inclusion of isoflavonoids in the human diet, mainly as soybean products, is linked to various health-promoting effects such as reduced incidence of breast and prostate cancers, and prevention of osteoporosis. Consequently, isoflavonoid preparations are popular as dietary supplements. While the various biosynthetic enzymes involved in isoflavonoid production have been identified, the regulation of their expression is still poorly understood. We aim to identify genes encoding regulators, such as transcription factors, that control isoflavonoid production in legumes. Our project involves the development of a Lotus transcription factor DNA micro-array in order to identify the transcription factors regulating isoflavonoid biosynthetic genes as well as genetic approaches to better understand the factors that contribute to isoflavonoid production. The results from this research provide tools necessary for the future improvement of isoflavonoid production in various legume crops.

Rational screening for health promoting effects of bioactive compounds in plants

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The general notion of plants having beneficial effects on human health is generally accepted, but much remains to be learned to fully explain and exploit the beneficial effects of plants and plant-derived compounds in the context of daily food intake and nutrition. Plants are a rich source of bioactive compounds that directly by influencing cellular metabolism and whole body homeostasis may promote human health and/or prevent typical life-style associated disorders such as obesity and the obesity related metabolic syndrome, inflammation and neurodegenerative diseases.

Because of their long history of human use, bioactive plant compounds are attractive as food supplements or possible ingredients in future functional food. In addition, plants constitute a still unexploited source of unusual molecules that may serve as leads for the development of novel pharmaceuticals. A consortium consisting of partners from Aarhus University, University of Southern Denmark, Developmental Center Aarslev and two Danish SMEs, Rheoscience A/A and Visiopharm A/S has recently obtained support from The Danish Council for Strategic Research, Program Commission on Food and Health to investigate the possible beneficial effects of bioactive molecules in plants. The work is based on previous work by several of the consortium members supported by local and international funding agencies where we have identified a number of promising plants. These plants will be further investigated in the present project which aims to i) provide novel in depth knowledge of the molecular basis for the health promoting effects of plant constituents, ii) provide information on the best cultivation methods to optimize the content of healthy ingredients, iii) provide information on compounds that may be included in future functional food, and iv) identify novel compounds that may serve as leads for the development of novel drugs.

The consortium comprises partners with expertise ranging from cultivation of plants, identification of bioactive compounds, cell based screening systems, image analysis to efficacy testing in animal models. We have established *in vitro* screening platforms that allow efficient and rational screening for compounds affecting metabolic performance, and inflammation. These platforms will be extended through novel innovative screening approaches using the nematode *C. elegans*, *in vitro* generated pig muscle cells and organotypic hippocampal slice cultures securing a much broader range of detectable biological activities. In this lecture I will briefly describe the organization of the consortium, our selection of plants, and the rationale behind the different approaches we will use.

Do bioactive compounds in food have disease reduction potentials?

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One of the major global challenges to our quality of life (a healthy, good and long life) and to the wellfare in future societies is the rapidly developing 'epidemic' of chronical and life-style related metabolic diseases (obesity, diabetes, cardiovascular diseases, allergies and certain cancer types). This 'life-style paradox' is one of the major drivers behind the strongest trend in the international food industry: new healthy food products that can help counteract this negative development in the health/disease balance at both individual and population levels.

The development of new health promoting and/or disease preventing foods will be based on two different approaches: (1) the documentation of intrinsic bioactive compounds in existing types of food, having positive or negative health effects on consumers, and (2) the addition of specific health-promoting bioactive ingredients to food products (commonly discussed as functional foods). Crucial for both approaches is the appropriate documentation of the specific health effects – not only as mode-of-action and proof-of-concept studies but also efficacy studies in human intervention studies and clinical studies. In most parts of the world, more transparent regulatory procedures are now being implemented for the approval of true health claims that may be used for future well-documented products of this kind.

It's now scientifically well-documented and increasingly accepted among consumers that a well-balanced immune system is one of the most important determinants for a sustained healthy condition of the human body. Our immune system plays a key role in the interactions between the food, the gut flora and the gradual development of a chronic low-grade inflammation. The latter seems to be causally involved in an increasing imbalance of the inflammatory and oxidative processes leading to obesity and diabetes and severe complications like cardiovascular diseases.

Research into food bioactives that may have protective or preventive effects on the immunological imbalance triggered by our modern life-style has received tremendous interest in recent years, and numerous concepts are being developed. However, from a documentation and efficacy point of view, a major challenge is the phenomenon that the causes of all the metabolic diseases are multifactorial – and, therefore, single bioactive compounds may not be the solution – unless they have multiple effects.

Three examples of bioactive components that may have multiple effects will be discussed:
(1) Probiotic bacteria and prebiotic compounds, (2) Milk peptides, and (3) Phenolic components from plants.

Modern NMR techniques for characterization of plant products and bioactive constituents

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Plant extracts, including pharmaceutical preparations from medicinal plants, are valued products that often represent extremely complex mixtures containing numerous chemical entities in strongly varying concentrations. It is important to obtain a detailed knowledge of chemical composition of such mixtures, i.e., plant metabolomes, for a number of reasons. One is that pharmacological activity of herbal medicine usually cannot be related to a single extract constituent, as many different compounds – directly or indirectly – may affect the biological effect. Commercial herbal preparations are often standardized using a single or a few constituents, but may be extremely variable with respect to other constituents. It is therefore important to achieve means of comprehensive description of these complex mixtures and to establish methods for rapid identification of constituents that account for observed variability. Another reason for seeking rapid identification of plant metabolome components, often present in very small amounts, is from the drug discovery perspective. Traditionally, natural-product based drug discovery has involved often very laborious preparative-scale fractionations, unfortunately often ending with purification of already known or trivial constituents. Exact knowledge of extract composition achieved at an early stage of an investigation, including rigorous structure determination of constituents without actually isolating them, can solve many frustrating problems inherent to natural product research. Finalally, studies of plant metabolomes are important in relation to clarification and engineering of metabolic pathways as well as geno- and phenotyping.

This lecture will describe how hyphenated NMR (nuclear magnetic resonance) methods, including extended HPLC-PDA-MS-SPE-NMR-CD hyphenation, as well as multivariate analysis of NMR data, can provide rapid and fairly comprehensive global description as well as in-depth knowledge of individual chemical structures present in complex mixtures of plant origin using minute sample amounts.

Cyanogenic glucosides in primary and secondary metabolism

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Cyanogenic glucosides (CNglcs) are a small group of bioactive natural products (secondary metabolites) present in crop plants like sorghum and in stone fruits like almonds. Upon damage of the plant tissue, the CNglcs are immediately hydrolyzed into hydrogen cyanide and ketones and thus provide an instant defence against herbivores and micro organisms. We discovered that CNglcs play a number of other important functions. In bitter almonds the CNglc amygdalin produced in the tegument is swiftly degraded when the kernel germinates and used as a nitrogen buffer in primary metabolism during seedling development. In sweet almonds, CNglc synthesis in the tegument is as active as in the bitter genotypes, but the CNglc is degraded upon passage of a □-glucosidase rich cell layer facing the nucellus and thereby provides nitrogen to the developing kernel. Nitrilases of the NIT4 type participate in the endogenous turn-over of the CNglcs. Different heteromers of the NIT4 nitrilases have different substrate specificity. This type of combinatorial biochemistry enables the plant to specifically control the endogenous turn-over of CNglcs without release of hydrogen cyanide. Some insects like Zygaenae species feed on cyanogenic plants and have acquired the ability to sequester the CNglcs or even to synthesize CNglcs *de novo*. The Zygaena female attracts males by emission of hydrogen cyanide and selects a male with a high CNglc content as mating partner. During mating, the male transfers CNglcs as a nuptial gift to protect the fertilized eggs. In an evolutionary context, the distinction between primary and secondary metabolism thus becomes blurred and eventually meaningless.

Molecular Plant Microbe Interactions

Presentation of the Centre for Carbohydrate Recognition and Signalling (CARB)

Jens Stougaard, Department of Molecular Biology, University of Aarhus

The centre aims at understanding the interactions between cells and organisms by investigating the role of cell wall glycans and polysaccharides exposed on cell surfaces, and polysaccharide signal molecules secreted as part of a complex interaction between organisms. The aim and perspective are to determine structural requirements for recognition of complex polysaccharides and the role of ligand-receptor interactions in the relationships between different cells and organisms. Carbohydrate signals and extracellular polysaccharides play an important role in cell-to-cell communication processes and are equally important for the organisation of multicellular organisms and the development of their specialised organs and tissues.

The Centre will undertake an integrated functional characterisation of receptor-ligand mechanisms mediating recognition of surface-exposed polysaccharides and subsequent signal amplification. Experimental approaches from chemical biology, bioinformatics, structural biology, functional genomics, proteomics, bioorganic chemistry, and nanobioscience tools will be used in an interdisciplinary environment combining experience from laboratories with complementary research expertise.

The Centre activities will focus on two model organisms: zebrafish and the legume plant *Lotus japonicus*, and their interactions with pathogenic and symbiotic microorganisms. The centre comprises a number of research groups at universities in Denmark, The Netherlands and New Zealand.

See www.carb.dk for additional information and a list of partners and participants.

Molecular Plant Microbe Interactions

Global spread of new wheat rusts

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On April 12th, 2007, FAO issued a warning that the worlds wheat production was threatened by wheat rust, which had emerged in Uganda in 1999, and since then spread to Kenya, Ethiopia and Yemen (http://www.fao.org/newsroom/en/news/2007/1000537/index.html). The alarm was based on a new mutant of wheat stem rust, *Puccinia graminis* f.sp. *tritici*, which was virulent to up to 80% of present wheat cultivars world-wide. So far, a range of stem rust resistances in wheat, introduced during the Green Revolution, has been very effective in controlling the stem rust disease. As consequence, international research institutions like CIMMYT and ICARDA has launched a "Global Rust Initiative" (http://www.globalrust.org), which contain new efforts on plant breeding and rust disease management, to prevent further spread and damage.

Stem rust is one of three wheat rusts, which are all biotrophic and capable of long-distance spread by wind and human activity. Although stem rust has got most attention in the media at present, the most damaging wheat rust on the global scale is *Puccinia striiformis* f.sp. *tritici*, causing yellow rust on wheat. A new strain of this fungus, adapted to warmer temperatures and extremely aggressive, has spread globally within three years or less. Since 2000, annual yield losses of more than 10M tons of wheat have been experienced in Northern America due to the new strain, and in West Australia, which was previously considered too warm for this fungus, yellow rust is now number one disease on wheat. The new strain has not yet caused epidemics in Europe, most likely due to the presence of disease resistance, at present highly effective but of non-durable types. The new challenges by the wheat rusts should be met by enhanced efforts on breeding for disease resistance world-wide and coordinated research in sustainable disease management strategies.

Molecular Plant Microbe Interactions

Colonisation of barley roots with DsRed expressing *Fusarium avenaceum* and GFP expressing *Fusarium culmorum*-competition and effect on toxin production

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Fusarium sp. are soil borne plant pathogens that are infamous for causing root rot, seedling and head blight in cereal species. The diseases have attracted a lot of attention due to loss of crop yield and price penalties because the fungi produce mycotoxins that are deposited in the grain. Compounds belonging to the trichothecene class of metabolites and zerealenone, a polyketide, cause diseases in mammals when present in feed or food grain products. *Fusarium culmorum* and *Fusarium avenaceum* are frequently found in Danish soils and are commonly found in cereal grains. At the phylogenetic level *F. culmorum* and *F. avenaceum* belong to different sections and produce different secondary metabolites.

A study was carried out to determine if *Fusarium* species commonly found together in the grain and with different toxin profiles are antagonistic. Two reporter strains: *F. culmorum* expressing the green fluorescent protein (GFP) and *F. avenaceum* expressing the red fluorescent protein (DsRed) were generated. A sterile hydroponic glass culture system was used to grow barley seedlings and for the application of spores from the fungi individually or together, to determine any competitive effects during infection. Confocal microscopy was carried out to register in real time the colonization process of these fungi in barley seedling roots. Infection of agar embedded barley roots allowed us for the first time to obtain confocal laser scanning microscopic recordings of *F. culmorum* and *F. avenaceum* hyphae growing inside root cells. The primary region of entry appears to be the root hair zone. Both fungi grow in the intercellular space and can directly enter living plant cells. The fungi do not appear to be antagonistic but *F. culmorum* appear to have a faster growth rate than *F. avenaceum*. Secondary metabolite profiles for *F. culmorum*, *F. avenaceum* and the combination of the two species under infection of barley roots were obtained by RP-HPLC. Aurofusarin were produced by *F. culmorum* under barley root infection while aurofusarin was not produced by *F. avenaceum*. The tendency was confirmed by qualitative RT-PCR of the *pks*12 gene the originator of aurofusarin.

Key words: *Fusarium species*, *DsRed*, *GFP*, reporter strains, fluorescence and confocal microscopy, root infection process, interaction

Mycorrhiza – for improving phosphate uptake

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Plants take up phosphorus (P) as soluble orthophosphate (Pi). Although the total amount of P in soil may be high, most of it is strongly adsorbed or chemically fixed, thereby rendering Pi concentrations in the soil solution low (~1 µM) and often growth-limiting. Plants have developed several adaptive mechanisms to overcome this Pi deprivation including enhanced expression of Pi transporters, and the association of roots with soil-borne fungi forming the arbuscular mycorrhizal symbiosis. Their extra-radical mycelium serves as an extension of the root system, thus enabling the host plant to access slowly diffusible Pi from a soil volume encompassing the mycorrhizosphere which greatly exceeds that of the rhizosphere. The formation of intracellular fungal structures in the root cortex triggers the expression of mycorrhiza-inducible Pi transporters and both are prerequisites for a functional symbiosis. Pi can be absorbed via two different pathways: the direct pathway through the epidermis including root hairs at the soil/root interface and the mycorrhizal pathway through the AM fungal mycelium and colonized cortex cells. The relative importance of the two pathways is under strong influence by the plant genotype and by the soil P status. Current research projects aims to investigate how Pi uptake can be optimized by manipulating the effectiveness and relative impact of the two Pi uptake pathways.

The power of genetics in signalling studies

Hans Thordal-Christensen, Dept. of Agricultural Sciences, LIFE-KU

The behaviour of living organisms is controlled by signalling processes. Traditionally, signalling is seen as linear pathways through which a stimulus activates a cell response. However, detailed studies of signalling often show that this is an oversimplification. Signals are more likely to be transmitted through networks that, after exposure to several stimuli, integrate and coordinate a shared response. Since such networks can be difficult to decipher, general methods for studying these are much needed.

In our work on pathogen defence signalling in plants, we explore the *Arabidopsis* syntaxin double mutant *syp121 syp122*. This mutant is lesion-mimic due to a wide suite of constitutively active pathogen defence signals. In order to learn more about these signals, we have re-mutagenised *syp121 syp122*. This has allowed us to collect 240 *syp121 syp122 ssd* triple mutants, where mutations in the "third" genes partially rescue the lesion-mimic phenotype. Through initial analysis of these triple mutants, we have map-based cloned six *SSD*-signalling genes and genetically demonstrated the identity of another two. In total we have identified 48 mutant alleles in these eight genes. In parallel, we have crossed mutant alleles of defence signalling genes that we expected to be required for the lesion-mimic phenotype, into *syp121 syp122*. This allowed us to identify another five genes required for *syp121 syp122* lesion-mimic phenotype.

In order to unravel how the known and the not yet identified defence signalling genes are positioned in the signalling network, we studied F_2 populations of crosses between triple mutants. Generally 1/16 of these F_2 plants are homozygous quadruple mutants. The performance of these quadruple mutants reveals the relative position of the mutated genes in the signalling network: if the quadruple mutant *syp121 syp122 ssdX ssdY* performs better than the triple mutant parents, then the SSDX and SSDY proteins are assumed to be distantly associated in the signalling network. Otherwise, if *syp121 syp122 ssdX ssdY* performs similarly to the triple mutant parents, then SSDX and SSDY are assumed to be closely associated. Using this to our knowledge novel approach, we have begun to build a scaffold for a signalling network.

We anticipate that this approach that could be of general value in many contexts, will help us to better understand the complex signalling in plant pathogen defence. More specifically, it will be fruitful in discovering novel signalling components, and in resolving how known and novel signalling components interact. Not least, the approach will help pointing out the most interesting novel components.

Final session

Globalization, biofuels and bioeconomics – implications for Danish agriculture

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Globalization means that trade, investments, mergers, labor movements etc. across borders are increasing. Globalization also means that international competitiveness becomes more and more important, and that comparative advantages among countries are revealed. Agriculture all over the world will persistently have to develop new competitive strengths in order to cope with the globalization pressure.

Biofuel production is an increasing business in many countries all over the world. High oil prices, new technologies in enzyme production, and security policies have increased the focus on production of ethanol based on cereals as raw materials. Furthermore the political pressure to reduce CO2-emission has even more increased the efforts to commercialize biofuel production.

In a global and liberalized world agricultural progress must be evaluated in both an economic and a biologic dimension to justify its sustainability. Biofuel has technical and biological potentials to be developed further, but using food as a resource for energy production has also economic limitations.

Danish agriculture has been a global or international business for many years. A very high export orientation and remarkably world market shares have been developed through competitive advantages. The question now is: Is biofuel a competitive and sustainable business for Danish agriculture at all?

Posters: Products

1. WallNet: Heterologous expression of Arabidopsis glycosyltransferases in *Nicotiana benthamiana*

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Plant cell walls are composed mainly of polysaccharides. The polysaccharides play essential roles in growth and development, response to the environment, and interactions with symbionts and pathogens. From the human point of view, cell wall polysaccharides are the most abundant source of biomass for food, fiber and biofuel.

Despite rather detailed information of the structure of the cell wall polysaccharides, little is known about their biosynthesis. The key process of the biosynthesis is catalyzed by the action of glycosyltransferases (GTs). Plants need an enormous amount of GTs to synthesize the complex polysaccharides present in the cell walls. However, only a few GTs have had their activity demonstrated. In the model plant, *Arabidopsis thaliana*, approximately 450 GT genes have been identified based on their sequence and deposited to the CAZy database (Carbohydrate Active Enzyme: www.cazy.org). In 'WallNet', an EU FP6 sponsored consortium, we selected 105 putative GT genes from Arabidopsis genome with an aim to elucidate biochemical activity of the corresponding proteins. Most of the cDNAs were cloned into Gateway entry vector and further moved to an expression vector for the Agrobacterium-mediated transient expression in *Nicotiana benthamiana*. We have expressed 10 cDNAs and identified novel GT activity for 3 cDNAs. We are currently working on more detailed characterization of the activity and synthesized products.

2. Characterization of CBM20 starch binding domains from three different origins

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Starch-binding domains (SBDs) from CBM family 20 display high phylogentic diversity, being encountered in archae, bacteria, fungi, and plants. Members of this family are typically found in amylolytic and related enzymes and contain around 100 amino acid residues folded as a well conserved anti parallel b-barrel [1]. A major function of the SBD is to bind onto granular starch thereby increasing the local substrate concentration, however, the precise impact of SBD on starch degradation remains to be elucidated.

Despite the high number of sequences and the diversity in family CBM20, it is not entirely clear if functional or specificity differences exist within the family. Hence, this study aims at casting light on this question by a comparison of representatives of CBM20 from various sources. To date, no CBM20 of plant origin has been characterised, but recently, we identified a putative CBM20 in *Arabidopsis thaliana* glucan water dikinase 3 (GWD3), an enzyme responsible for starch phosphorylation [2], and its starch binding function has been demonstrated. The GWD3-SBD will be compared with another CBM20 member, the SBD from the human glycogen phosphatase laforin (LAF-SBD) [3]. The SBD from glucoamylase (GA) of *Aspergillus niger* is included as a reference. The three SBDs were produced as GST-fusion proteins in *Escherichia coli.* The GWD3-SBD is also produced in *P. pastoris.* The interaction with soluble carbohydrates will be initially probed by surface plasmon resonance to determine *K*d values for cyclodextrins as model ligands. Binding experiments to insoluble supramolecular substrates will be evaluated using an array of starches including phosphorylated starches. Pivotal residues in the binding activity of the SBDs will be explored by site directed mutagenesis. Finally, the binding onto starch granules of different botanical sources and genotypes will be examined using confocal laser scanning microscopy.

References:
1. Giardina, T., Gunning, A.P., Juge, N., Faulds, C.B., Furniss, C.S.M., Svensson, B., Morris, V.J., and Williamson, G. 2001. Both binding sites of the starch-binding domain of *Aspergillus niger* glucoamylase are essential for inducing a conformational change in amylose. J. Mol. Biol. 313: 1149-1159.
2. Mikkelsen, R., Baunsgaard, L., and Blennow, A. 2004. Functional characterization of alpha-glucan, water dikinase, the starch phosphorylating enzyme. Biochem. J. 377. 525-532.
3. Ganesh, S., Tsurutani, N., Suzuki, T., Hoshii, Y., Ishihara, T., Gado-Escueta, A.V., and Yamakawa, K. 2004. The carbohydrate-binding domain of Lafora disease protein targets Lafora polyglucosan bodies. Biochem. Biophys. Res. Commun. 313: 1101-1109.

3. Production in *Pichia pastoris* and characterization of recombinant barley limit dextrinase

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The debranching enzyme limit dextrinase (LD) belongs to the glycoside hydrolase family 13 (GH13). It catalyses hydrolysis of α-1,6-glucoside linkages in limit dextrins, pullulan and amylopectin. The activity of limit dextrinase is important in the starch degradation during barley seed germination. The concerted action of limit dextrinase, α-glucosidase, α- and β-amylase facilitates the supply of glucose in the germinating seed as energy source for the embryo and growing plantlet (Kristensen et al., 1999). This study presents the first reported recombinant production of active barley limit dextrinase. The open reading frame of the gene encoding limit dextrinase was initially amplified using RT-PCR (Jensen, 2004). The gene fragment encoding the LD lacking its signal peptide was sub-cloned into the expression vector pPIC9K (Invitrogen) under the control of the AOX1 promoter. We optimized the feed batch protocol (Stratton et al., 1998) which enabled the production of active recombinant barley limit dextrinase in a 5 l Biostat B fermentor. Limit dextrinase was purified by a two-step purification strategy. Affinity chromatography using β-cyclodextrin (β-CD) crossed linked to a Sepharose matrix was followed by size exclusion chromatography (SEC) on a Sephadex-G200 column. The purification resulted in 18 mg pure protein. The molecular mass of the recombinant limit dextrinase was approximately 98 kDa which corresponds to the molecular mass of the native barley enzyme. The identity of the recombinant LD was verified by MALDI-TOF-MS MS/MS. The kinetics constant of the purified enzyme was determined using pullulan as substrate and they were consistent with the reported value of native LD.

The established system for the recombinant expression of limit dextrinase using *Pichia pastoris* facilitates future investigation of structure/function relationships using site-directed mutagenesis and truncations guided by information of family GH13 domain architecture as retrieved from the CAZy-database.

This work is supported by a Ph.D. scholarship from Biocentrum-DTU to MBVC, the Danish Natural Science Research Council and the Carlsberg Foundation.

References:
Kristensen, M.; Lok, F.; Planchot, V.; Svendsen, I.; Leah, R.; Svensson, B. (1999): Isolation and characterization of the gene encoding the starch debranching enzyme limit dextrinase from germinating barley. Biochim. Biophys. Acta 1431, 538-546.
Jensen, M. T. (2004): Structure and function of barley limit dextrinase and mutational analysis of barley α-amylase isozyme differences and interaction with branched oligosaccharides. Ph.D. thesis. University of Southern Denmark.
Stratton, J.; Chiruvolu, V.; Meager, M.(1998): High cell-density fermentation. In “Pichia Protocols” Edited by Higgings, D. R. & Cregg, J. M. Vol. 103, 107-120, Humana Press inc. Totowa, New Jersey, USA.

4. Mutational analysis of the "Sugar Tongs" carbohydrate binding surface site in barley α-amylase 2

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The two barley isozymes, AMY1 and AMY2 are among the best characterizeḍ α-amylases, the two isozymes share 80% sequence identity but differ distinctly in function and stability. Structural studies of both barley α-amylase 1 (AMY1) and the inactive catalytic nucleophile mutant D180A AMY1 with substrate and substrate analogues have shown that AMY1 comprises two surface carbohydrate binding sites in addition to the substrate binding cleft (Robert et al., 2003, 2005; Bozonnet et al., 2007). In the recently discovered "sugar tongs" site on domain C of AMY1, Tyr^{380} captures oligosaccharide ligands (Robert et al., 2003, 2005). The "sugar tongs" are reported to ensure binding onto starch and assist in disentangling the α-glucan chains facilitating the enzymatic attack, to guide the polysaccharide chains to the active site, and to be candidates for allosteric regulation of enzyme function through carbohydrate interactions. However, the "sugar tongs" site is empty in the crystal structure of the AMY2/acarbose complex (Kadziola et al., 1998), resulting in the proposal that Pro^{376}_{AMY2} corresponding to Ser^{278}_{AMY1}, hinders a conformational shift of Tyr^{378}_{AMY2} by rigidifying the preceding loop thus preventing oligosaccharide binding (Robert et al., 2005). M6 (A42P) was made to a problem of solve poor secretory expression of wild-type rAMY2. The mutant M6 was virtually identical with AMY2 wild-type in stability and enzymatic properties and was secreted from *Pichia pastoris* in 20-fold higher amounts than rAMY2 (Fukuda et al., 2005).

Tyr^{378} of the "sugar tongs" surface binding site in M6 was investigated in single Y378A, Y378M, and Y378F mutants; in addition P376S was examined as an AMY1 mimic. All sugar tong mutant showed very similar specific activity on insoluble blue starch compared to wild-type AMY2 and M6. K_d of β-cyclodextrin binding was determined by surface plasmon resonance and showed similar values for P376S and Y378A comparable to values of 0.18 mM for AMY2 and 0.2 mM for M6. However, Y378A showed reduced binding affinity to barley starch granules compared to M6. This analysis is currently in progress for the M6 other mutants.

This work was supported by H.C. Ørsted postdoc fellowship from the Technical University of Denmark, the Danish Natural Science Research Council, the Danish Research Council for Technology and Production Science, and the Korea Research Foundation Grant funded by the Korean Government (MOEHRD) (KRF-2005-214-D00275).

References:
Bozonnet, S., Jensen, M.T., Nielsen, M.M., Aghajari, N., Jensen, M.H., Kramhøft, K., Willemoës, M., Tranier, S., Haser, R., & Svensson, B. 2007. *Febs j.* **274**, 5055-5067.
Fukuda, K., Jensen, H.M., Haser, R., Aghajari, N., & Svensson, B. 2005. Protein engineering, design & selection. **18,** 515-526.
Kadziola, A., Søgaard, M., Svensson, B., & Haser, R. 1998. *J. Mol. Biol.* **278**, 205-217.
Robert, X., Haserm, R., Gottschalk, T., Ratajczak, F., Driguez, H., Svensson, B., & Aghajari, N. 2003. *Structure*, **11**, 973-984.
Robert, X., Haser, R., Mori, H., Svensson, B., & Aghajari, N. 2005. *J. Biol. Chem,* **280**, 32968-32978.

5. Characterization of the CSLH gene family

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The cell wall is comprised of different polymers of which polysaccharides are the most abundant. The polysaccharides can be divided into different types: cellulose, hemicellulose and pectin. The biosynthesis of these polymers is still poorly understood and only a small number of the glysosyltransferases have been characterized at the biochemical level. Grasses have type 2 walls that differ from the walls of most other plants. The major known differences are that grass walls are rich in feruloylated arabinoxylan and beta-glucan but low in xyloglucan and pectin. The *CSL* (Cellulose Synthase Like) superfamily of genes appears to encode backbone synthesizing enzymes involved in biosynthesis of mannans, glucans etc. Interestingly, grasses contain two groups of *CSL* genes, *CslF* genes and *CslH* genes, which are not found in Arabidopsis. We intend to carry out an investigation to determine the role of the *CslH* genes in *Oryza sativa* and *Brachypodium distachyon*.

The project described here is aimed at characterizing the role of CslH proteins in grasses, their biochemical function, and their potential interaction with other proteins. The genome of *O. sativa* contains three *CslH* genes. Two different knockout lines of *OsCslH1* showed a distinct pattern of stunted growth and development, with one line having a more severe phenotype. However, it has not yet been possible to unambiguously relate the phenotype to the knock-out in *OsCslH1*. Therefore, as an alternative strategy we plan the production of transgenic rice plants, with an RNAi construct targeting all three members of the *OsCslH* gene family. Work is also planned using the cereal model plant *B. distachyon*. This will involve the possible use of T-DNA and TILLING collections for identification of loss of function mutants to work as a comparison for the results obtained with *O. sativa*.

As a tool to investigate the distribution of CslH isoforms in rice tissues and examining potential protein complex formation, we have raised a polyclonal antibody against a region of OsCslH1 with high sequence similarity to OsCslH2 and OsCslH3. Characterization of the antibody is in progress. To investigate protein complexes, rice plants will also be transformed with tagged versions of *OsCslH1.* To investigate the biochemical activity and subcellular localization of CslH proteins, *OsCslH1* cDNA is cloned for transient expression of OsCslH1 in tobacco.

6. Structure-function relationship investigations and protein engineering of carbohydrate binding surface sites of barley α-amylase 1

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α-Amylases (EC. 3.2.1.1) catalyze the hydrolysis of internal 1,4-α-glucosidic linkages in starch and related oligo- and polysaccharides with retention of the anomeric configuration. Structural studies of both barley α-amylase 1 (AMY1) and the catalytically inactive D180A AMY1 with substrate and substrate analogues have shown that AMY1 comprises two secondary oligosaccharide binding sites (Starch binding site 1 and 2, SBS1 and SBS2) in addition to the substrate binding cleft (Robert et al., 2003; Robert et al., 2005). SBS1 on the catalytic domain A contains two contiguous tryptophans (Trp278 and Trp279). SBS2 is also called “a pair of sugar tongs” and located in the C-terminal domain where an essential tyrosine residue (Tyr380) has a key role in binding oligosaccharides.

Surface plasmon resonance showed a 7-fold increase in apparent K_d to β-cyclodextrin for the mutant Y380A and the pseudotetrasaccharide acarbose did not bind to SBS2 in the crystal structure of Y380A (Bozonnet et al., 2007). The joint role of SBS1 and SBS2 with the substrate binding cleft for hydrolysis of starch by barley α-amylases is not known. The aim of this project is to study a series of relevant structure-guided single and multiple AMY1 mutants of key aromatic residues Tyr380 at SBS2 and Trp278Trp279 at SBS1 in combination with the inactive catalytic nucleophile mutation (Glu180Ala). All AMY1 mutants were expressed in *Pichia pastoris*. K_d for malto-oligosaccharides, β-cyclodextrin (β-CD), and acarbose will be analysed by surface plasmon resonance (SPR) and fluorometric titration. Adsorption to the surface of starch granules will be examined using confocal laser scanning microscopy.

This work was supported by the EU 5th Framework Program to the project CEGLYC (QLK3-CT-2001-00149), the Danish Natural Science Research Council, and a Ph.D. scholarship from the Technical University of Denmark (to M.M. Nielsen).

References:
Bozonnet, S., Jensen, M.T., Nielsen, M.M., Aghajari, N., Jensen, M.H., Kramhøft, B., Willemoës, M., Tranier, S., Haser, R., & Svensson, B. 2007, *FEBS J.*, **274**, 5055-5067.
Robert, X., Haser, R., Gottshalk, T.E., Ratajczak, F., Driguez. H., Svensson, B., & Aghajari, N. 2003. *Structure*, **11**, 973-984.
Robert, X., Haser, R., Mori, H., Svensson, B., & Aghajari, N.2005 *J. Biol. Chem*. **280**, 32968-32978

7. Iron Bioavailability from Partially Degraded Phytate Globoids Measured in CaCo-2 Cells

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Most phosphate in rice grains is found in the aleurone layer of the grain and is chemically bound to inositol to form phytate (*myo*-Inositol-1,2,3,4,5,6-hexakisphosphate). Phosphate in this form is poorly digested by monogastric animals because they lack the enzyme (phytase) needed to remove phosphorus from the phytate molecule. Moreover, phytate is a strong chelator of proteins and cations such as calcium, magnesium, iron and zinc, resulting in small, spherical, insoluble complexes called phytate globoids.

In this work we have purified phytate globoids from rice bran and analysed the chemical composition of them by ICP-MS and HPLC. Furthermore, we have used a commercially available phytase from *A. Niger* to investigate if the phytase is actually able to degrade the phytate globoids. The intestinal cell line CaCo-2 was used to measure iron uptake from partially degraded rice bran and rice globoids. The level of degradation required to increase bioavailability of this mineral was thereby determined.

More information can be found in:
Quantitative analysis of phytate globoids isolated from wheat bran and characterization of their sequential dephosphorylation by wheat phytase. Bohn, L., Josefsen, L., Meyer, A.S., Rasmussen, S.K. (2007) *Journal of Agricultural and Food Chemistry* 55: 7547-7552.

8. Intricate interaction between pectins and pathogens

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The cell wall is one of the most important structural components of plants. The wall defines cell shapes, provides strength to withstand the turgor pressure, influences cell development, and serves as the last physical barrier against invading pathogens. Pectins constitute ca. 30% of the cell wall polysaccharides and fill space between the load-bearing cellulose-hemicellulose network.

Much of the previous work of pectin has focused on the structural characterization of these highly complex polymers and their properties as food ingredients. In contrast only limited examples are available where the biological roles of the polymers have been fully elucidated. One such example includes homogalacturonan. This polymer constitutes a backbone of some pectic polysaccharides. Previously studies have identified that oligogalacturonides released after digestion of homogalacturonan by pathogen-derived endopolygalactronase elicits defense response in the host, thereby functioning as an endogenous signal for the host defense activation.

We have recently identified two pectin mutants of *Arabidopsis thaliana*, *arad1* and *xgd1*, defective in pectic arabinan and xylogalacturonan biosynthesis, respectively. Detailed cell wall composition analyses have identified that these mutants lack ca. 70% of the respective polysaccharides in the pectic rhamonogalacturonan I fraction. Albeit these large changes in the wall, the mutants did not show visible growth phenotypes distinct from the wild type, indicating that arabinan and xylogalacturonan are not essential for plant growth under optimal growth conditions. When these plants were subjected to pathogen infections, however, they showed altered susceptibilities as compared to the wild type. *arad1* mutants showed increased susceptibility to the necrotic fungal pathogen *Botrytis cinerea*, while they appear to show the wild-type level of susceptibility to the bacterial pathogen *Pseudomonas syringae*. Preliminary results suggest that arabinan may be involved in signaling and elicitation of the host defense. In contrast, the *xgd1* mutant showed the wild-type level of susceptibility to *B. cinerea*, while it showed unusual induced and increased resistance to *P. syringae*. Exact molecular mechanisms underlining how two pectic polymers relate to two opposite pathogen responses are currently under investigation and will be presented. Our results add new insights into the intricate roles and functions of pectins in host-pathogen interactions.

9. Dietary Fibres and Natural Products; Interactions and Bioavailability

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Beta-glucans are known as hydrocolloid forming fibre polymers and are used as a texture promoting additive in the food industry. Numerous nutritional and clinical experiments indicate positive effects of dietary fibres including beta-glucans on digestion and cholesterol metabolism. In both animal and human models, an increased content in viscous non-starch polysaccharides in the diet is accompanied by an increase in faecal sterols, suggesting that these materials interact with the bile salts present in the gut lumen. This is supported by the significant reduction is in VLDL and LDL (cholesterol).

The interaction between beta-glucan and bile salts has been studied by simple dialysis studies. These results show that the interaction is most likely to be related to hygroscopic and hydrophobic properties of the viscous fibres. This raises the question: What happens to all the hydrophobic natural products which we ingest as part of our food? If natural products interact with fibres in a similar fashion as bile salts this may influence their uptake and metabolism and thus magnify or reduce the various health claims and the bioavailability of the natural products. The contradicting results obtained e.g. with respect to the health effects of isoflavonoids may reflect such interactions and may serve to increase individual variation.

We have studied binding of vanillin and a series of analogues to barley beta-glucan. The barley beta-glucan preparation is able to retain non ionic aglycons in our dialysis assay, whereas the glucosides of the same compounds are not. This support that the binding property of the beta-glucan is related to the hydrophobic properties of the fibres. The dialysis system we have developed is designed assess the binding under more complex experimental conditions that mimic the presence of enzymes and the different acidic conditions in the intestines and colon. Using this approach we hope to provide a better understanding of the role of plant fibre and natural product interaction for bioavailability and human health.

Part of the BEST (Build a Healthy Life) Project at LIFE.

10. Evaluation of Medicinal Plants from Chile

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Based on the ethnobotanical studies among Mapuches Indians in 1997 a group of researchers at the Faculty of Life Sciences and Faculty of Pharmaceutical Sciences has carried several ethnopharmacological in vitro and in vivo studies of these traditional medicinal plants.
Promising results regarding antidiabetic, antioxidant, antihypertension, and antimicrobial activities have been obtained, and secondary metabolites identified as responsible for the biological activity of these Chilean plants.

In this way, it was shown that plants used traditionally as antibacterial and antiviral remedies showed higher antimicrobial activities compared to the plants not used in this way. For examples plant-extracts and isolated natural compound of Lomatia hirsuta showed complete inhibition of Candida albicans. The highest activity against Penicillium expansum was also observed by leaf extract of Lomatia hirsuta. Other plants are currently being examined for their antimicrobial activity.
Medicinal plants used traditionally as diuretics or antihypertensive, Acaena argentea, Chusquea quila and Pseudopanax laetevirens have shown a complete inhibition of ACE, which supports the traditional use of these plants by Mapuches.

Crinodendron hookerianum, a species used traditionally by the Mapuches to treat diabetes has in vitro showed significant inhibition of insulin secretion from isolated islets of Langerhans. Additional studies of this plant is currently carried out to determine its antidiabetic activity RFS -fraction C isolated from the plant extract, showed to be more potent than insulin.

11. CYP703 is an ancient cytochrome P450 in land plants catalyzing in chain hydroxylation of lauric acid to provide building blocks for sporopollenin synthesis in pollen.

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CYP703 is a cytochrome P450 family specific to land plants. Typically each plant species contains a single CYP703. Arabidopsis thaliana CYP703A2 is expressed in the anthers of developing flowers. Expression is initiated at the tetrad stage and restricted to microspores and to the tapetum cell layer. Arabidopsis CYP703A2 knockout lines showed impaired pollen development and a partial male sterile phenotype. Scanning electron and transmission electron microscopy of pollen from the knockout plants showed impaired pollen wall development with absence of exine. The fluorescent layer around the pollen grains ascribed to the presence of phenylpropanoid units in sporopollenin was absent in the CYP703A2 knockout lines. Heterologous expression of CYP703A2 in yeast cells demonstrated that CYP703 catalyzes the conversion of medium chain saturated fatty acids to the corresponding mono-hydroxylated fatty acids with a preferential hydroxylation of lauric acid at the C-7 position. Incubation of recombinant CYP703 with methanol extracts from developing flowers confirmed that lauric acid and in chain hydroxy lauric acids are the in planta substrate and product, respectively. The data demonstrate that in chain hydroxy-lauric acids are essential building blocks in sporopollenin synthesis and enable formation of ester and ether linkages with phenylpropanoid units. This identifies CYP703 as a P450 family specifically involved in pollen development.

Posters: Productivity

1. Heterologous expression of plant cell wall glycosyltransferases in *Nicotiana benthamiana* as a source of pure recombinant protein

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Heterologous expression of plant glycosyltransferases involved in cell wall biosynthesis has caused considerable difficulties, and this has been a major obstacle in the study of these proteins. However, transient expression of the glycosyltransferases in *Nicotiana benthamiana* has a very high success. In this study we are investigating *N. benthamiana* as an expression system of Arabidopsis glycosyltransferases by the use of transient transformation.

RGXT2 (CAZy GT-family-77) was recently shown to be a (1,3)-alpha-D-xylosyltransferase involved in biosynthesis of pectic rhamnogalacturonan-II by xylosidating the fucose residue in side chain A (Egelund et al., 2006). We have successfully expressed RGXT2 in *N. benthamiana*. RGXT2 localizes to the Golgi vesicles and can be obtained from infiltrated leaf tissue from *N. benthamiana* as microsomes in significant amounts. Interestingly, RGXT2 can readily be assayed in crude solubilized microsomes by the “free sugar assay” method, i.e. xylosyltransferase activity from UDP-xylose onto free fucose. In this assay, xylosyltransferase activity is clearly detected in microsomes prepared from transformed leaves over the background activity in microsomes from non-transformed leaves. The activity determined in this way is comparable with activity determined with protein heterologously expressed in *Pichia* or insect cells. However, the *N. benthamiana* system is more reliable (see poster by Naomi Geshi et al. for examples of how the system is used to characterize other glycosyltransferases) and ensures plant-type post translational modifications.

For detailed characterization of the glycosyltransferases it is highly desirable to have pure protein in reasonable amounts. We have establish a simple and quick protocol using mild solubilization for purifying the RGXT2 protein from *N. benthamiana* by flag-tag affinity purification in amounts and purities highly suitable for activity studies. Further progress in this strategy will be reported.

Reference:
Egelund J, Petersen BL, Motawia MS, Damager I, Faik A, Olsen CE, Ishii T, Clausen H, Ulvskov P, Geshi N. (2006) Arabidopsis thaliana RGXT1 and RGXT2 encode Golgi-localized (1,3)-alpha-D-xylosyltransferases involved in the synthesis of pectic rhamnogalacturonan-II. Plant Cell 18, 2593-607.

2. Visualization of the *RRA* gene family in *Arabidopsis thaliana*: A bioimaging approach

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Two glycosyltransferases (GTs), RGXT1 and RGXT2, that are members of CAZy GT-family-77 have been shown to transfer xylose onto fucose, forming an &alpha-(1,3)-linkage that is a component of the rhamnogalacturonan-II domain of pectin. Together with the (1,3)-&alpha-D-galactosyltransferase from *Dictyostelium discoideum*, these are the only GTs in CAZy GT-family-77 with confirmed biochemical activity. However, it has recently been demonstrated that insertional mutants in two other genes assigned to CAZy GT-family-77, *RRA1* and *RRA2*, have an approximately 20% reduction in the arabinose content of the residual cell wall pellet after enzymatic removal of the pectic and xyloglucan polysaccharides. This preliminary data suggests that both these genes may encode arabinosyltransferases.

We will present further analysis of *RRA1*, *RRA2* and a homologous gene *RRA3.* In addition to a bioinformatic analysis of these genes, we will present data on their sub-cellular localization in *Arabidopsis thaliana* using a YFP fusion protein approach.

3. *RGXT3*: Regio- and stereochemistry of the *in vitro* formed product as revealed by NMR

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A putative glycosyltransferase encoding gene from *Arabidopsis thaliana*, At1g56550, homologous to two recently identified (1,3)-alpha-D-xylosyltransferases (*RGXT1* and *RGXT2*) from *Arabidopsis thaliana* implicated in the synthesis of pectic rhamnogalacturonan II (RG-II) (Egelund et al., 2006), has been heterologously expressed in *Pichia pastoris* and shown to possess xylosyltransferase activity transferring D-xylose from UDP-alpha-D-xylose onto L-fucose. The disaccharide formed was specifically hydrolyzed by alpha-xylosidase, and when analyzed by nuclear magnetic resonance the regio- and stereochemistry of the methyl xylosyl-fucoside disaccharide was shown to be an alpha-(1,3)-linked.

The specificity towards various L-fucoside derivates, designed to mimic the pattern in the A-chain of RG-II, were tested, and found to be similar to those of RGXT1 and –2. We therefore propose that At1g56550 encode a third member of the RG II specific xylosyltransferase (*RGXT*) family, and designate the gene, *RGXT3* accordingly. The apparent presence of three paralogous *RGXT*-genes in *Arabidopsis thaliana*, prompted us to instigate a expression analysis of *RGXT3* using promoter::*gusA* fusions and compared it with the expression profile of the closely related family members *RGXT1* and *RGXT2*.

4. Regulation of mixed-linkage-β-glucan biosynthesis during barley endosperm development

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A molecular and biochemical profile have been carried out to address the level(s) at which the biosynthesis of mixed-linkage (1→3, 1→4)-β-glucan (BG) is regulated during the endosperm development in barley. Specific barley mutants with low (*lys3* mutant) and high (*lys5* mutants) content of BG were used for this study.

In correlation with former studies a monosaccharide composition of the endosperm cell walls showed that within 38 days after flowering the *lys3* and *lys5* mutants contained reduced and increased levels respectively of BG compared to the parental line. As the differential incorporation of BG could be due to a differential regulation at the transcriptional level, a bioinformatic study was carried out in order to find the gene sequences of the BG-synthases in barley. A homology BLAST search in HarvEST Barley 1.58 database using the already known BG-synthase genes (*Os.CSLFs*) from rice resulted in identification of three potential homologs in barley. The genes, *Hv.CSLF3*, *Hv.CSLF6* and *Hv.CSLF9,* were named after their closest rice ortholog. The following quantitative real-time PCR analysis of *Hv.CSLF3*, *Hv.CSLF6* and *Hv.CSLF9* during the endosperm development of the parental line showed a very uneven expression of the three gene members. *Hv.CSLF6* followed by *Hv.CSLF9*, were significantly more expressed than *Hv.CSLF3.* Whether similar transcript levels and profiles of *Hv.CSLF3*, *Hv.CSLF6* and *Hv.CSLF9* can be found for the two *lys* mutants will be revealed in the nearest future.

As former studies of *lys5* mutant have indicated that increased incorporation of BG most like is a compensatory effect of a dysfunctional ADP-Glc transporter which causes a disruption of the starch synthesis, an additional analysis of the substrate levels (AGP-Glc and ADP-Glc) for BG and starch biosynthesis (respectively) are now being carried out. The preliminary data of the parental line show a significant increase in the availability of these two substrates from 8 to 20 days after flowering. Differences in substrate composition are expected between the parental line and the two mutants.

By using this experimental approach we hope to gain a more detail picture of the various levels at which the BG-biosynthesis is regulated, as well as more insight to the regulatory mechanisms of wall biosynthesis in general.

5. Characterization of the CSLH gene family

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The cell wall is comprised of different polymers of which polysaccharides are the most abundant. The polysaccharides can be divided into different types: cellulose, hemicellulose and pectin. The biosynthesis of these polymers is still poorly understood and only a small number of the glysosyltransferases have been characterized at the biochemical level. Grasses have type 2 walls that differ from the walls of most other plants. The major known differences are that grass walls are rich in feruloylated arabinoxylan and beta-glucan but low in xyloglucan and pectin. The CSL (Cellulose Synthase Like) superfamily of genes appear to encode backbone synthesizing enzymes involved in biosynthesis of mannans, glucans etc. Interstingly, grasses contain two groups of CSL genes, CSLF genes and CSLH genes that are not found in Arabidopsis. We intend to carry out an investigation to determine the role of the CSLH genes in Oryza sativa and Brachypodium distachyon.

Work in a previous project has focused on bioinformatics conducted on the three OsCSLH genes. Two different knockout lines of OsCSLH1 showed a distinct pattern of stunted growth and development, with one line having a more severe phenotype. However, it has not yet been possible to unambiguously relate the phenotype to the knock-out in OsCSLH1. Future experiments are aimed at determining the subcellular localization in native rice tissue and examining potential protein complex formation. This will be done by using both protein tags and a previously produced polyclonal antibody raised against a OsCSLH1 peptide with high homology to the OsCSLH2 and OsCSLH3 genes. Furthermore, OsCSLH1 cDNA will be cloned for heterologous expression of OsCSLH1 in tobacco for use in subsequent biochemical and other assay types, such as localization. Also planned for this project is production of RNAi rice plants, with a RNAi construct targeting the OsCSLH gene family. As a parallel to the Oryza sativa organism, work is also planned using the cereal model plant Brachypodium distachyon. This will involve the possible use of T-DNA and TILLING collections for identification of loss of function mutants to work as a comparison the results gained from the Oryza sativa experiments.

Posters: Nutrition

1. The ALA3/ALIS1 complex: a story about lipid flipping in the secretory pathway

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P_4-ATPases (also known as flippases) belong to a subfamily of P-type pumps and are believed to transport specific lipids between the two leaflets of biological membranes, thus establishing and maintaining lipid asymmetry in the bilayer (Axelsen and Palmgren, 2001). Based on phenotypes of yeast deletion mutants, these pumps have been implicated in the early phases of vesicle formation during endo- and exocytosis (Pomorski et al., 2006). Recently, it has been proposed that several members of the yeast Cdc50p/Lem3p family might be subunits required for proper targeting of P_4-ATPases (Saito et al., 2004).

In Arabidopsis 12 P_4-ATPase isoforms have been identified, named ALA1-12 for Aminophospholipid ATPase (Axelsen and Palmgren, 2001). So far, only one member of this family (ALA1) has been partially characterized and suggested to have a physiological relevance in chilling tolerance (Gomés et al., 2000).

Through a database search we have identified five Cdc50p/Lem3p homologues in Arabidopsis (ALIS1-5 for ALA Interacting Subunit). We have investigated the capacity of ALA3, alone and in combination with expressed ALIS proteins, to functionally complement a battery of yeast mutants carrying deletions in endogenous P_4-ATPases. Our results indicate that ALIS1 functions as a true β-subunit for ALA3, being required for ATP-dependent aminophospholipid transport and for genetic complementation of the yeast P_4-ATPase gene *Drs2*, which is involved in vesicle budding from the late Golgi

Together with ALIS1, we have found that one of the Arabidopsis P_4-ATPases, ALA3, localises to the Golgi apparatus in plant cells. Expression of a β-glucuronidase (GUS) gene under the control of the promoter for either ALIS1 or ALA3 showed a high expression level for both promoters at the columella root cap. Two independent lines of ala3 mutant were phenotypically analyzed. When grown under 24 h light conditions, mutant plants are significantly smaller than control wild type plants. A decrease in root growth is related to the inability of ala3 plants to release border-like cells from root caps that are damaged during root penetration of the growth medium. Electron micrographs show cellular defects in cells of the root cap of mutant plants. We propose that the flippase activity of the ALA3/ALIS1 complex in the Golgi apparatus is required for important secretory processes involved in shedding of damaged root cap cells.

References:
Axelsen KB, Palmgren MG (2001) Plant Physiol. 126:696-706
Gomes E, Jakobsen MK, Axelsen KB, Geisler M, Palmgren MG (2000) Plant Cell 12:2441-2454
Pomorski T, Menon AK (2006) Cell Mol Life Sci. 63:2908-2921

2. Biotechnology for improved nitrogen use efficiency (NUE) in perennial ryegrass *Lolium perenne* L.

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Ammonium (NH_4^+/NH_3) is a central intermediate in nitrogen assimilation and remobilisation within plants. Recent studies have shown that tonoplast intrinsic proteins (TIPs) TIP1 and TIP2 from Arabidopsis and wheat are able to transport ammonia across biological membranes. TIPs are mainly localised in the vacuolar membrane where they may serve a role in sequestration of toxic NH_4^+, or in releasing NH_4^+/NH_3 from the vacuoles to the cytosol under nitrogen deficient growth conditions[1]. Some studies have reported a possible localisation of the TIPs in the plasma membrane, which could suggest a role in uptake of nitrogen[2,3].

In the present study we investigate the possible role of TIP1 and TIP2 in nitrogen metabolism in the forage and turf grass species *Lolium perenne* L. We wish to investigate the effect on nitrogen use efficiency (NUE) after manipulating *Lp*TIPs and the enzyme glutamine synthetase (GS), the latter which is responsible for the assimilation of ammonium into amino acids.

We have used the coding sequences of TIP1, TIP2 and GS from Arabidopsis and wheat to identify homologous sequences in a *Lolium* specific EST-library. The EST-sequences were used as templates for designing primers for RACE-PCR and genomic walking, to obtain full length sequences of TIP1, TIP2 and GS. Functional complementation tests, using a yeast strain deficient in ammonium uptake, have shown that the TIP-homologues isolated from *Lolium perenne* are able to restore ammonium transport. Also the GS-homologues are able to restore glutamine synthesis in a yeast strain deficient of endogenous GS. Further experiments include over-expression and silencing of LpTIP1, LpTIP2 and LpGS and subsequent analysis of transformant lines under different nitrogen regimes.

References:
1. Jahn TP et al. (2004) FEBS Lett. 574: 31-36
2. Liu L-H et al. (2003) Plant Physiology. 133: 1220-1228
3. Marmagne A et al. (2004) Moll Cell Proteomics. 3(7): 675-91

3. Membrane transport of arsenite and other uncharged metalloids through aquaporins of Arabidopsis thaliana

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Metalloids are elements with characteristics intermediate between metals and non metals. The function of metalloids in biological systems ranges from essential, via beneficial to highly toxic. In plants, two aquaporin homologues have recently been identified as the bottle neck for the uptake of silicon and boron (Ma et al. 2006 Nature 440: 688-691, Takano et al. 2006 Plant Cell 18: 1498-1509), respectively. In rice the low silicon 1 locus (lsi1) was identified as a NIP homologue. Rice plants lacking *Lsi1* are characterised by a strong reduction in silicon content which leads to a reduction in seed number, increased chlorosis and pathogen susceptibility. Similarly, in *Arabidopsis*, NIP5;1 has been shown to represent the bottle neck for boron uptake. These two studies represent the most clear-cut examples of a molecular function of single aquaporin isoforms in plants to date.

Arsenic poses a threat to human health via drinking water and the food chain. Improved phytoremediation and the development of safe crops are two immediate targets in plant biotechnology. Therefore, it is important to study the uptake and internal distribution of arsenic in plants. Transport of uncharged metalloid species of arsenic (AsIII) and antimony (SbIII) via aquaglyceroporins is well characterised in bacteria, yeast, protozoan parasites and mammals but not yet in plants.

Using yeast as a model system for arsenite transport and detoxification, we have now identified 2 aquaqlyceroporins from *Arabidopsis* as bi-directional arsenite channels. *AtNIP5;1* and *AtNIP6;1* functionally complement the deletion of *fps1* in yeast. Expression of *NIP5;1* and *NIP6;1* decreased the growth rate of mutants with di_erent sensitivities towards arsenic to a similar extent as the expression of *rAQP9* and *Scfps1*. Conversely, the same transformants improved resistance towards intracellular arsenite produced after the reduction of As(V). AtNIP5;1 and AtNIP6;1 belong to the same sub group of NIP homologues in plants with identical substitutions in the aromatic arginine constriction region. Interestingly, expression of *AtNIP7;1* rendered yeast more sensitive to Sb(III) but not As(III). NIP7;1 is the closest plant homologue to mammalian AQP9 which channels both As(III) and Sb(III).

Further studies are under way to reveal whether these aquaporins are potential targets for improving the phyto-remediation of toxic metalloid species.

4. Map based cloning of symbiotic genes in Lotus japonicus including genes responsible for a spontaneous nodulation phenotype

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Symbiotic nitrogen fixation is very import for assimilation of nitrogen in nitrogen poor environments. With the help of the model legume *Lotus japonicus* we are isolating the plant genes that are needed for this symbiosis. The *L. japonicus* symbiotic community has provided map positions of 37 symbiotic loci (Sandal *et al*. 2006).

In our laboratory we are doing map based cloning of two symbiotic genes (*sym40* and *sym67*) giving in addition to a symbiotic phenotype also a trichome phenotype with short distorted trichomes.

Additional plant loci for symbiotic nitrogen fixation were found by a screen for plant mutants that were able to develop spontaneous nodules in the absence of Rhizobia. These nodules were similar to wild type nodules in respect to nodule structure, upregulation of early nodulin genes and repression of nodule formation in the presence of nitrogen in the growth medium. Map based cloning of spontaneous nodule formation 1 (*snf1*) lead to the identification of a calcium calmodulin dependent kinase (*CCaMK*) gene with a particular mutation resulting in *snf* phenotype (Tirichine *et al*., 2006a).

A second plant mutant (*snf2*) with a spontaneous nodulation phenotype was found. By map based cloning we have shown that it is caused by a gain of function mutation in a cytokinine receptor (Tirichine *et al*., 2007). Further characterization of the relation between cytokinine and nodule primordium initiation will be presented.

References:
Sandal et al. 2006 MPMI 17, 80-91
Tirichine et al. 2006a Nature 441, 1153-1156
Tirichine et al. 2007 Science 315, 104-107

5. A genetic approach to dissect the function of exopolysaccharides in the legume *Lotus japonicus*

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The symbiotic relationship between legume plants and the soil bacteria rhizobia is agronomically important, as legumes produce over half of all biologically fixed nitrogen. The process is initiated by the bacterium invading the root tissue and a controlled developmental program begins which leads to the formation of a specialised organ – the nodule.

The formation of symbiotic nodules require a number of cellular events, such as root hair deformation, infection thread formation, Ca^{2+} spiking and cell proliferation to occur in a coordinated manner. First, the legume root secretes flavonoid compounds to attract the bacterium. Once proximity has been established the rhizobia releases a chitin compound, the Nod factor, to initiate a conserved signaling cascade mediated by the Nfr receptors.

In addition to the Nod factor signal recent results have shown that other signals are as well needed for the proper formation of symbiotic nodules. When *Lotus japonicus* plants are inoculated with rhizobia mutated in the exopolysaccharide biosynthetic pathway no functional nodules are formed. Early events mediated through Nod factor signalling such as root hair deformation is normally initiated suggesting that exopolysaccharide signal occurs downstream of or in parallel to these events. Often a failure to form infection threads is observed and the mutant bacteria are predominantly found in the epidermal cell layer of the root.

Here we describe a genetic screen to elucidate the function of exopolysaccharides during the formation of symbiotic root nodules.

6. PCR based genome walking technique for promoter mapping of barley genes

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Whole genome sequencing of model plants has increased our knowledge of the composition and organization of plant genomes. Most crop species have very large genomes which are time and cost intensive to sequence. Significant progress has been made to unlock the gene content of crop plants by sequencing expressed sequence tags (EST) or BAC clone libraries. However, new methods are needed to facilitate the access to genes considered as major targets for crop improvement in biotechnology, including regulatory elements and promoters.

Genome walking is a molecular biology technique basically used to determine an uncharacterized DNA sequence flanking a known region and to cover gaps in genome sequencing. Here we adapt a fast and inexpensive PCR based genome walking technique, originally developed for microorganisms and modified for application in cereal genomes. Unknown 5'- and 3' flanking regions of barley (*Hordeum vulgare* L.) metallothionein and phosphoenolpyruvate carboxylase genes were cloned by using four different partially degenerate primers in combination with a set of nested gene specific primers. By performing three successive rounds of nested PCR, we successfully amplified unknown sequence regions flanking the known coding sequences. PCR products were subsequently cloned and sequenced.

Compared to other genome walking techniques the new approach is shown to be very specific and consistent in allowing high success rates even for difficult genomic templates such as those of crop plants. The new strategy allows a fast and convenient mapping of whole promoter regions or, more generally, loci that are not yet fully sequenced. Thereby, the method is a good alternative to the use of tedious library screening or expensive genome walking kits. It may contribute to increase our knowledge of the genome structure of cereals and the regulation of their genes, facilitating crop improvements through breeding programs and new gene transformations strategies.

7. The C-terminus of the Arabidopsis P_{1B}-ATPase, HMA4, functions as an autoinhibitory and metal-binding domain

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P_{1B}-ATPases are important transporters of heavy metals such as Zn, Cu, Cd and Pb. In *Arabidopsis thaliana*, the plasma membrane located P_{1B}-ATPase, HMA4, has been shown to be involved in translocation of Zn from root to shoot[1] and in metal detoxification[2]. Characteristic of HMA4 is a long cytoplasmic C-terminus of approximately 470 amino acid residues, which contains 13 cysteine pairs and a terminal histidine stretch. The function of the C-terminus has not yet been elucidated but it may act as an autoinhibitory as well as a metal-binding domain.

To study the potential autoinhibitory role of the C-terminus of HMA4, full-length HMA4, HMA4 without the His-stretch (hma4Δ18), as well as several C-terminal deletion mutants lacking one to 13 Cys-pairs (hma4Δ32-459) were expressed in a Zn-sensitive yeast strain, *zrc1 cot1*. Removal of the His-stretch resulted in a slight increase in yeast Zn-tolerance compared to the full-length pump, while removal of the His-stretch and the Cys-pairs gradually increased the Zn-tolerance, with the highest level observed by removal of 150 or more residues. These results were supported by identification of spontaneous mutants of yeast expressing hma4Δ18 on high Zn concentration (500 µM).

All the isolated mutants showed frame-shift mutations in the HMA4 C-terminus region, thereby introducing stop-codons that caused C-terminal truncation of more than 150 amino acid residues. The observed yeast Zn-tolerance was due to transport activity, as mutation of an essential amino acid residue involved in the catalytic cycle in the mutant ATPases resulted in loss of Zn-tolerance. Expression levels were analysed for HMA4, and hmaΔ18-Δ263. The expression levels were similar for HMA4 and hmaΔ18-Δ53; hereafter the expression levels increase with the number of residues removed. Furthermore, localization in yeast was studied for HMA4, hma4Δ18 and hma4Δ459. All showed similar localization: in the plasma membrane and to the ER. Thus, at least the last 53 amino acid residues have a role in autoinhibition

HMA4 C-terminus alone (HMA4-C_t) was also expressed in *zrc1 cot1*. Interestingly, HMA4-C_t was able to support growth of yeast on 500 µM Zn. This could be explained by a potential role of the C-terminus in chelation of Zn, removing toxic levels of free Zn from the cytosol. To investigate the potential Zn-binding capacities of the C-terminus, Zn bound to a purified C-terminus was stained with dithizone (DTZ), which indicated that the C-terminus specifically binds Zn. Further investigations to determine the number of Zn bound to the C-terminus are under progress.

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[1] Hussain *et al*. (2004), Plant Cell *16*, 1327-1339
[2] Mills *et al.* (2005) Febs Lett. *579*, 783-791

Based on these results, we suggest that the HMA4 C-terminus functions as an autoinhibitory as well as metal-binding domain. An autoinhibitory domain has not previously been identified for a Zn-ATPase.

8. Characterizing receptors and their ligands involved in development and regulation of Legume-*Rhizobium* symbiosis

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In all multicellular organisms one of the fundamental mechanisms by which cells communicate over distance is through the secretion of ligands. Subsequent binding and recognition at protein receptors localised in the cell surface initiates downstream signalling pathways. Approximately one third of all genes in multicellular organisms encode membrane bound proteins and a large part of these encode putative receptors. To date the biological role has been determined for only a limited number of receptors. For most of these it is not known whether they are arranged in protein complexes, the ligand is unknown and the mechanism by which ligand/signalling molecule perception triggers downstream functions is not described.

Symbiotic interaction between legumes and rhizobia results in the development of a new organ, the root nodule. The development and restriction of nodule number involves a complex exchange of signals not only between the two symbiotic partners but also over long distances such as between the root and shoot of the plant. To date several different receptor molecules involved in these processes have been identified (ex. *Lj*NFR1, *Lj*NFR5 and *Lj*HAR1). Elucidating how these receptors function can give us an understanding of how symbiotic interactions are established and controlled.

The goal of this project is to establish a platform for determination of proteins interacting in a receptor complex followed by detection of receptor-ligand interaction using the Biacore technology.

9. Unravelling the full complement and potential of the cereal phytases

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Phytic acid (phytate, $InsP_6$, *myo*-inositolphosphate 1,2,3,4,5,6-hexakisphosphate) is known as the primary storage form of phosphate and inositol in plant seeds. Moreover, phytic acid is considered to be the single most important anti-nutritional factor for the bioavailability of minerals in human nutrition. During germination, phosphate and minerals are released from the phytic acid in a hydrolytic reaction catalyzed by the enzyme phytase (*myo*-inositol hexaphosphate phosphohydrolase). However, in most dry seeds and in the human and animal digestive tracts, insufficient or no phytase activities causes low phosphate and mineral bioavailability. In consequence, large amounts of undigested phosphates are excreted in the manure and contribute significantly to the environmental phosphate load.

In spite of their importance, only very little is known about the plant phytases, their genes, the corresponding enzymes and the mechanisms regulating the cereal seed phytase activity. The phytase activities in cereals varies considerably and ranges from 74 and 15 FTU/kg in rice and maize respectively to 400-900 in barley, 690-1400 in wheat an to astonishing 1900-5200 FTU/kg in rye. In order to unravel and exploit the cereal phytase potentials, we are isolating and characterizing the full complement of phytases in the most important cereal species.

Candidates belonging to the families of the histidine acid and purple acid phosphatases have been isolated and after characterization of *Escherichia coli* and *Pichia pastoris* expressed proteins, our studies focus on four wheat and three barley isogenes encoding histidine acid phytases and four wheat and two barley isogenes encoding purple acid phytases. After defining a purple acid phytase consensus motif it is now possible to identify purple acid phytases within the very large group of purple acid phosphatases.

It is obvious that the expression of the individual phytases during plant growth and seed development and the biochemical properties of the phytases vary considerably. In example the redox potential of the ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron in the binuclear metallic center of the purple acid phytase is capable of regulating the catalytic activity of the phytase. In our next step, we will identify and characterize the phytase complement of rye, the cereal with the highest known phytase activity.

10. Ferric reductase activity and pH changes in response to iron starvation in two closely related *Lotus species*

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Iron is very abundant in the Earth's crust, but due to its low solubility at neutral and alkaline pH, it is not readily available for plants. Plants use two different strategies for the acquisition of iron. Graminaceus monocotyledons rely on the secretion of siderophores from the roots. These compounds will chelate Fe^{3+} and the plants will take up the chelated iron.

Dicotyledons and nongraminaceous plants are only able to uptake ferrous ions and therefore they have developed a strategy to reduce Fe^{3+} to Fe^{2+}. Root plasma-membrane ferric chelate reductase (FRO) plays an essential role in iron reduction. The activity of FRO decreases when iron is sufficient and increases when plants suffer from iron starvation. Moreover, under iron deficiency roots are capable of reducing rhizospheral pH and increase Fe (III) solubility. Plants acidify the rhizosphere by the secretion of protons. Root H^+ ATPases and cell membrane metal ion anti- and symporters are responsible for this activity. In addition root cells are able to secrete organic acids that may both reduce pH and chelate ions. In this study we investigate whether there are any differences in either FRO or pH reducing activity between two Lotus species: *Lotus japonicus* Gifu and *Lotus filicaulis.* These two species are closely related, they can be crossed, but they differ in how much iron accumulates in their leaves and seeds.

We have grown *L. japonicus* and *L. filicaulis* plants in 6 different conditions. We have compared plants grown in the presence or absence of *Rhizobia* and we have added three different concentrations of iron to the growth media. We have studied the levels of iron (III) reduction activity in the roots of these plants using Fe (II)-bathophenanthrolinedisulfonic disodium salt chromophore (BPDS) and we have regularly measured the pH of the growth media.

We have observed that the responses to iron starvation are different between *L. japonicus* Gifu and *L. filicaulis*. We have measured differences in the iron (III) reduction activity between the two species and our results indicate that the presence of *Rhizobia* affects iron (III) reducing activity and the acidification of the growth media.

11. A vacuolar aquaporin is potentially involved in ammonia homeostasis

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We have recently identified specific aquaporin isoforms from plant and mammals with the capacity to channel NH3 gas when expressed in yeast or Xenopus oocytes (Jahn et al. 2004 FEBS letters 574, 31-6). The results were confirmed by a number of laboratories. Recently, Saparov et al. (2007 JBC 282, 5296-301) provided data from *in vitro* measurements after purification and reconstitution of human AQP8 to suggest that this channel is even more specific for NH3 than for water.

Here we show that AtTIP1;1 also channels NH3 with high capacity when expressed in yeast. It is able to complement the growth deficiency of a yeast strain, lacking its endogenous ammonium transporters (*mep1,2,3*). Additionally, stopped flow experiments using a pH sensitive GFP variant in yeast cells were applied to show that ammonia is the channelled substrate. Albeit increasing evidence on the molecular level for the existence of aquaammoniaporins, direct evidence for a physiological role of plant TIPs and AQP8 in ammonium handling is still lacking.

In a recent study, down regulation of TIP1;1 in *Arabidopsis* by RNAi was shown to result in a dramatic phenotype including early senescence and death (Ma et al. 2004 Plant J. 40, 845-59). RNAi plants showed changes in various transcripts in categories of defence, signalling, redox control and carbon metabolism. Seedlings accumulated starch and showed changed sugar content. In this study we identified a transposon insertion line called attip1;1-1. Homozygotes were collected and RT-PCR verified that the insertion line lacks the transcript of *TIP1;1*. An antibody raised against the N-terminal domain of TIP1;1 recognizes TIP1;1 in wt *Arabidopsis* but not in attip1;1-1 verifying the complete absence of TIP1;1 in the insertion line. However, in contrast to the conclusions by Ma et al. (2004), the insertion line appears just as healthy as wt. In addition, differences in starch and sugar content shown in Ma et al. (2004) for RNAi knock down lines could not be confirmed with the insertion line.

We are now in the process of applying quantitative real time PCR and microarray hybridisation to analyse if other genes potentially substitute for the lack of TIP1;1 in the insertion line. Microarray analysis will also be used to study expression profiles of wt *Arabidopsis* under various N-nutrition regimes.

12. Iron and Zinc in legumes

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Legumes are important staples in the developing world. They are frequently grown in soil with limited nutrient availability. There is a natural variation of seed micronutrient content among legumes of the same species even when plants are grown under similar conditions (Mohamed AI., et al 1991). This indicates that a genetic factor affects the nutritional value of these staples. Consequently it is possible that during breeding for specific traits, the micronutrient value of seeds is reduced. To analyze progeny from each cross for micronutrient content is very tedious and costly. Therefore it is important to have genetic markers that will follow the high micronutrient traits during selective breeding.

Plants use finely tuned mechanisms to keep appropriate levels of iron and zinc in each of their organs. Several genes involved in iron and zinc homeostasis have been described in yeast, and a few orthologs have been studied in plants (Grotz N. and Guerinot ML. 2006). The goal of this project is to find ways to improve the nutritional value of legumes by identifying genes and proteins important for iron and zinc regulation in the model legume *Lotus japonicus*. The knowledge obtained in *L. japonicus* will be used to develop genetic markers that will follow the trait of high micronutrient content in common bean. The use of these genetic markers will reduce the time and cost required to develop new cultivars with improved nutritional content.

Methods:
We have used sequences of genes that are known to play a role in iron and zinc metabolism in other organisms to search for both L. japonicus ESTs and genomic loci. Identified sequences are being used for gene regulation studies.

Furthermore, the micronutrient content of different tissues from four Lotus ecotypes/species (Lotus japonicus Gifu, L. japonicus Miyakojima (MG-20), L. burttii, and L. filicaulis) was analysed using ICP-MS (inductively coupled plasma mass spectrometry). We are performing mapping and QTL analysis of recombinant inbred lines from a cross between L. japonicus Gifu and L. filicaulis. Additionally, we are studying the distribution of iron and ferritin in the seeds of different legumes.

Results and discussion:
Sequences with high similarity to most of the known genes involved in iron and zinc homeostasis have been identified in the model legume *Lotus japonicus*. For example *L. japonicus* ESTs and genomic loci corresponding to ferritins, ferric reductases, ATPases, FER transcription factor, ITP, metal transport proteins of the ZIP family, and cation transporters of the NRAMP family where found.

The content of iron and zinc in the seeds of the two analysed *L. japonicus* ecotypes and *L. burttii* were similar, but we found significantly different levels of several micronutrients between *Lotus filicaulis* and *L. japonicus* Gifu. These differences are being used to map

genes important for iron and zinc homeostasis using *L. filicaulis* x *L. japonicus* Gifu recombinant inbred lines.

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References:
Grotz N. and Guerinot ML. (2006) Molecular aspects of Cu, Fe and Zn homeostasis in plants. *Biochim Biophys Acta*. 1763(7):595-608.
Mohamed AI, Mebrahtu T, Rangappa M. (1991) Nutrient composition and anti-nutritional factors in selected vegetable soybean (Glycine max [L.] Merr.). *Plant Foods Hum Nutr*. 41(1):89-100.

13. Transport and Deposition of Zinc in the Developing Barley Grain

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The primary aim of this project is to study the genetic and molecular mechanisms underlying zinc transport and deposition in the cereal grain. Barley is used as a model due to the available post-genomic tools.

In small-grained cereals, zinc and iron are primarily stored together with phytate in protein storage vacuoles in the aleurone layer and the embryo. The deposition pattern of zinc and iron has far reaching consequences for human mineral nutrition. During milling the outer tissues and the embryo are removed from the grain, which results in a virtual depletion of zinc and iron.

Zinc and iron deficiencies are the most widespread micronutrient deficiencies in humans. These deficiencies are particularly common in developing countries where diets are rich in cereal-based foods, which serve as excellent suppliers of carbohydrates but with low concentrations of bioavailable zinc and iron. Some of the major health consequences of these deficiencies include retardation of growth, anaemia and impaired immune function. This can be explained by the fact that about 3% of all genes in humans encode proteins with zinc binding motifs and in more than three hundred enzymes zinc is required as a co-factor.

Elucidation of the mechanisms responsible for transport and deposition of zinc in the barley grain is approached by performing transcript profiling of different tissues of the grain. For that a barley zinc related transcriptome microarray has been established which comprises the majority of genes known to be involved in zinc transport, deposition and homeostasis. The zinc transcriptome array contains unique oligonucleotides representing 236 different ESTs belonging to 18 different gene families.

The array is used for expression studies of the zinc transcriptome in different fractions of the grain at different developmental stages. In order to identify relevant genes the expression is induced by applying 5 mM $ZnSO_4$ to the leaves.

The laser capture microdissection technique is used to isolate transfer cells from the unloading region, aleurone cells, cells from the embryo and cells from the endosperm. RNA from the isolated cells is subsequently extracted, amplified and used for microarray hybridizations and for real-time PCR analyses to establish the temporal and spatial expression patterns of relevant genes.

References:
Kerk, NM, Ceserani, T, Tausta, SL, Sussex, IM, Nelson, TM: Laser capture microdissection of cells from plant tissues. *Plant Physiology* 132: 27-35 (2003)

14. *Lotus japonicus* nodulation requires two GRAS-domain regulators, *NSP1* and *NSP2*

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A nodulation-defective mutant of *Lotus japonicus* does not initiate nodule cortical cell division in response to *Mesorhizobium loti*, but induces root hair deformation, Nod factor-induced calcium spiking, and mycorrhization. We have shown that this mutant has a premature stop in the *NSP1* (Nodulation Signalling Pathway 1) gene (*Ljnsp1-1*) resulting in loss of the C-terminal 23 amino acids (aa) and we recently identified another mutant in *NSP1* (*Ljnsp1-2*) with a truncated protein of 341 aa. Additionally, we have sequenced and isolated a mutant in *LjNSP2* (*Ljnsp2-3*) that has a premature stop codon showing a similar phenotype to the *Ljnsp1* mutants, whereas another mutant (*Ljnsp2-4*) showed increased nodulation.

Both *Lj*NSP1 and *Lj*NSP2 are predicted GRAS (GAI, RGA, SCR) domain transcriptional regulators. Transcript steady-state levels of *LjNSP1* and *LjNSP2* initially decreased and then increased following infection by *M. loti*. In hairy root transformations, *LjNSP1* and *MtNSP1* complemented both *Mtnsp1-1* and *Ljnsp1-1* mutants, demonstrating that these orthologous proteins have a conserved biochemical function. A *Nicotiana benthamiana NSP1*-like gene (*NbNSP1*) was shown to restore nodule formation in both *Ljnsp1-1* and *Mtnsp1-1* mutants, indicating that NSP1 regulators from legumes and non-legumes can propagate the Nod factor-induced signal, activating appropriate downstream targets. The *L. japonicus* nodules complemented with *NbNSP1* contained infected cells and could fix nitrogen. However, the *NbNSP1*-complemented *M. truncatula* nodules did not fix nitrogen and contained very few bacteria released from infection threads. These observations suggest that NSP1 could also be involved in infection, bacterial release, and normal bacteroid formation in nodule cells.

15. Genes involved in phosphate uptake

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Phosphorus is an essential plant nutrient but phosphate (Pi), the form of phosphorus taken up by plants, is poorly accessible in most soils. Therefore plants have developed strategies for obtaining the phosphorus they need, including expression of genes encoding phosphate transporters, MYB-related transcription factors, RNAs containing short non-conserved reading frames, and microRNAs (miR-399).

Barley and wheat are very economically important plants. We want to identify genes involved in phosphate acquisition in barley and wheat based on homology to genes known from Arabidopsis and other plant species. Candidate genes will be silenced using a gene silencing technique based on *Barley stripe mosaic virus* (BSMV) in barley and wheat plants. Furthermore, we will look for and describe correlations between particular genes and their regulations.

We have isolated fragments of several phosphate transporter genes and some putative regulatory genes from barley or wheat and performed a preliminary expression analysis in plants grown under different phosphate conditions. Currently we are preparing BSMV constructs for silencing experiments with phosphate transporter genes in barley.

Collaboration:
Tom Hamborg Nielsen, Faculty of Life Sciences, University of Copenhagen.

Project supported by:
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Posters: Diseases

1. Emergence of a New Mycosphaerella graminicola Population in Winter Wheat

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Foci (hotspots) of Mycosphaerella graminicola were observed in an unsprayed winter wheat in 2005. To investigate potential factors for focal formation, we analysed for aggressiveness defined as latent period and number of days before maximum percentage of disease was reached, EC50-values for epoxiconazole, genotypes of CYP51-mutations, variability in terms of population clonality and genetic differentiation, measured by means of specific micro-satellite markers, and frequency of mating type idiomorphs. The results did not show any correlation between any of these parameters. The aggressiveness parameters chosen gave no indications of the focal population being more aggressive. Microsatellite analysis revealed a significant segregation into two subpopulations, and furthermore that the focal population was not clonal. Fungicide resistance parameters for both genotypic (CYP51) and phenotypic (EC50-values) revealed significant differences. However, it is unclear whether these effects are in some way responsible for focal formation since we did not find any correlations to other parameters. Similarly, although significant differences in mating types were found, lack of correlation to other parameters makes it unclear whether this parameter had an effect. In conclusion, we can report for the first time M. graminicola hotspots in unsprayed winter wheat fields. However, what has caused these hotspots is unclear and further studies are thus needed in future.

2. Identfication and expression analysis of potato genes induced by the late blight pathogen, *Phytophtora infestans*

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Late blight, caused by the oomycete pathogen *Phytophtora infestans*, is the most devastating disease of potato resulting in massive losses of potato cultivation every year. The very high amount of fungicide used for potato protection could be markedly reduced by utilizing the host resistance.

The goal of the project is to reveal the molecular mechanisms behind the high resistance of the Sarpo Mira cultivar and to develop tools for marker assisted breeding programs that use this cultivar as a donor for late blight resistance.

To indentify genes that are differentially expressed in response to *P. infestans* infection, a cDNA amplified fragment length polymorphism (cDNA-AFLP) was performed on *P. infestans*-infected and non-infected *in vitro* grown plants of the resistant Sarpo Mira and of the susceptible Bintje cultivars. 16 of 45 identified regulated bands were analyzed using real time Reverse Transcriptsase-PCR (RT-PCR).

The expression levels of nine genes increased more than 400% under blight infection. Most of them showed similarity to genes previously found to respond to pathogen attack or stress. The expression of these *P. infestans* regulated genes was different in uninfected resistant plants compared to susceptible ones.

The results from this project will improve the general knowledge about the mechanisms that plants use to respond to pathogens and will assist the development of late blight resistant potato cultivars, increasing yields of ecological potato farming and reducing the use of fungicides in traditional potato farming.

3. USER friendly™ construction of vectors for targeted gene replacement in fungi

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Functional genetics in filamentous fungi have always been dependent on the isolation or construction of mutant strains. The genome sequencing of several different filamentous fungi has changed the experimental approach from forward to reverse genetics. This has speeded up research in focus areas such as secondary metabolism and pathogenicity, thereby increasing the need for faster methods to carry out targeted gene replacement for generation of deletion mutants and overexpression mutants. To accommodate this we have developed a new vector system that allows single-step construction of vectors for targeted gene replacement, thereby reducing vector construction time from ten to only three days.

The vector system is dependent on the Uracil-Specific Excision Reagent cloning technology (USER friendly™), which offers high efficient directional cloning of a single PCR amplicon. However, our research shows that USER friendly™ cloning also can be used for the simultaneous directional cloning of several PCR amplicons and vector fragments, with a cloning efficiency of 85 %, thus allowing single-step construction of replacement vectors.

The new vector system includes vectors for: gene replacement (pRF-HU2), promoter exchange (pRF-HU2E), ectopic overexpression (pRF-HUE) and general purpose cloning (pRF-HU). All compatible with both protoplast and *Agrobacterium tumefaciens* mediated transformation technologies.

We have used the system to replace and overexpress several of the polyketides synthases genes found in *Fusarium graminearum*.

4. Syntaxins in pathogen defence

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The Arabidopsis mutant *syp121* (*pen1*) displays a delayed penetration resistance to the non-host barley powdery mildew fungus. The *SYP121* gene encodes a syntaxin that is involved in vesicle trafficking and secretion. Mutations in the closely related syntaxin gene *SYP122* do not cause any evident phenotype. However, in the *syp121 syp122* double mutant, multiple plant defence mechanisms are activated, which leads to pronounced resistance against for instance host powdery mildew fungi. This resistance is mediated by a multicellular programmed cell death reaction, the hypersensitive response (HR). We are currently unravelling the mechanisms of this resistance.

syp121 syp122 develops a lesion-mimic, dwarf phenotype, accompanied by a constitutively elevated salicylic acid (SA) level and *PR1*-gene expression. The *syp121 syp122* dwarf phenotype can be partially rescued by introducing specific mutant-alleles of genes in the SA-pathway, such as *SID2*, *EDS5* and *NPR1*, without affecting the resistance to powdery mildew.

We used a suppressor mutant screen to identify genes important for the syntaxin double mutant phenotype. These genes were named *Suppressors of Syntaxin-related Death* (*SSD*). Some of those are known genes from the SA-pathway, and map-based cloning of other genes revealed that *FMO1* and *ALD1* are important for the development of the *syp121 syp122* double mutant phenotype. Among our predominantly occurring *SSD*s, we confirmed ten alleles of *FMO1*, six alleles of *ALD1* and seven novel alleles of *PAD4*.

The discovery of defence signalling pathway genes is classically done in screens using pathogens. The knockout mutant *syp121 syp122* is an excellent alternative to discover novel defence genes where use of pathogens is not needed.

5. The role of autophagy in *Fusarium graminiarum* during infection of barley

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Recycling of nutrients is a strategy for fungi to survive stress and starvation. Autophagy is thought to be one of the primary mechanisms for relocation of resources within fungal mycelia. The nutrients that are made available through this process are used to renew proteins and organelles in the cell. It is dependent on the vesicular enclosement of portions of the cytosol resulting in the formation of autophagosomes (double layered vesicles). The autophagosomes in turn fuse with the vacuole, which contains digestive enzymes that upon fusion is activated and starts to degrade the contents of the autophagosome.

F. graminearum is a phytopathogenic fungus that infects cereal crops. The infection is a very rapid process that starts with spore germination and invasion of the plant tissue where it turns into a necrotrophic relationship. Our theory is that autophagy plays an essential part in this process by allowing the fungus to recycle nutrients during germination and later by retrieving nutrients from established mycelium to the points of new infections.

Atg8 is an essential protein involved in autophagy and the gene has been used as a marker for autophagy in other organisms. Using an *Agrobacterium*-mediated transformation technique we have produced *F. grameniarum* Atg8-deletion mutants. When grown on nutrient rich media the Atg8-deletion mutants and the WT had a similar phenotype. When the mutants were grown on minimum media they grew slower indicating that autophagy has been inactivated and they have lost their ability to reallocate nutrients within the mycelium. Infection assays with the Atg8-deletion mutants will be performed on barley and preliminary results presented.

6. Intricate interaction between pectins and pathogens

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The cell wall is one of the most important structural components of plants. The wall defines cell shapes, provides strength to withstand the turgor pressure, influences cell development, and serves as the last physical barrier against invading pathogens. Pectins constitute ca. 30% of the cell wall polysaccharides and fill space between the load-bearing cellulose-hemicellulose network. Much of the previous work of pectin has focused on the structural characterization of these highly complex polymers and their properties as food ingredients. In contrast only limited examples are available where the biological roles of the polymers have been fully elucidated. One such example includes homogalacturonan. This polymer constitutes a backbone of some pectic polysaccharides. Previously studies have identified that oligogalacturonides released after digestion of homogalacturonan by pathogen-derived endopolygalactronase elicits defense response in the host, thereby functioning as an endogenous signal for the host defense activation.

We have recently identified two pectin mutants of *Arabidopsis thaliana*, *arad1* and *xgd1*, defective in pectic arabinan and xylogalacturonan biosynthesis, respectively. Detailed cell wall composition analyses have identified that these mutants lack ca. 70 % of the respective polysaccharides in the pectic rhamonogalacturonan I fraction. Albeit these large changes in the wall, the mutants did not show visible growth phenotypes distinct from the wild type, indicating that arabinan and xylogalacturonan are not essential for plant growth under optimal growth conditions. When these plants were subjected to pathogen infections, however, they showed altered susceptibilities as compared to the wild type. *arad1* mutants showed increased susceptibility to the necrotic fungal pathogen *Botrytis cinerea*, while they appear to show the wild-type level of susceptibility to the bacterial pathogen *Pseudomonas syringae*.

Preliminary results suggest that arabinan may be involved in signaling and elicitation of the host defense. In contrast, the *xgd1* mutant showed the wild-type level of susceptibility to *B. cinerea*, while it showed unusual induced and increased resistance to *P. syringae*. Exact molecular mechanisms underlining how two pectic polymers relate to two opposite pathogen responses are currently under investigation and will be presented. Our results add new insights into the intricate roles and functions of pectins in host-pathogen interactions.

7. Proteome Analysis of stored carrots for evaluating changes in susceptibility to the post harvest pathogen *Mycocentrospora acerina* causing liquorices rot.

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During post harvest storage, a large amount of carrots (sometimes more than 50%) are discarded due to the development of liquorice rot caused by *Mycocentrospora acerina*. This fungus is soil borne and over winters in the soil as chlamydospores. *M. acerina* enters the cold store with soil adhering to the root. Control of liquorice rot is mainly related to physiological or structural resistance of carrot, and to other factors such as storage conditions and cultural practices. It is believed that, at the beginning of storage carrots, can resist disease development due to host defence mechanisms. Some proteins and peptides are considered to be important in these mechanisms.

The hypothesis for this newly started project, is that proteome changes during storage of carrots are related to the susceptibility to *M. acerina*. The carrots used in this study are grown under four different agricultural practices (one conventional and three organic), in order to investigate the effect of the cropping system on the susceptibility to liquorice rot.
We are developing bioassays for infection studies of *M. acerina* on conventionally and organically cropped carrots in order to determine the critical time points in the infection process. The proteome of the carrots and of *M. acerina* will then be investigated at these different time points using two dimensional gel electrophoreses and mass spectrometry. Finally bioinformatics will be applied to shed light on the processes of infection and resistance during storage.

Posters: Breeding

1. Application of molecular markers for variety protection of ryegrass (Lolium perenne L.)

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Before seed of most major agricultural and vegetable varieties can be sold in the EU, the variety must be included on a National List (NL) of a member state or on the Common Catalogue (a compilation of the NLs of the member states). In many countries, particularly in the EU, the controlling legislation complies with the Convention of the International Union for the Protection of New Varieties of Plants (UPOV 1991). The corner stone of the UPOV system is that in order to qualify for protection, a newly bred plant variety has to be shown to be distinct (D), uniform (U) and stable (S).

At the moment, the DUS criteria are assessed in the EU member states mainly using a series of replicated field tests and trials. DUS testing takes a minimum of two years and requires considerable resources. Thus, there is much interest in reducing the time, resources, land use and hence cost, of variety registration systems. Although DUS testing currently employs mostly visually observable characteristics that are expressions of the phenotype of a variety, there is much interest in the use of molecular markers.

The overall objective of this project is to examine the potential use of molecular markers for the description of genetic variation in ryegrass varieties and to evaluate their possible use in DUS examination.

Sixteen reference varieties, four control varieties and one candidate variety were selected for the project. The reference varieties were found to look similar to the candidate variety based on the morphological characterization from the DUS trial. 18 SSR markers were selected based on their genome distribution, reproducibility, level of information and ease of scoring. It was found, that for variety discrimination, reducing the number of SSR markers from 18 SSR markers with 262 alleles to six SSR markers with 140 alleles gives the same level of information. Furthermore, number of genotypes per variety can be reduced to 20 compared to the original dataset containing 60 genotypes when using all 18 SSR markers but not when using only six SSR markers. Significant association was found between the molecular data and the morphological data, indicating that SSR markers can be used for variety identification in ryegrass.

2. VIGS in Kalanchoë: Finding and cloning of a vector suitable for VIGS in Kalanchoë

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Producers of ornamental plants in Denmark face increasingly hard competition from producers in other countries both now and in the future. Their ability to compete relies on pricing as well as constant improvement of the quality of their products. The traditional methods in classical plant breeding are usually very labor intensive hence a very costly affair especially in countries like Denmark where manual labor is expensive. Alternative less labor-intensive methods are therefore needed.

The discovery of the mechanism of virus induced gene silencing (VIGS) in combination with gene manipulation offers a promising alternative to the traditional methods used in plant breeding. The development of a vector for VIGS in Kalanchoë would provide Kalanchoë breeders with a valuable tool for relatively fast testing of “specific genes of interest’s” phenotypic traits in Kalanchoë prior to gene manipulation.

This project currently focuses on obtaining full-length infectious clones of two different isolates (KV-1-mut and KV-2) of Kalanchoë Latent Virus (KLV). KLV is a positive sense single stranded RNA virus belonging to the Carla virus group. The virus causes no visible symptoms in Kalanchoë upon infection. The virus genome is one component and sequencing has so far revealed a full length of approximately 8.5 kb of isolate KV-2. Full-length cDNA clones of the two virus isolates will be made from total RNA isolated from Kalanchoë to better ensure that the clones will be infectious. When the full-length clones have been obtained they will be equipped with the appropriate accessories according to the chosen means of introduction into the plant. The infectious clones will then be manipulated to work as VIGS vectors in Kalanchoë.

3. Phenotypic and molecular characterization of genetic resources of Nordic timothy (*Phleum pratense* L.)

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Timothy is the most important forage grass species in the northern parts of the Nordic countries. Serious winter damages, which significantly lower persistency and yields, occur regularly. The Nordic plant breeders have so far been quite successful in developing new, high-yielding varieties. However, the challenges today are to further improve winter hardiness and quality, especially in view of changing climatic conditions, as well as to produce varieties that might be exported to new markets.

The Nordic Gene Bank (NGB) has a large collection of timothy accessions. However, neither the genetic structure nor the value of the collection for plant breeding purposes has been studied based on modern molecular tools. For plant breeding such information would make the collection more accessible and make it possible to improve specific traits more targeted and develop new varieties more efficiently.

In this project, field trials in three Nordic countries and application of DNA marker techniques will be used to study the phenotypic and genetic variation of Nordic timothy germplasm in terms of distribution, dispersion history and important adaptive traits such as vernalization response and frost tolerance. The bio-geographical history of Nordic timothy will be studied using exotic germplasm, and the genetic basis of breeding materials broadened by identification of heterotic groups and new sources for improving important traits. The resulting data will be utilized both for improving genetic resource management of timothy and for breeding purposes.

4. Cisgenenic barley and wheat for animal feed

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Genetic transformation is currently met with substantial scepticism among the general public and consequently also by the growers, the agro industry and the retailers. One major concern is the mingling of genetic material between species. In the light of this we have initiated a collaborating project with different partners based on the *Cisgenesis* concept with the financial support from the Danish Directorate for Food, Fisheries and Agro-business.

In contrast to *Transgenesis, Cisgenesis* implies that the plants are transformed only with its own or very closely related genetic material. Furthermore, all 'helper' genes and gene sequences of foreign nature are removed from the transformed plant lines. The *Cisgenesis* concept allows for the introduction of extra gene copies of a particular gene whereby a particular trait can be accentuated. Transgenic crops generated by the *Cisgenesis* concept are accordingly very similar to those generated by conventional breeding.

In our part of the project we are focusing on barley and wheat phytases as candidate genes for *Cisgenesis*. Recently, Dionisio et al (2007a,b) have cloned and characterized phytases in barley and wheat belonging to the purple acid phosphatases (PAPs) and the multiple inositol polyphosphate phosphatases (MINPPs). We are currently isolating genomic clones of these cDNA's including 1-2 kb of the promoter region and 600-700 bp of the terminator region using a barley lambda library. These clones are sequenced and characterized and will subsequently be inserted into *Agrobacterium* vectors and used for transformation according to the *Cisgenesis* concept.

References:
Dionisio G, Holm PB and Brinch-Pedersen H (2007a) Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) multiple inositol polyphosphate phosphatases (MINNPPs) are phytases expressed during grain filling and germination. Plant Biotech. J. 5: 325-338

Dionisio G, Holm PB and Brinch-Pedersen H (2007b) Cloning and biochemical characterization of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) purple acid phosphatases (PAPs). In prep.

5. Linkage disequilibrium and associations with forage quality at loci involved in monolignol biosynthesis in breeding lines of European silage maize (*Zea mays* L.)

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During recent decades, breeding efforts have lead to a substantial increase in whole plant yield of silage maize. However, during the same period of time there has been a steady decrease in cell wall digestibility, and, consequently, in feeding value of elite hybrids. Cell wall digestibility is influenced by both lignin content and lignin structure. Thus, genes involved in the lignin biosynthetic pathway are considered promising candidate genes for improving digestibility of silage maize.

Partial genomic sequences of 10 genes involved in biosynthesis of monolignols have been obtained in a number of inbred lines currently employed in European silage maize breeding. Different levels of nucleotide diversity and linkage disequilibrium (LD) were found, indicating different levels of selection pressure on individual genes of the monolignol pathway. Individual polymorphisms were tested for association with four quality-related traits to identify candidate functional markers for forage quality. Significant associations were identified, both when including and excluding population structure in the analysis. However, discrimination of effects of individual polymorphism was in some cases not possible due to extended LD. Studies in larger and/or broader sets of maize germlasm could decrease LD and validate candidate functional markers for forage quality identified in the present study.

6. Dwarfism by introducing *rol*-genes from *Agrobacterium rhizogenes* into *Kalanchoe*

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Kalanchoe blossfeldiana is an economically important potted plant in North America and Europe and the Danish production of *K. blossfeldiana* was estimated to be 40 million plants with a value of 29 million EUR in 2006. Denmark is one of the leading countries in the production of potted ornamental plants and around 85 percent of the Danish production is exported, and this imposes a great demand on plant quality.

One important quality criterion is that the plants should be compact, but most potted plants like *K. blossfeldiana* have an elongated natural growth habit, which necessitates growth control through the application of chemical growth retardants. As chemical growth retardants are hazardous to human health and environment, some chemical growth retardants can no longer be used in many countries and most likely more of these compounds will be banned in near future. An alternate strategy to the application of chemical growth retardants could be the production of compact genotypes by inserting the *rol*-genes from the naturally occurring soil born bacterium *Agrobacterium rhizogenes* into the plants.

Leaf explants of *Kalanchoe blossfeldiana* were inoculated with wild-type *A. rhizogenes* strain and transgenic hairy roots were induced at the site of infection due to the insertion of *rol*-genes. These transgenic roots were selected based on the hairy phenotypic characteristics without using any selection marker. From the transgenic root lines, plants were regenerated in tissue culture and in a number of lines, the presence of *rolC* was confirmed by PCR and copy number of the insert was determined by Southern blotting. These transgenic lines were grown and evaluated under greenhouse conditions and exhibited various degrees of the Ri-(Root inducing) phenotypic characteristics such as changes in leaf number and morphology. The internodes of the transformants were clearly shorter giving a compact growth habit, and the inflorescences were denser compared to that of control plants. These characteristics are useful in creating new genotypes in ornamental species, especially to create compact plants.

Posters: Systems Biology

1. Organelle markers in *Lotus japonicus*

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Fluorescent proteins have become an important tool in molecular biology. They allow for the localisation of proteins and the detection of expression patterns in live cells and tissues. Subcellular localisation studies require specific markers for the different cellular organelles. Recently, a set of multicolored organelle markers was developed for *in vivo* colocalisation studies in plants (Nelson et al, 2007). These markers use different fluorescent proteins, CFP, GFP, YFP and mCherry with targeting sequences characteristic for a number of organelles – ER, Golgi, tonoplast, peroxisomes, mitochondria, plastids and the plasma membrane. In the present study the usefulness of these markers was tested in the model system *Lotus japonicus.*

References:
Nelson, B.K., Cai, X., Nebenführ, A. 2007. A multicolored set of *in vivo* organelle markers for co-localisation studies in Arabidopsis and other plants. *The Plant Journal* **51(6):** 1126-1136

2. Oncological Target Genes For Human Diagnostics And Therapeutics Identified In Plants

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Cancer is a serious and common disease influencing a wide range of organs and tissues in the human body. Many initiatives are taken in order to find efficient treatments, but the complexity of cancer makes the process of finding cures difficult.

This project attacks cancer research from a unique and novel angle, operating in the cross field between plant and medical science. The aim of the project is design of new cancer therapeutics and diagnostic kits based on human tumor suppressor genes that have been identified using the plant *Arabidopsis* as a model system. The reasoning behind employing a plant system to identify target genes is the strong evolutionary conservation of plant and human cancer relevant genes. In addition plants may be able to circumvent cancerous growth simply by following alternative developmental pathways giving rise to novel phenotypes.

Our strategy has already been successfully applied as a first round screen demonstrated that five of six types of cancerous plants contained mutations in genes homologous to different human cancer-related genes. This project suggests the processing of the identified putative human tumor suppressor genes into diagnostics products and further development of the core technology leading to additional diagnostics and therapeutics opportunities.

3. The expression level of the highly dynamic chromatin-associated HMGB1 protein influences development and stress response in *Arabidopsis*

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After the histones, the high mobility group (HMG) proteins are the most abundant chromosomal proteins. Members of the plant HMGB family are characterised by an HMG-box DNA-binding domain that flanked by a basic N-terminal and an acidic C-terminal domain. *Arabidopsis* HMGB1 is a typical member of the plant HMGB family that displays a high dynamics in the cell nucleus, interacting with chromatin only transiently. Accordingly, HMGB1 may play both a global role in chromatin architecture and site-specific effects.

We have examined the growth and stress response of *Arabidopsis* plants lacking HMGB1 and of plants overexpressing HMGB1. The plants with altered levels of HMGB1 exhibit some developmental abnormalities (root growth, germination). Moreover, they are affected in stress responses. Therefore, the level of *Arabidopsis* HMGB1 protein is critical for normal development and stress response. Transcript profiling by microarray analysis of seedlings lacking HMGB1 revealed that a large number of genes is misexpressed in the mutant plants compared to control plants. In summary, our experiments demonstrate that despite the presence of several other members of the HMGB family, both lack and overexpression of HMGB1 have various consequences during *Arabidopsis* development.

4. Barley plasma membrane proteomics: identification of protein targets for improvement of crop plants

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Almost all contact with the surroundings of a plant cell is initially perceived via the plasma membrane embedded proteins that are known to act as sensors and facilitators of transport. Knowledge of the plasma membrane protein profile is therefore needed, if we are to use targeted breeding or gene technology to develop crop plants that tolerate soils of poor quality, have enhanced seed quality and nutritional value or to breed plants that are resistant to pathogenic attacks.

We have optimized the aqueous polymer two-phase systems for isolation of plasma membranes from barley seeds, roots and leaves. By employing an efficient reversed-phase chromatography strategy, which combined with SDS-PAGE and tandem mass spectrometry has proved valuable for enrichment of integral membrane proteins (Hynek et al., 2006), we were able to characterize the plasma membrane of the aleurone layers and embryos from barley seeds. Data showed that the plasma membrane protein profiles were very different, reflecting different roles of the aleurone layer and embryo during seed germination.

At the moment, we are focusing on isolation and characterization of plasma membrane proteins from barley leaves, because plasma membrane proteins play an important signaling role in the response to pathogens. Pathogens such as the powdery mildew fungus have been suggested to induce both expression and activation of a plasma membrane proton pump in barley epidermis (Finnie et al., 2002). Furthermore, mediators of resistance to the fungus have been identified in the plasma membrane.

In order to compare the plasma membrane proteome of infected and non-infected barley leaves, we are currently optimizing and implementing a 2-DE method based on benzyldimethyl-n-hexadecylammonium chloride (16-BAC) electrophoresis in the first dimension and SDS-PAGE in the second, for obtaining a much improved separation of membrane proteins. This 2-DE gel method, when combined with tandem mass spectrometry is a powerful tool for plasma membrane proteomics.

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5. DeepSAGE SOLEXA

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Digital transcriptomics produced by DNA sequencing-by-synthesis technology (SOLEXA, Illumina) of monotags provides high sensitivity and cost-effective gene expression profiling. Sample preparation and handling are greatly simplified compared to Serial Analysis of Gene Expression (SAGE) (Velculescu et al. 1995). Here we introduce the DeepSAGE SOLEXA sample preparation protocol and compare it with the protocols of DeepSAGE 454 (Nielsen et al. 2006) and LongSAGE (Saha et al. 2002) to emphasize the improvements and to demonstrate the power of detection and multiplexing of samples derived from varies organisms.

6. Transcriptome Analysis of Potato Tuber Life Cycle

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Solanum tuberosum (potato) is the fourth major crop worldwide and used for food, feed and biotechnological applications. To fully realize the biosynthetic potential for production of starch, protein and metabolites, we conducted an extensive quantitative profiling of the expressed genes of five different developmental stages of the potato tuber cv. Kuras by Serial Analysis of Gene Expression (SAGE) (Velculescu et al. 1995) and DeepSAGE (Nielsen et al. 2006).

A total of 213,419 tags were generated by LongSAGE (Saha et al. 2002) 167,331 by DeepSAGE from 8 week old plants (Mini tuber, MIT), from plants at the end of flowering (Mature tuber, MT) (Crookshanks et al. 2001), at the time of harvest (HAR), at dormancy after 60 days of storage at 10°C (Dormant, DOR) and tuber tissue just below the shoot of a sprouting tuber (Sprouting Eye, SE). The resulting 19 nt tags were matched to genes using the 219,407 ESTs assembled into 30,255 tentative consensus sequences known for potato and stored at compbio.dfci.harvard.edu. A multiple comparison of SAGE libraries was conducted and significantly regulated genes were identified using strict Bonferroni correction. These were clustered into 24 clusters using a Poisson-based clustering algorithm (Cai et al. 2004) after SAGE library data were normalized to a tag count of 100,000.

The potato tuber transcriptome analysis shows that the tuber is a highly dynamic tissue where at least a thousand genes are regulated in the course of development. The loading of storage proteins happens early in tuber development and thus the protein pool produced is stored for some nine months before it is catabolized by the new plant. During maturation, transcripts encoding protease inhibitors dominate the expression profile, but these transcripts are at least a hundred fold down regulated at the time of sprouting, which also sees the emergence of the cystein protease family, Vacuolar Processing Enzymes. Most likely this facilitates the mobilization of amino acids from the storage proteins. Furthermore, during sprounting the expression of metallothioneins is extremely abundant.

7. Characterization of the thioredoxin system in barley seeds: cloning and expression of two NADPH-dependent thioredoxin reductase (NTR) isozymes

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Thioredoxins are small (12 kDa) proteins catalyzing reduction of disulfide bonds and are involved in regulation of the cellular redox environment. Plants contain different types of thioredoxin systems. Chloroplast thioredoxins are reduced by a ferredoxin-thioredoxin reductase (FTR), whereas the cytosolic or mitochondrion h-type thioredoxins are reduced by NADPH in a reaction catalyzed by NADPH-dependent thioredoxin reductase (NTR). Trx h isoforms from barley seeds (HvTrxh1 and HvTrxh2) were characterized previously [1,2].

In the current study we describe isolation and cloning of two cDNAs encoding plant cytoplasmic or mitochondrial type NTR from barley seeds. The polypeptides deduced from these two cDNAs, NTR1 and NTR2, have 88% sequence identity. Both isoforms were expressed in *E. coli* as His-tagged proteins and exhibited virtually the same affinity towards HvTrxh1 and HvTrxh2, whereas HvNTR2 has slightly higher catalytic activity than HvNTR1 with both Trx h isoforms, and HvNTR1 has slightly higher catalytic activity towards HvTrxh1 than HvTrxh2. Notably, both NTRs reduced Trx h at the acidic conditions residing in the starchy endosperm during germination. Interspecies reactions between the barley proteins and *E. coli* Trx or *Arabidopsis thaliana* NTR, respectively, occurred with 20-90 fold weaker affinity. In addition, the gene expression profiles and protein appearance patterns of enzymes involved in the thioredoxin system were studied in embryo of barley seeds during germination and in aleurone layer in response to hormone signals. Although mRNA encoding both Trx h isoforms is present in embryo and aleurone layer, the corresponding proteins differed in spatio-temporal appearance [1,3]. HvNTR2, but not HvNTR1 gene expression seems to be regulated by gibberellic acid.

This first investigation of regulation and interactions between members of the NTR/Trx system in barley seed tissues suggests that different isoforms are differentially regulated but may have overlapping roles, with HvNTR2 and HvTrxh1 being the predominant isoforms in aleurone layer.The heterologous expression of both NTR and Trx h isoforms provided a basis for design and characterization of mutants using site directed mutagenesis to investigate the interaction between NTR and Trx h at the level of molecular structures.

References:
[1] Maeda et al., (2003). Eur J Biochem 270: 2633-2643
[2] Maeda et al., (2006). Structure 14: 1701-1710
[3] Bønsager et al., Proteomics (in press)

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8. A new holistic exploratory approach to Systems Biology by Near Infrared spectroscopy evaluated by chemometrics and data inspection

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There is a need for an improved theoretical and mathematical interpretation of Near Infra-Red Spectral (NIRS) fingerprints from tissues that could contribute with holistic overview to fine-grained detail modelled in Systems Biology. The concept of gene expression in self-organised networks was experimentally tested in a barley endosperm model with molecularly defined and undefined mutants. Surprisingly reproducible phenomenological gene specific NIRS fingerprints were observed directly in log1/R MSC pre-treated spectra that could not be accurately represented by destructive mathematical methods. A mutant spectrum from an isogenic background represents the physiochemical effect of the gene on the whole network (tissue). The necessary holistic overview that is needed experimentally to introduce Ilya Prigogine´s theory on self-organisation in Systems Biology was supplied by defining the spectral phenome. Interval spectral information on genotypes and environment was classified by iECVA (interval Extended Canonical Variates Analysis). Genetic changes in spectra were interpreted by iPLSR (interval Partial Least Squares Regression) correlations to chemical variables. The finely grained "bottom up" modelling of molecular and chemical data from pathways requires a coarsely grained exploratory "top down" overview by NIRS to account for the outcome of self-organisation.

The amplification of expression from a gene to the phenome (pleiotropy) can now for the first time be quantified as a whole by NIRS and compared to other gene spectra. It explains published findings that transformed and mutated genes in GMO´s and cancer patients respectively can be detected unsupervised from the tissues by spectroscopy, chemometrics and data inspection.

Keywords:
Self-organisation in an endosperm mutant model. The NIR spectral phenome. Interval iECVA/iPLSR physiochemical spectral interpretation. "Top down-bottom up" data modelling. Indeterminacy in Systems Biology

Reference:
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SDU: University of Southern Denmark

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Inst: Institute