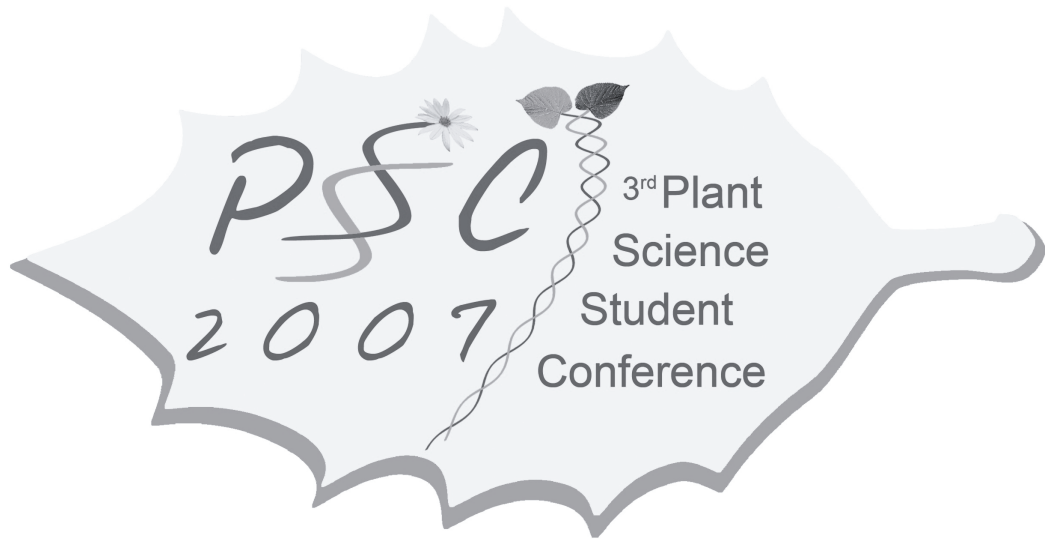


Plant Science Student Conference



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Leibniz Institute of Plant
Biochemistry
Halle
June 5 – 8, 2007

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Program

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	Isolation and Characterisation of MEKK1 interacting proteins in <i>Arabidopsis thaliana</i>	
11:10 - 11:35	Ernst Metzner	
	Transcriptome and proteome dynamics of pathogen-attacked barley epidermis	
11:35 - 12:00	Franziska Handmann	
	Characterization of a necrosis-inducing protein from <i>Phytophthora sojae</i>	
12:00 - 13:00	lunch break	
13:00 - 13:25	Daniela Floß	
	The importance of the plastid-located methylerythritol phosphate (MEP) pathway for the arbuscular mycorrhizal (AM) symbiosis in <i>Medicago truncatula</i>	
13:25 - 13:50	Gerit Bethke	
	Identification of a MAPK substrate in <i>Arabidopsis thaliana</i>	
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	The role of 9-oxylipins for plant defense	
14:15 - 14:30	coffee break	

Session II: Methods and applications (chair: Tino Unthan)

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14:55 - 15:20	Christian Kuenne	
	DiSTo - The Diversity Studies Toolkit	
15:20 - 15:45	Anja Scholz	
	Research and production of polyhydroxyalkanoates in plants and yeasts	
15:45 - 16:15	coffee break	

16:15 - 16:40	Felix Bollenbeck Quantifying Biodiversity in Histological Cross-Sections towards fast Tissue Prediction for Inter-Individual 3D Models
16:40 - 17:05	Doreen Manuela Floß Plantibodies against HIV: A proof of concept study
17:05 - 17:30	Felix Stehle Towards the Mystery of Beeing no Hydrolase of a Serine Carboxypeptidase-like Acyltransferase
17:30 - 18:00	break
18:00 - 19:30	poster session (even numbers)
19:30	dinner / leisure time

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11:35 - 12:00	Nicole Staroske The role of AtMPK3 and AtMPK6 in the female gametophyte development
12:00 - 12:15	coffee break
	Session IV: Genetics (chair: Silke Pienkny) 29
12:15 - 12:40	Navina Hamilton Understanding Apomixis in <i>Hypericum perforatum L.</i>
12:40 - 13:05	Jens Keilwagen Predicting the number of haplotypes by an integration of marker, passport, and phenotypic data
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Friday, 8th of June

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- 09:00 - 09:25 **Thekla Pleines**
An AFLP phylogeny of the diploid New World *Hordeum* species
- 09:25 - 09:50 **Dirk Meißner**
The role of Hydroxycinnamate Glucosyltransferases (HCA-GTs) in *Arabidopsis thaliana*
- 09:50 - 10:15 **Thomas Thiel**
Inferring barley genome duplications using the synteny to rice
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- 10:35 - 11:00 **Silke Pienkny**
Specialization of biosynthetic enzymes exemplified by the evolution of substrate specificity of *O*-methyltransferases
- 11:00 - 11:25 **Markus Benderoth**
Biochemical Characterization of MAM Synthases in *Arabidopsis* and Friends
- 11:25 - 11:45 **coffee break**
- 11:45 - 12:10 **Heike Riegler**
The Pyrimidine Salvage Pathway - a Recycling System in Plants
- 12:10 - 12:35 **Yvonne Pöschl**
Analysis of Metabolomics Data
- 12:35 - 13:00 **Kathleen Weigelt**
Repression of ADP-glucose pyrophosphorylase in developing seeds of transgenic pea (*Pisum sativum*) changes starch and protein metabolism
- 13:00 - 14:00 **lunch break**

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- 14:00 - 14:25 **Anja Paschold**
CO(I)-ordination of Jasmonate biosynthesis and local defense responses to herbivory in *Nicotiana attenuata*
- 14:25 - 14:50 **Anja Hanemann**
Towards the Map-Based Cloning of the Scald Resistance Gene *Rrs2* in Barley (*Hordeum vulgare* L.)
- 14:50 - 15:15 **Daniel Peisker**
Phytochelatin synthase - a major enzyme in heavy metal detoxification
- 15:15 - 15:35 **Simone Altmann**
The role of 13-lipoxygenase-derived oxylipins for pathogen defence in potato
- 15:35 - 15:55 **coffee break**

15:55 - 16:20 **Annika Johrde**
An association-genetic approach to durable powdery-mildew resistance in barley

16:20 - 16:45 **Khalil Zaynali Nezhad**
Post-anthesis drought tolerance assessment on bread wheat

16:45 - 17:10 **Stefanie Ranf**
Role of the vacuolar cation channel AtTPC1 in biotic and abiotic stress responses: calcium or potassium?

17:10 - 17:30 **break**

17:30 - 19:00 **poster session (odd numbers)**

20:30 **award ceremony**

21:00 **party**

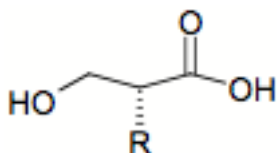
Invited Speakers

White Biotechnology at BASF

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The presentation will give an overview of the activities in the area of white or industrial biotechnology at BASF. Our research strategy comprises four areas: fermentation, biocatalysis, biopolymers and performance biologicals. Several examples will be used to explain the specific fields. In a second part the talk will focus on the development of a biocatalytic process for a pharmaceutical intermediate. BASF is applying biocatalysis at large scale to produce chiral synthons as advanced intermediates or in custom synthesis. The use of lipases for the racemic resolution will be described at the example of β -hydroxycarboxylic acids.



The findings of the first enzyme screening will be introduced together with a laboratory synthesis deduced from it. Finally, an optimized synthesis was scaled up successfully to 4000 liter scale.

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The Charming Complexity of Cullins

H. Hellmann

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Ubiquitination is a fascinating regulatory tool for various biological processes, mostly for the control of rapid and selective degradation of important regulatory proteins involved in cell cycle and development, among others. The superfamily of cullin-RING finger protein complexes is the largest known class of E3 ubiquitin ligases in eukaryotes and several substrates have been described in different organisms. In plants, cullins can be grouped at least into four subfamilies, and each subfamily associates with a specific class of substrate receptors that often belong to large protein families. Consequentially, the corresponding complex interaction patterns indicate that numerous substrate proteins are ubiquitinated by plant E3 ligases and that most aspects in plant development are influenced by cullin-based E3 ligase activities. This talk will provide a brief overview about the current knowledge on the different cullin-based E3 ligases present in higher plants, including description of their complex composition, regulatory mechanisms controlling their activity, but also perspectives on what we have to expect from them in the future.

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The regulatory code for transcriptional response diversity in *Arabidopsis thaliana*: Increased breadth of differential gene expression response to external stimuli correlates with greater regulatory potential in gene promoter regions

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Regulation of gene expression via specific *cis*-regulatory promoter elements has evolved in cellular organisms as a major adaptive mechanism to respond to environmental change. Assuming a simple model of transcriptional regulation, genes that are differentially expressed in response to a large number of different external stimuli should harbor more distinct regulatory elements in their upstream regions than genes that only respond to few environmental challenges. We tested this hypothesis in *Arabidopsis thaliana* using the compendium of gene expression profiling data available in AtGenExpress and known *cis*-element motifs mapped to upstream gene promoter regions. We observed highly significant positive correlations between the density of *cis*-elements in upstream regions and the number of conditions in which a gene was differentially regulated. Multi-stimuli response genes were observed to be associated with significantly longer upstream intergenic regions, retain more paralogs in the *Arabidopsis* genome, are shorter and contain fewer introns, and are more likely to contain TATA-box motifs. In abiotic stress time series data, multiple-stimuli response genes were found to be overrepresented among early responding genes. Genes involved in the regulation of transcription, stress response and signalling processes were observed to possess the greatest regulatory capacity. Our results suggest that greater gene expression regulatory complexity appears to be encoded by an increased density of *cis*-regulatory elements and provide further strong evidence for an evolutionary adaptation of the regulatory code at the genomic layout level. Larger intergenic spaces preceding multi-stimuli response genes may have evolved to allow greater regulatory gene expression potential.

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Talks

Isolation and Characterisation of MEKK1 interacting proteins in *Arabidopsis thaliana*

T. Unthan, C. Spielau, R. Schlichting, G. Bethke, J. Lee, and D. Scheel

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Mitogen-activated protein kinase (MAPK) cascades are important components of signal transduction in plants. In response to abiotic and biotic stresses the components of the cascades can be activated via phosphorylation. A MAPK cascade consists of at least 3 parts. A MAPK is activated by a MAP2K, which in turn is activated by a MAP3K. In *Arabidopsis thaliana* 20 putative MAPK, 10 putative MAP2K and 60 putative members of the MAP3K family were found. These numbers show the complexity of the possible combinations of the components but also highlight the fact that some members must cater to different pathways; thus raising the question of signal specificity maintenance.

MEKK1 is one of the best characterised MAP3K and was described as activator of the MAP2Ks MKK4/MKK5 after recognition of the bacterial elicitor Flagellin or of MKK1/MKK2 after cold or salt stress. Furthermore, MEKK1 is required for plant development, because mekk1 mutant plants show an early senescence phenotype, are perturbed in the development of true leaves and die at early stage.

The organisation and regulation of the MAPK cascade components in plants is still poorly understood.

One of the aims of our research is to understand MAPK cascade signal specificity by identifying MEKK1 interacting proteins. One approach is the native purification of MEKK1 protein complexes via protein tags. We want to create transgenic plants expressing MEKK1 with a TAP (tandem affinity purification) tag or a Strep tag. The TAP method enables the native isolation of proteins with high purity in two affinity steps. In comparison, the Strep purification is more rapid but may yield lower purity. The second approach for the identification of MEKK1 interacting partners is the use of a Yeast-2-hybrid (Y2H) system.

Preliminary data for these two approaches will be reported.

Topics: Biochemistry

Keywords: MAPK cascade, MEKK1, protein purification, TAP tag, Strep tag

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Transcriptome and proteome dynamics of pathogen-attacked barley epidermis

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Barley (*Hordeum vulgare*) is an important cultivated plant, which can be infected by various pathogens. Powdery mildew is a widespread disease of barley caused by the obligate biotrophic fungus *Blumeria graminis* sp. *Hordeum*. This pathogen invades exclusively epidermal cells after penetrating directly through the cell wall. Besides this barley can be infected by *Rhynchosporium secalis*. In our project we want to identify novel cellular factors mediating resistance against fungal diseases. The new approach of this venture is to compare the cellular responses of a susceptible barley cultivar infected with the biotrophic fungus *Blumeria graminis* and the necrotrophic fungus *Rhynchosporium secalis* in parallel, working in close cooperation with the Institute of Plant Biochemistry (Wolfgang Knogge, IPB, Cellular Signaling) in Halle. Because of the crucial role of the epidermis in defense against fungal diseases epidermal tissues were analyzed. In three harvests we dissected epidermis from leaf. With the use of several points of time we are able not only to identify differential transcriptional and translational effects, but also to get an insight of the kinetics over three biological replicates.

For each time point we compared the protein pattern of epidermis of control and infected plants with the DIGE-method. By this method protein samples are labeled prior to 2D PAGE with up to three fluorescent dyes such that samples can be co-separated and visualized on a single 2D gel. That reduces the problems arising from gel-to-gel variation during image analysis. For each time point 2 gels were made, while the dyes are altered over the samples. Quantitative differences were determined by comparative image analysis. Spots that are differentially expressed between control and infected epidermis tissue are picked by automated spot-picking and are in identification process using a combination of MALDI-TOF/MS and ESI-Q-TOF/MS. First results will be shown.

Topics: Biology

Keywords: barley, powdery mildew, susceptible, proteomics, 2D PAGE

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Characterization of a necrosis-inducing protein from *Phytophthora sojae*

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The family of NLPs (necrosis- and ethylene-inducing protein-like proteins) exists in a wide range of phytopathogens including oomycetes and bacteria. These proteins are discussed as positive virulence factors during the necrotrophic phase of pathogen attack by triggering cell death possibly as a toxin. However they also have elicitor functions since they activate plant defense responses, such as callose deposition, ROS, ethylene, camalexin and salicylic acid production.

Here we want to characterize defense responses of *Arabidopsis thaliana* and *Petroselinum crispum* upon treatment with the necrosis inducing protein of *Phytophthora sojae* (PsojNip). Since the heterologous expression of PsojNip in *E. coli* results in formation of inclusion bodies, refolding conditions for active protein had to be optimized. The refolding efficiency was estimated by necrosis formation.

The results obtained with PsojNip will be compared to those obtained with the highly homologous protein PaNie from *Pythium aphanidermatum*.

Topics: Biochemistry

Keywords: necrosis inducing protein, defense responses

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The importance of the plastid-located methylerythritol phosphate (MEP) pathway for the arbuscular mycorrhizal (AM) symbiosis in *Medicago truncatula*

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The plastidial MEP pathway produces precursors for isoprenoid biosynthesis. In *M. truncatula* and many other plants 1-deoxy-D-xylulose 5-phosphate synthase (DXS), the first enzyme of the MEP pathway, is encoded by two distinct genes. The *DXS1* isogene is required for primary isoprenoids, whereas the *DXS2* isogene encodes the enzyme for secondary isoprenoid biosynthesis. Transcript analysis of *MtDXS* in AM roots revealed an increase in *MtDXS2* transcripts correlated with the accumulation of carotenoid cleavage products (apocarotenoids). However, the functional significance of these compounds for the AM symbiosis is not known so far. To reveal a potential importance of MEP pathway activation and/or (apo)carotenoid biosynthesis we reduced the *MtDXS2* transcripts in roots of *M. truncatula* by an RNAi approach.

The suppression of *MtDXS2* resulted in a significant reduction of apocarotenoid levels in mycorrhizal transgenic roots harboring the RNAi-constructs in comparison to plants transformed with the empty vector. In roots with strongly silenced *MtDXS2* transcript a reduction in the transcript levels of the AM-specific phosphate transporter *MtPT4* was observed along with an increase in degenerating and dead arbuscules. However, roots with a moderate suppression in *MtDXS2* transcripts surprisingly revealed a marked increase in *MtPT4* transcript levels indicating a parallel increase in functional arbuscules, the main organs of the symbiosis. These results suggest an important multiple role of MEP pathway end products for the functionality and/or abundance of arbuscules.

Topics: Biology

Keywords: mycorrhiza, symbiosis, isoprenoid biosynthesis, methylerythritol phosphate pathway, RNAi

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Identification of a MAPK substrate in *Arabidopsis thaliana*

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Due to their sessile life style plants have evolved sophisticated mechanisms to detect changes in their environment. Mitogen-Activated Protein Kinase (MAPK) cascades play important roles in various cellular signalling pathways including defence responses to pathogen attack. In *Arabidopsis thaliana*, activation of the MAPKs, AtMPK3, AtMPK6 and AtMPK4, in response to the flagellin-derived peptide elicitor flg22 has been reported.

In a yeast two hybrid screen, we identified several putative MAPK interactors. With the aim of verifying these interactors *in vivo*, we established a Fluorescence Resonance Energy Transfer (FRET) assay based on transient expression in *Arabidopsis* protoplasts.

We report here the interaction between AtMPK6 and a putative EREBP-like transcription factor (abbreviated as EL1 hereafter). This interaction occurs within the nucleus and can be disrupted by elicitation of the protoplast with flg22. This disruption is dependent on the activity of AtMPK6, since kinase-inactive mutants of AtMPK6 show continuous interaction with EL1 under flg22 treatment. AtMPK3 and AtMPK4, the other flg22-activated MAPKs, show no interaction with EL1. Hence, among the known flg22-activated MAPKs, interaction with EL1 appears to be specific to MPK6.

The flg22 disruption of AtMPK6 and EL1 interaction prompted us to test, if EL1 is a MAPK substrate. To this end, we found that recombinant EL1 can be phosphorylated by active MPK6. However, phosphorylation had no obvious impact on the ability of EL1 to bind a GCC promotor element. Overexpression of EL1 in plant cells leads to the activation of a synthetic GCC-Promotor.

The interaction of EL1 and AtMPK6, in addition, is disrupted by 1-aminocyclopropane-carboxylic acid (ACC), an ethylene precursor. Since the flg22-dependent disruption of EL1-MPK6 interaction did not occur in ethylene signalling mutants, we suggest that the *in vivo* EL1-MPK6 protein-protein interaction is modulated by ethylene signalling.

Topics: Biology

Keywords: MAPK, signalling, protein-protein-interaction

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The role of 9-oxylipins for plant defense

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Oxygenated polyunsaturated fatty acids are known to play an important role in plant defense against pathogens. Direct antimicrobial effects as well as functions in defense signaling could be shown. Starting with the fatty acids linoleic (La) or linolenic acid (LnA), the oxylipin pathway leads to the production of a large variety of fatty acid derivatives. Two main branches of the pathway can be induced during pathogen attack, the 9- and 13-lipoxygenase (9-/13-LOX) pathways, depending on the position at which molecular oxygen is introduced into La or LnA in the first step (Rosahl and Feussner, 2004).

In potato plants infected with virulent *Phytophthora infestans*, the 9-LOX pathway is preferentially induced, leading to the accumulation of (tri)hydroxy- and divinyl ether-derivatives of La and LnA (Göbel et al., 2001). Additionally, in solanaceous plants a specific role for the 9-LOX pathway in lipid peroxidation processes during hypersensitive cell death in response to elicitor treatment could be shown (Rustérucchi et al., 1999; Göbel et al., 2003). To functionally address the importance of 9-oxylipin formation for basal defense as well as *R* gene-mediated resistance against *P. infestans*, potato *9-LOX*-RNAi plants were generated in the susceptible cultivar Désirée (*r*) and in *R1* resistance gene carrying Désirée (*R1*).

One of several metabolic routes following the initial 9-LOX step is the 9-divinyl ether synthase (9-DES) branch, leading to the production of the divinyl ethers colneleic and colnelenic acids which were shown to have direct antimicrobial effects (Prost et al., 2005). To investigate the impact of divinyl ethers on plant defense, *9-DES*-RNAi Désirée (*r*) plants were generated.

References:

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Göbel, C. et al., 2003. J. Biol. Chem. 278, 52834-52840.
Rosahl, S. and Feussner, I., 2004. Plant Lipids: Biology, Utilisation and Manipulation, Blackwell, Oxford, 329-354.
Rustérucchi, C. et al., 1999. J. Biol. Chem. 274, 36446-36455.

Topics: Biology

Keywords: oxylipins, potato, *Phytophthora*, RNAi

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Thermal analysis of the water status in explants of potato (*Solanum tuberosum* L.) comparing two cryopreservation methods

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Cryopreservation (storage in liquid nitrogen) is used for long term maintenance of potato in the IPK. Hereby the dimethyl sulfoxide (DMSO) droplet method is applied and so far over 1000 accessions are cryopreserved with an average regeneration rate of 40%. The aim of most cryopreservation methods is the formation of a so-called glassy state of cell solution during rapid cooling. In this state no chemical and physical reactions occur and the conservation of germplasm is stable for an indefinite period. Formation of ice crystals within cells should be avoided, because they destroy the plasma membrane and other cell structures. Characteristic thermodynamic processes like glass transition and crystallization of water can be analyzed by differential scanning calorimetry (DSC). Two methods of cryopreservation were compared, the droplet vitrification and the DMSO droplet method. DSC analysis for the vitrification method showed glass transition at a temperature range from -95 °C until -105 °C. Some crystallisation and melting of ice was also found. In contrast, in the DMSO droplet method, only ice melting was observed with a melting peak at -0.7 °C. Using this method, no glass formation was measured. These results showed that the two compared methods are based on two different principles. During vitrification, formation of glass occurred, whereas during the DMSO droplet cooling formation of very small ice crystals may happen, which do not destroy the cell structure of shoot tips. With this DSC measurement it was proven that ice formation proceeds during DMSO droplet cooling. When compared with non-protected shoot tips, DMSO treated shoot tips showed melting of ice in lower temperatures and with a smaller heat flow. Therefore, the cryoprotectant lowers freezing temperature and amount of freezable water in the shoot tips. Further ultrastructural studies should be made to get a clear view on the structure of glass and small ice crystal formation.

Topics: Biology

Keywords: cryopreservation, differential scanning calorimetry, *Solanum tuberosum* L.

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DiSTo - The Diversity Studies Toolkit

C. Kuenne, K. J. Dehmer, A. Graner, U. Scholz, T. Sretenovic Rajcic, J. Tetz, E. Willner, and I. Grosse

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To support DNA fingerprinting of a collection of ryegrass accessions (*Lolium* spp.) within the Genebank fusion project of the IPK and the BAZ Braunschweig we developed a database to store pyrosequencing experiment data. As a prerequisite to build a comprehensive analysis platform for studying genetic diversity, it provides a structure to store sequence information, SNP marker data, allele frequencies, and further experimental data from pyrosequencing the collection of 3,187 *Lolium* accessions. These data are integrated into the Plant Data Warehouse together with sequence data from the IPK Sequence Database, passport data from the IPK Genebank Information System (GBIS), and phenotypic data measured in different geographic locations. All data necessary for diversity studies are combined in an application specific "Diversity" data mart. Based on this dedicated data storage unit we developed the Diversity Studies Toolkit (DiSTo) facilitating data queries, comprehensive and multivariate data analysis, as well as visualisation of results. In this context DiSTo provides various features including (i) accession filtering, (ii) colouring and labelling, (iii) genetic distance calculation, (iv) phenogram construction, and (v) principal component analysis together with 2D and 3D visualisation.

Topics: Bioinformatics

Keywords: statistical analysis, diversity studies, fingerprinting, tool, database

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Research and production of polyhydroxyalkanoates in plants and yeasts

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Polyhydroxyalkanoates (PHA) are polymers which offer very attractive commercial properties like thermoplastic characteristics, biocompatibility and biodegradability. The most important property of the biodegradability could aid to solve problems like the environmental pollution and our waste management. Equally in medical science it can be used inside of human bodies during surgeries.

The most examined kind of PHA was poly(β -)hydroxybutyrate (PHB). PHB is a potential biodegradable alternative to petrochemical-derived plastics. In the nature only wild types of bacteria are able to produce these polyesters. We want to transfer the bacterial gene expression system (constructed *E. coli* plasmids) into eukaryotic yeast cells. The integration into the bakery yeast *Saccharomyces cerevisiae* was successful. However, the yield is still less. So now we want to force up the rate of the PHB-production by transformations into the unconventional yeast *Blastobotrys adeninivorans* (formerly: *Arxula adeninivorans*) which shows unusual biochemical activities.

Nevertheless this field of research is also applied for transformations into plants. But after transformation often obvious effects to the growth rate could be observed. Successful integrations could be carried out by *Agrobacterium*-mediated transformations, e.g. in *Arabidopsis thaliana*, cotton or Alfalfa.

Topics: Biochemistry

Keywords: polyhydroxyalkanoates, PHB, *Arxula*, yeast, transformation

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Quantifying Biodiversity in Histological Cross-Sections towards fast Tissue Prediction for Inter-Individual 3D Models

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Current technologies in life-science offer the exploration of living organisms on genomic and metabolomic scale in high-throughput manner.

The spatio-temporal resolution of these assays is essential for the understanding of growth and development of organisms - interesting topics especially in investigating crop plants.

Therefore techniques for 3-D and 4-D modeling and visualization are indispensable and have been widely used in biomedical applications, facilitating the integration of multi-modality data, and furthermore serving as a basis for the accession of new data.

Since individual 3-D models are derived from data gathered on unique organism, they are not statistically representative.

To overcome this limitation, the reconstruction process, i.e. segmentation, registration, and surface generation is done jointly on data sets from several organisms in order to obtain a generalized model or map.

We introduce an approach for 3-D histology-reconstruction of barley seeds at different developmental stages based on inter-individual serial sections.

Automated tissue detection for image segmentation using Artificial Neural Networks has proven to be robust and are used for detecting relevant materials.

Image alignment and 3-D reconstruction is solved using Principal Axis Transform and non-rigid image registration algorithms with Finite Element (FEM) solvers.

Averaging 3-D models are constructed by jointly registering inter-individual serial sections to achieve robust tissue predictions on unknown organisms. To quantify and visualize inter-individual biodiversity, we introduce a model based on deformation fields obtained by B-Spline image registration methods.

We propose the described pipeline as a prototype for the generation of biologically sound 3-D reference maps for automated cryo-dissection. The algorithms are promising both in terms of computational demands and achieving high degrees of certainty in predicting spatial locations of tissues in unseen instances.

Topics: Bioinformatics

Keywords: biomedical image registration, pattern recognition, computergraphics

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Plantibodies against HIV: A proof of concept study

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Plants are promising vehicles for the expression of recombinant proteins with regard to medical and veterinary purposes. Since the first report of a recombinant antibody derived from transgenic plants, various antibody derivatives have been produced in different crop species using several expression systems. In order to enhance the accumulation of two HIV neutralising antibodies (2F5 and 2G12) in tobacco leaves and seeds, synthetic repeats of elastin-like polypeptides (ELP) were C-terminally fused to both antibody chains. Transgenic plants expressing either the light or the heavy chain

with/without fusion were generated and combined by subsequent crossing of respective lines.

Constructs for ubiquitous and seed-specific expression of light and heavy chains with/without fusion of both anti-HIV antibodies were used for the transformation of tobacco *via Agrobacteria*. Stable transformed plants expressing the non-assembled chains with/without the synthetic protein in the ER were selected. The accumulation of light or heavy chain ELP fusion proteins was increased compared to the corresponding chains without ELP. Transgenic tobacco lines with single chromosome insertions were crossed to obtain the complete anti-HIV antibodies. The successful assembly of both chains as fusion proteins was verified in tobacco leaves as well as in seeds. The ELP fusion does not influence the assembly of the full-length antibodies. Furthermore the recombinant proteins will be purified and the functionality of plant-derived antibodies tested. In addition they will be compared to the corresponding animal cell produced antibodies regarding their binding to the antigens and their capacity to neutralise HIV.

Topics: Biochemistry

Keywords: antibody, fusion proteins, HIV, tobacco, ELP

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Towards the Mystery of Beeing no Hydrolase of a Serine Carboxypeptidase-like Acyltransferase

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In plant phenyl propanoid metabolism acyltransferases were found which accept energy-rich hydroxycinnamoyl glucose esters (β -acetal esters) as acyl donors instead of CoA-activated thioesters. Sequence features indicate homology of these transferases with hydrolases of the serine carboxypeptidase type defining them as serine carboxypeptidase-like (SCPL) acyltransferases. Belonging to the highly diverse group of α/β hydrolase fold enzymes, SCPL acyltransferases apparently make use of a catalytic triad formed by a seryl residue as nucleophile, an aspartyl and histidyl residue.

A gene encoding the enzyme of interest, 1-*O*-sinapoylglucose:malate sinapoyltransferase (SMT, EC 2.3.1.92) from *Arabidopsis*, had been identified previously. However functional analysis of the cloned cDNA was basically confined to mutant complementation. As our work aimed at unravelling the mechanistic aspects which drive the functional shift from hydrolase to acyltransferase activity requires structure elucidation, we had to develop a robust heterologous expression system for this enzyme class. After a long period of expression optimization we are now able to produce enough protein in *S. cerevisiae* for site-directed mutagenesis studies and biochemical characterization. Hence, we established new expression constructs to raise the protein yield to a level appropriate for crystallization trials.

Nevertheless initial structural modeling studies of SMT were performed accompanying site-directed mutagenesis examinations which helped to identify sequence elements of functional importance. Additionally, enzyme kinetic and substrate specificity analyses provide a first clue of the mechanism of water exclusion.

Topics: Biochemistry

Keywords: acyltransferases, serine carboxypeptidase-like protein (SCPL), structure-function relationship, molecular evolution, tuning of heterologous expression

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Genome-wide detection of ABI3 target genes in *Arabidopsis thaliana* from ChIP/chip data using Hidden Markov Models

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Chromatin immunoprecipitation (ChIP) coupled with hybridization to promoter arrays (chip) is a powerful technique for the identification of potential transcription factor (TF) target genes. In the trilateral project ARABIDOSEED, this technology is applied to seed-specific TFs of *Arabidopsis thaliana*, such as ABI3, ET1, ET2, FUS3, LEC1, LEC2, MYB44, or MYB77 with the goal of identifying the regulatory network of these TFs controlling seed development and maturation.

The analysis of thousands of measurements of TF-DNA interactions resulting from such ChIP/chip experiments is one of the current challenges of bioinformatics. Here, we present an approach based on Hidden Markov Models (HMMs) for analyzing ChIP/chip data in the context of whole-genome sequence data and biological a-priori knowledge about ChIP/chip measurements.

In the future, this approach will be the first step of a currently developed pipeline for *de-novo* discovery of *cis*-regulatory elements in the detected target genes [1].

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[1] Michaela Mohr et al., Analysis of binding sites of seed expressed transcription factors in *Arabidopsis thaliana*, Plant Science Student Conference 2007

Topics: Bioinformatics

Keywords: ChIP/chip, seed-specific transcription factors, *Arabidopsis thaliana*, Hidden Markov Models

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Analysis of binding sites of seed expressed transcription factors in *Arabidopsis thaliana*

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In the context of the trilateral project ARABIDOSEED involving laboratories from Spain, France, and Germany, we develop bioinformatics tools for studying the transcriptional regulatory network underlying seed development in *Arabidopsis thaliana*. With the goal of identifying putative target genes of seed expressed transcription factors, we take two approaches. For a motif-to-gene approach, we developed the program CoMoFinder that allows the prediction of target genes for known composite motifs. For a gene-to-motif approach, we developed a MEME-like algorithm based on variable order Markov models and variable order Bayesian trees with the goal of improving the prediction of *de-novo cis*-regulatory elements in the studied promoters. We analyze data sets of promoters obtained from ChIP/chip analysis of the transcription factors ABI3, FUS3, LEC1, LEC2, MYB44, MYB77, ET1, and ET2, which are key regulators of seed development in *Arabidopsis thaliana*, and present first results for ABI3. The data sets of significantly enriched target promoters are compiled using an approach based on hidden Markov models and whole-genome sequence data [1].

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Topics: Bioinformatics

Keywords: seed development, *Arabidopsis thaliana*, ChIP/chip, analysis of TFBS, variable order Bayesian trees

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The role of AtMPK3 and AtMPK6 in the female gametophyte development

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Mitogen-activated Protein Kinase (MAPK) cascades are highly conserved signal transduction modules in eukaryotes, which link extracellular stimuli to a wide range of cellular responses. The MAPKs AtMPK3 and AtMPK6 are known to be involved in responses to a variety of environmental stresses (Nakagami et al., 2005). Interestingly, they also seem to play a role in developmental processes. During the attempt to produce double knock-out plants, which failed due to lethality, it was observed that homozygous MPK6 knock-out plants which are heterozygous for *MPK3* (*mpk3*-Hz/*mpk6*-KO) show a female sterile phenotype. Microscopic examination illustrates an arrest in ovule development. Furthermore, although the plants are male fertile, the pollen sacs are undersized and contain fewer pollen grains. In contrast, the opposite genotype (*mpk3*-KO/*mpk6*-Hz) does not show any developmental defects. The *mpk3* or *mpk6* single mutant plants show no developmental phenotype as well, suggesting overlapping but still distinct functions of both MAPKs. Our aim is to elucidate the molecular basis of the female sterility of *mpk6*-KO/*MPK3*-Hz plants. One strategy is the identification of target proteins of MPK3 and MPK6, respectively, which may reveal the role of these stress-activated MAPKs in female gametophyte development.

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Topics: Biology, Biochemistry

Keywords: MAPK, signal transduction, gametophyte development

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Understanding Apomixis in *Hypericum perforatum* L.

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Hypericum perforatum L. (St. John's wort) is a species that can reproduce both sexually or by apomixis (asexual reproduction through seeds). *Hypericum perforatum* L. exhibits one form of apomixis called apospory, in which a random somatic cell from the nucellus or the chalaza changes its fate to generate an unreduced embryo sac through mitosis. *Hypericum perforatum* L. is a facultative apomict species where a single individual can produce varying degrees of both sexual and apomictic seeds. Wild populations of *Hypericum perforatum* L. consist of sexual diploid and aposporous, polyploid (mostly tetraploid, $2n = 4x = 32$) plants. It is believed that apospory is not an independent or novel trait, but rather results from the deregulation and reprogramming of genes involved in sexual reproduction pathway, which may be stimulated by the asynchronous expression of duplicate genes during past hybridization and polyploidization events. We have initiated a gene expression analysis of apomixis in *Hypericum perforatum* L., in which the transcriptomes of developing ovules in the sexual and aposporous phenotypes will be compared. Ovules at different developmental stages of both aposporous and sexual individuals have been identified and isolated using microscopy and microdissection. Total mRNA has been isolated followed by cDNA amplification (normalized). High throughput 454 sequencing will be used to generate a cDNA library consisting of approximately 200 bp sequences of the whole transcriptome. This sequencing information will be used to produce microarrays that will be used to measure gene expression patterns in microdissected cells from both sexual and apomictic plants.

Topics: Biology

Keywords: apomixis, apospory, apomeiosis, parthenogenesis, 454sequencing

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Predicting the number of haplotypes by an integration of marker, passport, and phenotypic data

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Resistance to soil-borne Bymoviruses BaMMV and BaYMV in barley is controlled by an allelic series of the *eukaryotic translation initiation factor 4E* (*Hv-eIF4E*). Screening of genetic resources for new variants of the gene bears the potential of identifying new functional alleles providing alternative sources of resistance for breeding. The German federal *ex-situ* germplasm collection stores more than 10,000 different barley accessions, a resource of potentially new alleles not only for the above-mentioned trait.

Allele mining is of increasing interest to plant researchers and breeders, but little is known about the potential of genetic resources with respect to the following questions. How complex is the diversity present in an *ex-situ* collection for selected candidate genes? How many accessions need to be screened for revealing a new variant or for covering the complete genetic diversity? We use approaches derived from sequence analysis and pattern recognition for predicting the diversity of the barley *eIF4E* gene in the entire collection based on a subset of approximately 700 sequenced accessions. We study several models, including Markov models and Bayesian networks, and for each model we evaluate its prediction power by a 10,000-fold stratified holdout sampling procedure. Finally, we provide results showing to which degree different combinations of marker data, passport data, and phenotypic data improve the prediction, and which subpopulations of the germplasm collection bear the greatest potential for finding not yet sequenced haplotypes.

Topics: Bioinformatics

Keywords: allele mining, probabilistic models

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An AFLP phylogeny of the diploid New World *Hordeum* species

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The South American species of the barley genus *Hordeum* form a monophyletic group of closely related species, which evolved through a rapid radiation during the last 2 million years. To find out more about the processes involved in the evolution of the South American species, a profound knowledge of species relationships is necessary. However, phylogenetic relationships within this group could up to now not be clarified, due to small DNA sequence differences among the species or incomplete sorting of ancient alleles. To overcome these problems we analyzed 45 samples covering all 13 diploid New World species with amplified fragment length polymorphism (AFLP) analysis. Six primer combinations yielded 870 fragments, 657 of which were parsimony informative. North American *Hordeum brachyantherum* subsp. *californicum* was used as outgroup for the analysis of the South American taxa, following the results from a phylogenetic analysis on nuclear markers. AFLP was able to resolve species relationships, resulting in three major phylogenetic groups. These are a central Argentinean and northern Andean group, a mainly central to northern Argentinean group including two North American species, and a group occurring mainly in Chile or southern Patagonia. Within these groups sister species could be defined with high bootstrap support, while statistical support for the branches defining the relationships among these groups is relatively low. The AFLP results support the monophyly of the only three annual New World species. They question, however, the proposed initial colonization of South America via long-distance dispersal from California, as southward migration along the Andes instead of a Californian-Chilean dispersal event seems also possible from the resulting trees.

Topics: Biology

Keywords: *Hordeum*, AFLP, biogeography, phylogeny, South America

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The role of Hydroxycinnamate Glucosyltransferases (HCA-GTs) in *Arabidopsis thaliana*

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HCA glucose esters are important energy-rich intermediates of plant secondary metabolism, thus playing a pivotal role in biological functions. In *Brassicaceae* 1-O- β -D-Sinapoylglucose is the precursor for Sinapoylcholine in seeds and Sinapoylmalate that is localized in epidermal leaf tissues and thought to function as UV screen. Additionally, HCA conjugates are known cell wall constituents, whose UV B stress-mediated increase has been assigned to a role in shortening leaf elongation. The genome of *Arabidopsis* harbours 4 genes encoding HCA-GTs (UGT84A1-4) which share a low basic expression level. However, induced expression was detected during seed development and early seedling growth. In accordance with the proposed role of sinapate esters as UV screen, we detected an increase in transcription of all four HCA-GTs under UV-B stress conditions.

Topics: Biology

Keywords: *Brassicaceae*, UV screen

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Inferring barley genome duplications using the synteny to rice

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Previous studies based on cross-hybridization experiments suggest similar genome structures within the grass family comprising many important crop plants, such as barley, wheat, rye, or rice. So far, the complete genomic sequence is available only for rice suggesting 18 distinct pairs of duplicated segments covering almost two thirds of its genome. All but one of these duplicated segments could be dated back before the divergence of the grasses. We show on the example of barley that current genetic maps containing only a few thousand mapped ESTs are not sufficient to investigate segmental genome duplications using a Blast-versus-all approach alone. However, using the synteny to rice we find indications for at least six ancestral genome duplications still present in the two species rice and barley.

Topics: Biology, Bioinformatics

Keywords: synteny, genome duplication, barley, rice

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Specialization of biosynthetic enzymes exemplified by the evolution of substrate specificity of *O*-methyltransferases

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The benzyloquinoline alkaloids such as the analgesic morphine, the antimicrobial berberine, the vasodilator papaverine and the antitussivum codeine are the major secondary metabolites of the plant order of the *Ranunculales*, and especially of the plant family of the *Papaveraceae*. There are about 2,500 known benzyloquinoline structures, which are all derived biosynthetically from a central core structure. This structural complexity is achieved by a multitude of specific oxidations, reductions / dehydrogenations and methylations. Three cDNAs coding for *O*-methyltransferases (OMTs) have previously been isolated and characterized from *Papaver somniferum* with respect to substrate specificity. The presence of enzymes methylating benzyloquinolines as well as phenolic substances among those specific for benzyloquinolines led to the assumption that the benzyloquinoline specific OMTs are evolutionary derived from methyltransferases of phenylpropanoid metabolism which adopted their present specificity by modification of the substrate binding site. The objective of the project is the identification of critical amino acids conferring the substrate specificity of benzyloquinoline methylating OMT from *P. somniferum*. A new OMT with high homology to benzyloquinoline OMT was recently isolated based on its specific expression in *P. somniferum* compared to other *Papaver* species. A model of the tertiary structure of the unknown *P. somniferum* OMT was created followed by substrate docking experiments in order to obtain hints about its substrate specificity. Assays performed with the heterologously expressed enzyme confirmed the results of the docking studies. The project involves the extension of the modelling approach to other benzyloquinoline OMTs as a basis for site directed mutagenesis studies to evaluate the evolutionary relationship between OMTs based on substrate recognition sites.

Topics: Biochemistry

Keywords: *O*-methyltransferase, evolution, substrate specificity, benzyloquinolines, homology modelling

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Biochemical Characterization of MAM Synthases in *Arabidopsis* and Friends

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Methionine-derived glucosinolates are the predominant glucosinolate class in *Arabidopsis thaliana* and its close relatives, comprising more than 20 different structures. MethylthioAlkylMalate-Synthases (MAM) catalyze an early step in the biosynthesis of these compounds, the condensation of 2-oxo-acids with acetylCoA. Therefore, MAM enzymes are central to the diversity of aliphatic glucosinolates.

The MAM cluster comprises a small gene family in cruciferous plants, consisting of MAM2, MAM1, and MAM-L in *A. thaliana*, and of MAMa, MAMb, and MAMc in *Arabidopsis lyrata* and *Boechera divaricarpa* which shared a common ancestor with *A. thaliana* approximately 5 and 10 million years ago, respectively. Phylogenetic reconstruction showed that MAM1 and MAM2 arose by gene duplication of MAMa after *A. thaliana* diverged from its congeners. Furthermore, statistical tests from molecular evolution indicated positive selection acting on MAM1 but not MAM2 or MAMa.

To test which property of MAM1 was subject to positive selection, we expressed MAM1, MAM2, and MAMa cDNAs in *E. coli*, and examined the biochemical properties of the encoded proteins, i.e. pH-optimum, cofactor- and ATP-dependency, and acceptance of native and artificial substrates of different chain lengths.

In contrast to MAMa and MAM2 which were only capable of catalyzing the condensation reaction in the first cycle of methionine chain elongation, MAM1 was also able to catalyze the condensation reaction in the second elongation cycle, leading to the accumulation of dihomomethionine derived glucosinolates and, upon herbivore attack, to an alteration in the composition of glucosinolate hydrolysis products. Since these breakdown products govern the encounter with insect herbivores, we conclude that expanded substrate acceptance of MAM1 was the target of positive selection

Topics: Biology, Biochemistry, Bioinformatics

Keywords: *Arabidopsis thaliana*, glucosinolates, evolution, positive selection

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The Pyrimidine Salvage Pathway - a Recycling System in Plants

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Nucleotides are the allrounder among biomolecules. They are involved in structure building (glycolipids and glycoproteins), energy metabolism (ATP as "energy currency" of the cell) and as nucleotide polymers they are the basis for information storage (DNA) and realisation (RNA). This work is focused on the pyrimidine nucleotide metabolism in the model plant *Arabidopsis thaliana*. Although the biosynthesis of UMP and following phosphotranfer reactions are well investigated, the role and course of recycling of uracil and uridine to UMP in plants are unclear.

In the first year of this PhD project *Arabidopsis* candidate genes possibly involved in the pyrimidine salvage were identified by sequence analyses and appropriate T-DNA insertion lines were selected. Some candidate genes seem to be tissue specific expressed, others show striking changes in expression under nutrient treatment. Metabolite profiling of the T-DNA insertion mutants and comparison with wild-type plants shall reveal the in vivo importance of each gene. Metabolite feeding experiments will give insights into regulation and capacity of the salvage pathway in *Arabidopsis*. Therefore a quantification method which covers the range of nucleobases, nucleosides and nucleotides is needed. As experiments showed that GC-MS is unsuitable for that purpose an ion-pair reversed-phase HPLC system was set up.

Topics: Biology

Keywords: *Arabidopsis*, pyrimidine salvage, nucleotides

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Analysis of Metabolomics Data

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In biological systems there is a relationship between the transcript, the protein, and the metabolite level. If a gene is silenced or even knocked out, its transcript level and consequently its protein (enzyme) level is reduced. As this protein may be involved in metabolic pathways, metabolite levels may be affected. Two techniques, gas chromatography and liquid chromatography mass spectrometry (GC and LCMS), are used to measure the metabolite contents in biological systems e.g. in *Arabidopsis thaliana* and rapeseed.

Statistical methods are used to analyze the metabolomics data obtained with these methods. The goal is to find significantly low and high abundant metabolites and to get hints for new pathways, especially secondary pathways, from these metabolomics data. These statistical results are interpreted with the knowledge about the transcript level. The statistical methods employed are e.g. the students t-test, Pearson correlation and Bayesian networks.

Topics: Bioinformatics

Keywords: correlation, Bayesian networks, metabolomics

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Repression of ADP-glucose pyrophosphorylase in developing seeds of transgenic pea (*Pisum sativum*) changes starch and protein metabolism

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Synthesis of storage protein is regulated at different levels during seed maturation. The availability and distribution of assimilates and nitrogen compounds are mostly important. During seed growth, sugar and nitrogen compounds confer regulatory control on storage activities. Thus, seed storage production could be regulated by the supply of nutrients. We created transgenic pea lines where ADP-glucose pyrophosphorylase (AGP) has been repressed by RNAi approach in the seeds. The plastidial enzyme AGP catalyzes the reaction of glucose-1-phosphate and ATP to pyrophosphate and ADP-glucose, which is the substrate for starch synthase. We show that AGP activity and transcript levels are strongly decreased in transgenic seeds. Repression of AGP results in a wrinkled seed phenotype obviously due to transient accumulation of free sugars during maturation. Mature seeds have reduced starch content whereas the protein concentration is higher due to increased fractions of albumins and globulins. We conclude that repression of AGP interferes with starch and storage protein metabolism and alters fluxes of carbon and nitrogen during seed growth. In further analysis we test the influence of decreased AGP on altered gene expression in developing cotyledons using cDNA-microarrays. Furthermore we are comparing amino acid composition in growing seeds as well as assimilate partitioning on the whole plant level.

Topics: Biology

Keywords: ADP-glucose pyrophosphorylase, legume seed development, starch biosynthesis, storage protein synthesis, metabolic regulation

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CO(I)-ordination of Jasmonate biosynthesis and local defense responses to herbivory in *Nicotiana attenuata*

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CORONATINE INSENSITIVE 1 (COI1) is an F-box protein that mediates jasmonate (JA) responses. COI1-deficient *Arabidopsis* and tomato mutants are more susceptible to herbivores in laboratory trials but the exact mechanisms of COI1-mediated resistance are not known. We silenced COI1 by transformation with an inverted repeat construct (*ir-coi1*) in *Nicotiana attenuata*, a plant whose direct and indirect defenses against various herbivores have been well studied. *Ir-coi1* plants are male sterile and impaired in JA-elicited direct (nicotine, caffeoylputrescine, and trypsin proteinase inhibitor [TPI] activity) and indirect (*cis*- α -bergamotene emission) defense responses; responses not elicited by JA treatment (ethylene production, flower TPI activity) are unaffected. Larvae of *Manduca sexta*, a common herbivore of *N. attenuata*, gained three times more mass feeding on *ir-coi1* than on WT plants in glasshouse experiments. By regularly moving caterpillars to unattacked leaves of the same plant, we demonstrate that larvae on WT plants can grow and consume leaves as fast as those on *ir-coi1* plants, a result which underscores COI1's role in mediating locally induced resistance in attacked leaves and the importance of herbivore movement in avoiding a plant's induced defenses. When transplanted into native habitats in the Great Basin Desert, *ir-coi1* plants suffer greatly from damage by the local herbivore community, which includes herbivores not commonly found on *N. attenuata* WT plants. Phytohormone analyses revealed that JA as well as JA-Ile amounts in herbivore-induced *ir-coi1* plants differ from that of WT indicating a regulatory function of COI1 in the octadecanoid pathway. In addition, we demonstrate that COI1 regulates the expression of some JA biosynthetic genes, while others are COI-independent.

Topics: Biology

Keywords: *Nicotiana attenuata*, jasmonates, *Manduca sexta*

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Towards the Map-Based Cloning of the Scald Resistance Gene *Rrs2* in Barley (*Hordeum vulgare* L.)

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The *Rrs2* gene which confers dominant resistance to scald (*Rhynchosporium secalis*) is located at the telomeric end on the short arm of chromosome 7H. To fine map the resistance gene 9920 F2-plants of the cross Atlas (res.) x Steffi (sus.) were screened for recombination events. A total of 41 recombinants could be identified between the flanking markers AFLP14 and A-D23R, indicating a genetic distance of 0.21 cM for the interval. By utilizing the synteny between rice and barley two BAC-contigs flanking the resistance gene have been established using mainly a BAC library of the variety Morex. The proximal contig of three Morex BAC clones, was fully sequenced and assembled and has a size of 220 kb. The minimal tiling path for the distal contig consists of eight BACs, one of which originates from a BAC library of the variety Cebada Capa which was used to bridge a gap in the Morex library. Of the distal contig three BAC clones were fully sequenced and assembled yielding a sequence of 230 kb in length. The additional BACs of the distal contig are estimated to account for at least another 300 kb. Knowing the actual physical distance between markers on the two contigs the correlation between the genetic and the physical distance was analysed. 1 cM corresponds to 1,4 Mb on the distal contig and to 2,6 Mb on the proximal contig. This is in agreement with previous studies which found that 1 cM at the telomeric end of the short arm of chromosome 7H corresponds to 1-4.4 Mb (KUENZEL *et al.*, 2000). Markers at the end of both flanking contigs cosegregate with the resistance gene. Due to the lack of recombination events close to the resistance gene in the mapping population the identification of candidate genes is complicated. Therefore additional populations segregating for the resistance gene are being analysed for recombination events in the vicinity of the *Rrs2* gene. Furthermore the chromosome walking is being continued with the aim to eventually join the two flanking contigs.

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Topics: Biology

Keywords: barley, map based cloning, *Rhynchosporium secalis*, BAC library

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Phytochelatin synthase - a major enzyme in heavy metal detoxification

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“All substances are poisons; there is none which is not a poison. The right dose differentiates a poison. . .” (Paracelsus). This well known fact easily describes one of the main problems that all forms of life have to deal with. Heavy metals like Zn, Cu, Mn and Fe are key components of many enzymes involved in both respiration and photosynthesis and therefore essential for normal plant growth and development. Beside this there are also some non-essential heavy metals in the environment, e.g. Cd and As. They are already toxic in low concentrations and use the same uptake pathways as essential metals. Phytochelatins are the main mechanism to detoxify Cd and As in plants [1]. These small, thiol-rich peptides are synthesized by phytochelatin synthase (PCS) and play an important role in chelating and transporting essential heavy metals such as Zn. PCS is an important actor in metal homeostasis and heavy metal detoxification. Understanding the catalytic activity and cellular regulation of PCS will provide a deep view into metal metabolism, which includes metal chelation, distribution, storage and detoxification *in planta*.

The finding of a PCS in the animal *Caenorhabditis elegans* (CePCS) [2] and a phytochelatin synthase-like protein in the cyanobacterium *Nostoc spec.* (NsPCS) [3] offered new tools to compare the activities of phylogenetically very different PCSs and to learn more about the catalytic centre as well as the structure of this enzyme. The 3D-structure of NsPCS is known [4], but the structure of a “real” PCS is missing, to date. The use of NsPCS as a matrix seems to be a promising approach to change this fact.

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Topics: Biology

Keywords: phytochelatins, heavy metals, detoxification, metal homeostasis, cadmium

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The role of 13-lipoxygenase-derived oxylipins for pathogen defence in potato

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Oxygenated derivatives of polyunsaturated fatty acids, so called oxylipins, possess direct antimicrobial activity as well as functions in several signalling processes of plants including defence responses.

In our work we are interested in the role of the 13-lipoxygenase- (13-LOX) derived oxylipins jasmonic acid (JA) and 12-oxo phytodienoic (OPDA) acid during pathogen defence of potato. Transcripts of the 13-LOX *StLOX3* accumulate in leaves of potato challenged with the late blight pathogen *Phytophthora infestans* (Göbel et al. 2002). Also, upon infiltration of Pep13, an elicitor of *Phytophthora* species that induces defence responses, potato plants react with an increase in JA levels (Halim et al. 2004). In the non-host interaction of potato with *Pseudomonas syringae* pv. *maculicola*, local accumulation of JA and OPDA and systemic increases in OPDA levels are detectable (Landgraf et al. 2002).

In this study, an RNAi approach was used to analyse the implication of JA and OPDA for defence responses in potato. RNAi constructs against potato *OPR3*, *AOC* and *COII* transcripts were expressed in potato plants to interfere with OPDA and JA biosynthesis or signalling, respectively. The increase in *P. infestans* biomass on these plants was measured using a Realtime-PCR-based method (Eschen-Lippold et al. 2007). However, reducing the ability to accumulate OPDA and/or JA and interfering with OPDA/JA signalling does not lead to a significant change in the growth of *P. infestans* on potato leaves or tubers. On the other hand, alterations in defence responses following Pep13 infiltration can be observed in the respective RNAi potato plants.

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Topics: Biology

Keywords: oxylipins, potato, basal defence, *Phytophthora*, RNAi

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An association-genetic approach to durable powdery-mildew resistance in barley

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The obligate biotrophic fungal pathogen *Blumeria graminis f.sp. hordei* (Bgh) can cause considerable yield losses in barley cultivation. Resistance genes to Bgh can be placed into two main categories:

- 1) Natural or induced, loss-of-function alleles of the Mlo-gene that give broad-spectrum resistance and
- 2) Major resistance (R) genes that confer race-specific resistance.

Both types of R-genes are depending on an arsenal of signalling and downstream effector genes that – by themselves – may be responsible for complex patterns of quantitative resistance loci (QTLs). The aims of the project are the estimation of haplotype diversity of 60 selected candidate genes and their association with strong race-nonspecific resistance to Bgh in a set of barley (*Hordeum vulgare ssp. vulgare*) accessions from the IPK *ex-situ* collection that include exotic genotypes and landraces. We tested 112 spring barley accessions in a detached leaf assay for the verification of previously determined race-nonspecific resistance to Bgh and could confirm 36 resistant accessions. By using a transient complementation assay with a Mlo-containing BAC clone we could classify the accessions into non-complementing, partially and fully complementing groups. A final set of 63 accessions (33 resistant and 30 susceptible) have been selected for the production of single seed descent lines and for genotyping. The genetic diversity present in this customized barley core collecting was estimated using 46 microsatellite markers and revealed no indication of grouping according to the resistance phenotype.

Analysis by re-sequencing of the selected candidate genes is in progress and may reveal new Mlo haplotypes from the partially complementing genotypes as a first result with the prospect to discover new, subtle alleles of this key regulator defence that might be less associated with pleiotropic side effects.

Topics: Biology

Keywords: association study, durable powdery-mildew resistance, barley

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Post-anthesis drought tolerance assessment on bread wheat

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This research has been conducted to study tolerance against post-anthesis drought stress in wheat. It contains (1) investigations of the genetic diversity to identify tolerant accessions and (2) the mapping of quantitative trait loci (QTL) in order to locate genes of interest in the wheat genome. During the genetic diversity study, 145 Iranian wheat accessions including both spring and winter type were tested separately in field experiments. Chemical desiccation was applied to induce post-anthesis drought stress; the stress tolerance index was calculated. The number of tolerant accessions and also the degree of tolerance in spring wheat accessions were much better than in winter wheat accessions. For the performance of the molecular studies one tolerant and one sensitive accession were selected as parental lines and these accessions were crossed to generate a F₂ mapping population. Parental screening was carried out by using SSR markers. 300 polymorphic markers out of 650 Gatersleben wheat microsatellite markers were identified. The population genotyping has been performed using the best polymorphic markers on each linkage group. Data from population genotyping after testing for segregation distortion have been utilized to construct wheat linkage groups. Finally, data from population phenotyping will be used to map QTLs responsible for drought tolerance.

Topics: Biology

Keywords: bread wheat, drought stress, QTL mapping, genetic diversity

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Role of the vacuolar cation channel AtTPC1 in biotic and abiotic stress responses: calcium or potassium?

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Ca²⁺ as a ubiquitous internal second messenger plays a pivotal role in diverse signaling processes in plants. Signals are translated into spatio-temporal concentration changes, so called Ca²⁺ signatures. These arise from the combined action of Ca²⁺ fluxes into and out of the cytosol, either from the extracellular space or internal stores. Although some of the Ca²⁺ release channels involved have been characterized electrophysiologically, less is known about the encoding genes.

A new type of cation channel, two-pore channel 1 (TPC1), a putative voltage-gated Ca²⁺-permeable channel, was found in rats and subsequently cloned from different plant species. Data from tobacco and rice cell cultures suggest that TPC1 plays a role in responses to several abiotic stimuli, as well as in pathogen defense by acting as mediator of Ca²⁺ entry through the plasma membrane. In contrast, AtTPC1 was recently reported to be localized in the tonoplast and to cause slow vacuolar (SV) currents. Therefore, AtTPC1 is assumed to mediate voltage- and Ca²⁺-dependent cation release from the vacuole and participate in Ca²⁺ homeostasis.

We show here that AtTPC1 co-localizes with the vacuolar K⁺ channel, AtTPK1. A loss of TPC1 abolished SV currents and overexpression of TPC1 led to an increase compared to the wild type. Ca²⁺-responses to several biotic factors and a set of abiotic stimuli, for which the involvement of intracellular Ca²⁺ stores has been documented, were measured in aequorin-expressing wild-type, knockout, and overexpressing plants. The Ca²⁺ signatures, as well as Ca²⁺-dependent downstream gene activation were not affected by alterations in *TPC1* expression. In addition, kinetics of ABA- and CO₂-induced stomatal closure was similar in wild-type and knockout plants. In summary, we conclude that AtTPC1, under physiological conditions, functions as a vacuolar K⁺/Na⁺ channel without impact on cytosolic Ca²⁺ homeostasis.

Topics: Biology

Keywords: TPC1, calcium, aequorin, SV channel, stress responses

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Posters

P/1 – Characterisation of NIMA-like kinases of *Arabidopsis thaliana*

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NIMA (Never In Mitosis gene A) like kinases are a large family of serine/threonine kinases that plays a key role in the regulation of chromosome condensation and mitosis in different organism. NIMA was first discovered in the fungus *Aspergillus nidulans*, where it is involved in the phosphorylation of histone H3 at serine 10, while its misfunction can block the cell in G2 phase with uncondensed DNA. In mammalian organisms the closer homologs of NIMA are proteins Nek 2 and Nek 6. The *Arabidopsis thaliana* genome encodes a NIMA-like gene family with at least four members (described as *AtNIMA*).

We aim to characterize the functions of three NIMA-like kinases in *A. thaliana*. Especially, we are interested to test whether a functional link exists between the cell cycle dependent phosphorylation of histone H3 and the action of NIMA-like kinases. First we analysed the expression levels of *AtNIMA* 2, 3 and 5 genes in different parts of the plant, with a focus on active dividing tissues (young leaves and flowers), using semi-quantitative RT-PCR. To identify the function of *AtNIMAs* the analysis of *Arabidopsis* mutant plants with altered activity of *AtNIMA* genes is in progress. A homozygous line with T-DNA insertion in *AtNIMA5* resulted in complete inactivation of this gene, although no obvious difference was observed in the level of H3S10 phosphorylation. No plant containing a homozygous T-DNA insertion in *AtNIMA2* gene could be identified, suggesting a lethal phenotype of mutated *AtNIMA2*. Other experiments scheduled involve the in planta overexpression and RNAi-based inactivation of *AtNIMA* genes, the localization of *AtNIMAs* using different reporter constructs (GFP, GUS) and *AtNIMA* antibodies.

Topics: Biology

Keywords: *Arabidopsis*, NIMA kinases, histone phosphorylation, cell cycle

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P/2 – The role of invertases in phloem unloading of sucrose in *Petunia* during Adventitious Root Development

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The new generation and subsequent growth of adventitious roots in stem tissues of excised leafy cuttings is a crucial physiological process in propagation of many ornamental plant species. Despite intensive control of environmental factors in the modern propagation industry, high economic losses still occur due to insufficient rooting. It has been repeatedly shown that adventitious root formation can be improved by the control of mineral supply to the stock plants, of storage conditions and also by application of microorganism such as arbuscular mycorrhizal fungi. *Petunia* is of high economic importance in German horticulture and meanwhile a substantial proportion of this bedding and balcony plant is propagated vegetative by rooting of leafy cuttings. Since photoassimilate partitioning and allocation of assimilates between source and sink organs are important processes in plant development, a detailed analysis of reactions involved in unloading of sucrose has been started to elucidate the function of sucrose-degrading enzymes during adventitious root development in *Petunia*. Sucrose, one of the primary products of photosynthesis and the major transport carbohydrate in higher plants, is unloaded by following mechanisms, (a) along a concentration gradient, (b) unloading into the apoplast by a sucrose carrier and (c) symplastic transport. Sucrose, once imported into sink organs, may be stored as sucrose in the vacuole, rapidly metabolized to provide energy or converted into a storage polysaccharide such as starch. In order to study the role of invertases in sucrose unloading process in *Petunia* we combined molecular and biochemical investigations. In particular, different developmental stages of adventitious root formation are analysed regarding carbohydrates and metabolites involved in sucrose mobilisation and glycolysis. Results and possible explanations for the involvement of invertases in the development of adventitious roots are presented and discussed.

Topics: Biology

Keywords: invertase, *Petunia*, adventitious root development

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P/3 – Expression Profiling of Cold Responsive Genes in Barley, Wheat and Rye

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Temperature is one of the limiting factors for the geographical distribution of plants and plant growth. Especially, low temperature is the most important abiotic stress in regions with severe winters and could have serious consequences for the crop yield. In terms of the ongoing climatic change with uncertain local effects, more abiotic stress tolerant cereals are to be developed for a safe food production.

During cold acclimation, changes occur in the transcriptome, proteome and metabolome yield into improved cryoprotection and reduced freezing injury. In spite of their conserved and collinear genomes, barley, wheat and rye differ in their nature to cope with low temperatures. The trait frost tolerance is under control of a few conserved major loci *Vrn-1*, *Fr-1* and *Fr-2* in cereals. Therefore, an evolutionary overlap could be expected in the regulatory response to cold. Differences in gene expression are becoming more obvious if comparing barley, wheat and rye and different genotypes of each species. The expression of the full potential of freezing tolerance will be achieved only after a period of cold acclimation. Therefore, a set of six genotypes of each species (three freezing-tolerant and three sensitive genotypes) were selected for tissue sampling during cold acclimation under controlled phytotron-conditions. To elucidate differences in cold-responsive gene expression, a 12K-cDNA macroarray is designed from IPK barley cDNA libraries. The array comprises a comprehensive set of transcription factor and known cold-responsive structural genes. This tool provides a novel opportunity for a comprehensive survey of candidate genes across related cereal species and their regulatory networks in cold responses.

Moreover, this comparative approach could provide new insights into common and species-specific aspects of cold stress responses in cereal crops.

Topics: Biology

Keywords: low temperature, macroarray, abiotic stress, cereals, transcriptome profiling

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P/4 – Analysis of the genetic structure of two Southeast Asian tropical ant-plants *Macaranga winkleri* and *M. tanarius* (Euphorbiaceae)

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The genus *Macaranga* Thou. consists of about 300 shrub and tree species with paleotropical distribution from western Africa to some Pacific islands in the east. Within three Southeast Asian sections of the genus a wide variety of ant-plant mutualisms exist, ranging from the attraction of opportunistic ants to obligate myrmecophytism. In this project we analyze the impact of mutualism on speciation rates in *Macaranga*.

Both species under study are pioneer trees, flowering throughout the year. While the obligate myrmecophyte *M. winkleri* is occurring both in open areas in primary rainforest and disturbed sites along the edges of secondary forest, myrmecophilic *M. tanarius* is not found in primary forest. Dealing with pioneer trees we expect efficient long-distance seed and pollen dispersal in both species. However, as *M. winkleri* has always to co-disperse with their specific ants this species should be a poorer colonizer, compared to *M. tanarius*, which does not rely on specific ants to mature. Remote populations should, thus, be effectively isolated in *M. winkleri*, while gene flow might connect *M. tanarius* populations. On the other hand primary forest might be a migration barrier for *M. tanarius*, but is more permeable for *M. winkleri*. To analyze gene flow within these species we use nuclear and chloroplast microsatellites. Due to the differences in colonization ability we expect to find distinct patterns of population genetic and geographic structures, reflecting differences in colonization abilities.

Topics: Biology

Keywords: population genetics, microsatellite

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P/5 – Prosystemin in *Nicotiana attenuata*: Defense or development?

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Internal signaling in plants is essential for the regulation of growth and development, the coordination of metabolic processes, and the fine-tuning of defense responses. Systemin, which is derived from the precursor protein prosystemin, is a well studied signaling peptide in *Lycopersicon esculentum*, and has been shown to be involved in anti-herbivore defense. To determine whether prosystemin from the native tobacco *Nicotiana attenuata* mediates anti-herbivore responses, we transformed plants to either silence (IRsys) or over-express (OVsys) *N. attenuata*'s prosystemin gene, and determined defense traits in glasshouse and field experiments. We found a correlation between prosystemin and defense traits, but compared to the susceptibility of plants impaired in their jasmonic acid signaling, such as plants silenced in *NaLOX3*, the resistance of plants silenced in prosystemin was largely indistinguishable from that of wild-type plants. We propose that the small effects on herbivore resistance are indirect effects of another yet-to-be discovered function of prosystemin, rather than a direct influence of prosystemin on mediating defense. Current experiments concentrate on the appearance of changes in flower morphology in plants silenced in prosystemin. A possible role for prosystemin in flower development, and influences on plants fitness are under investigation.

Topics: Biology

Keywords: *Nicotiana attenuata*, prosystemin, defense, development

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P/6 – Optimization of assay procedures for screening of crude plant and fungal extracts to find new leads against prostatic diseases

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Prostate cancer is one of the most common type of cancer among men in western countries. Plant extracts have been used to improve the state of prostatic diseases. We screened 120 fractions of crude plant and fungal extracts to find new naturally derived leads to fight prostatic diseases.

The extraction was performed automatically using the Dionex ASE® 200 system. In a first round 7 plants and 2 fungi were extracted with tert-butylmethylether/ethanol (80%/20%) followed by methanol. The crude extracts were prefractionated by gradient extrography.

We chose the LNCaP and PC-3 cell line as prostate cancer models. The LNCaP cell line is the most widely used prostate cancer in vitro model and shows an androgen dependent growth. In comparison to the LNCaP cells, PC-3 cell growth is not influenced by androgens. To visualise any hormonal effects of the extracts or fractions tested, the assay conditions were chosen to reveal differences on the growth of both cell lines.

The investigation of cell viability is one of the major screening parameters. The cells were seeded in 96 well plates and, while adding of substances or extracts, incubated over 5 days. Finally the viability of the cells was determined using XTT detection. Positively tested fractions have been further separated by HPLC and these fractions were tested again. The isolation and structure elucidation is in progress.

Topics: Biochemistry

Keywords: prostate cancer, screening, extraction, LNCaP, PC-3

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P/7 – One-Pot Synthesis of N-substituted Diketopiperazines

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Multicomponent reactions (MCRs) are convergent reactions in which three or more starting materials react to form a product.¹

Diketopiperazines (**DKPs**) are of high importance in drug discovery,² e. g. as inhibitors of many enzymes, as bradykinin antagonists, or as opioid receptor agonists and antagonists.³

In this project we apply the UGI-4 MCR to the synthesis of a variety indole substituted 2,5 diketopiperazines, in a one pot procedure with good yields.

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Topics: Biochemistry, Chemistry

Keywords: multicomponent reactions, diketopiperazines, tryptophane, indole alkaloids

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P/8 – Metabolite profiling via Gas Chromatography / Mass Spectrometry (GC/MS)

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Metabolites can be viewed as final products of gene expression (Fiehn 2000). Metabolite profiling aims at the identification and the relative quantification of the metabolites present in an organism (Hall 2006).

Our aim is to characterize metabolite profiles of plants with genetic differences or changes in their environmental conditions (e.g. heavy metal stress or pathogen infection).

The large structural chemical diversity of metabolites requires different analytical tools. A LC/MS platform capable for profiling of most plant secondary metabolites was established in our laboratory recently (von Roepenack-Lahaye 2004). In order to enlarge the spectrum of detectable metabolites a GC/MS-based platform is established additionally.

GC/MS is able to identify and quantify many key primary metabolites. Such metabolites are either naturally volatile compounds which are thermostable (e.g. alcohols, monoterpenes and esters) or nonvolatile polar metabolites, which can be converted by chemical derivatization into volatile and thermostable compounds (e.g. amino acids, sugars and organic acids).

First steps of establishing such an analytical tool is to validate extraction procedures and derivatization conditions as well as performing technical replicates.

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Topics: Biochemistry

Keywords: GC/MS, metabolite profiling

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P/9 – Expression of influenza A (H5N1) vaccine in barley grains for oral bird immunization

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Outbreaks of highly pathogenic avian influenza A (H5N1) virus killed thousands of wild and domestic birds in Asia. Single human fatalities caused by the H5N1 strain have also been reported recently. The H5N1 strain has spread further, and animals infected by the virus, probably through contact with migratory birds, have been found in Europe. The development of a cost-effective vaccine for the immunization of both domestic and wild birds is mandatory. Furthermore, control of H5N1 through vaccination in the avian population will greatly reduce the risk of virus transfer across species. It is of great interest that a major outbreak in humans, as was observed in 1918, will be avoided. Our strategy to generate a vaccine against the H5N1 influenza A virus is based on the expression of hemagglutinin (HA), a major virus surface antigen, in plant tissue that may be used for massive oral immunization of birds. Various transient and stable plant expression systems were tested. Among those, a codon-optimized HA1 antigen driven by the seed specific gliadin promoter of wheat resulted in the highest expression. Representative molecular and biochemical analyses of transgenic barley have been performed. Western blot analysis revealed a particularly high expression of HA1 in the seeds of two out of 84 transgenic lines. Immunological evaluations of recombinant H5N1 hemagglutinin antigen are in progress.

Topics: Biology

Keywords: oral bird immunization, avian influenza A (H5N1), HA1 antigen, plant expression systems, transgenic barley

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P/10 – The Sinapine Esterase from *Brassica napus* is a Lipase-like Enzyme

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Members of the *Brassicaceae* accumulate sinapate esters with sinapoylcholine (sinapine) and sinapoylmalate as major compounds. Sinapine is a characteristic seed component found mainly in the embryo and sinapoylmalate in the cotyledons of the seedling. During early stages of seed germination sinapine is hydrolyzed to sinapate and choline by an esterase activity (SCE). The enzyme has been described biochemically¹, but the protein structure and the corresponding gene have not been characterized.

Based on enzyme purification from germinating seeds of oilseed rape (*Brassica napus*), peptide sequences of SCE were generated and used to clone a full-length cDNA. Heterologous expression of this cDNA in *Nicotiana benthamiana* conferred SCE activity to the leaf protein extract. Sequence analysis of the purified rape SCE reveals homology of the protein with a newly described group of GDSL lipases of Arabidopsis giving rise to the hypothesis that SCE has been recruited from lipolytic enzymes of primary metabolism in the course of evolution.

Further biochemical experiments indicate that the SCE has broad substrate specificity towards choline esters including phosphatidylcholine. Future work includes promoter analyses, studies on gene expression and protein localization as well as evaluation of the evolution of this lipase-like enzyme family.

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Topics: Biology, Biochemistry

Keywords: sinapate ester metabolism, sinapine esterase, *Brassicaceae*, GDSL-lipase, molecular evolution

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P/11 – Fast approximate Duplicate Detection for 2D-NMR Spectra

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2D-Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical method to elucidate the chemical structure of molecules. In contrast to 1D-NMR spectra, 2D-NMR spectra correlate the chemical shifts of ¹H and ¹³C simultaneously. To curate or merge large spectra libraries a robust (and fast) duplicate detection is needed. We propose a definition of duplicates with the desired robustness properties mandatory for 2D-NMR experiments. A major gain in runtime performance wrt. previously proposed heuristics is achieved by mapping the spectra to simple discrete objects. We propose several appropriate data transformations for this task. In order to compensate for slight variations of the mapped spectra, we use appropriate hashing functions according to the locality sensitive hashing scheme, and identify duplicates by hash collisions.

Topics: Bioinformatics

Keywords: 2D-NMR, duplicate detection, hashing

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P/12 – Investigation of the natural diversity of metal tolerance and accumulation in *Arabidopsis thaliana* ecotypes (Core Collection 24)

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Soil and water contamination pose a major environmental and human health hazard that is still waiting for an effective and affordable technological solution. An idea that has gained popularity in recent years is the use of plants to clean up contaminated sites – the so called phytoremediation. Unfortunately, very little is known about genetic and biochemical processes involved in metal uptake, transport and storage in plants, and this knowledge is essential to improve this technology (Clemens et al., 2002).

Our work is aiming at elucidating the molecular mechanisms of plant metal tolerance and homeostasis. We are studying *Arabidopsis thaliana* ecotypes collected from different sites in the northern hemisphere (Core Collection 24; Mollhann et al. 2004). The Core Collections were assembled based on sequencing a limited number of loci and attempt to maximize genetic diversity in a workable number of accessions. We used this diversity to select ecotypes that show interesting phenotypes when treated with heavy metals. Growth assays on agar plates that contained Zn, Cu and Cd allowed us to select 4 ecotypes (Bur-0, Cvi-0, St-0, Can-0) that differ significantly in metal tolerance responses. It is important now to characterise accessions at the molecular level. To localise QTL (Quantitative Trait Loci) responsible for metal tolerance and accumulation differences we use established RIL populations (recombinant inbred lines). For one ecotype so far we have been able to identify a locus for Copper tolerance.

Topics: Biochemistry

Keywords: heavy metals, RIL, core collection, *Arabidopsis thaliana*

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P/13 – Preprocessing Affymetrix microarrays

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High density oligonucleotide arrays are popular in plant biology, as they allow parallel expression measurements of thousands of genes. Each gene is represented by a set of 11 - 20 probe pairs consisting of a perfect match probe and a mismatch probe with the central base changed. Before analyzing experimental results, several preprocessing steps including background correction, normalization, and summarization need to be performed. For Affymetrix oligonucleotide arrays, there are a number of algorithms performing one or several of the preprocessing steps, such as MAS5.0 developed by Affymetrix, RMA (Robust multi-array analysis)[1], or dChip[2]. Here, we present a systematic comparison of the most popular preprocessing algorithms based on expression data of *Arabidopsis thaliana* obtained from the ATGen-Express repository.

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Topics: Bioinformatics

Keywords: Affymetrix, gene expression, preprocessing, normalization, microarrays

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P/14 – Natural product enzyme families in seeds of *Brassicaceae*

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Final reactions in plant secondary product biosynthesis are mostly performed by the enzymes of the transferase families, e.g. glucosyl-, methyl-, glutathione-S- or acyltransferases, which enable the formation of complex conjugates. *Brassica napus* seeds contain a variety of glycosylated- and/or acylated compounds, like flavonoids and the characteristic sinapate esters, e.g. sinapoylcholine (sinapine), derived from the general phenylpropanoid biosynthesis. We have initiated a combination of affinity chromatography and 2D-electrophoresis techniques together with mass spectrometry (MALDI-TOF MS, nanoLC-ESI-MS) to characterize complete subclusters of development- and tissue specific transferases in seeds.

Topics: Biochemistry

Keywords: *Brassica napus*, proteomics, affinity chromatography, mass spectrometry, 2D gel electrophoresis

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P/15 – Comparative analysis of the barley GA insensitive dwarfing locus *sdw3* to its colinear region in rice and *Brachypodium sylvaticum*

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Brachypodium species with their small genome size and close evolutionary relationship to the temperate cereals is believed to provide a suitable bridge in comparative genome analysis and gene isolation attempts in Triticeae cereal species. Here we report about a highly conserved synteny between rice and *Brachypodium sylvaticum* at the colinear region to the GA insensitive dwarfing *sdw3* locus in barley. In our study, the *sdw3* gene has been located to a 0.04 cM region on barley chromosome 2H (genetic resolution 0.02 cM). By using genome colinearity to rice chromosome 7L, we succeeded in developing 19 molecular markers to delimit the *sdw3* interval between two flanking TC149841 and TC149567 marker. Marker TC147542, encoding for a GA response GRAS protein, was found to be co-segregating with the *sdw3* gene. After exploiting all capacity of the rice orthologous genomic region, approximately 300 kb genome sequence of *Brachypodium sylvaticum* was determined in order to support for both further marker saturation and the detection of possible further *sdw3* candidate genes. Twenty four genes of 39 rice annotated genes were found colinearly orthologous with those of Brachypodium genome, excepted for a 60 kb inversion. The average inter-gene distance in the *sdw3* interest region is 6.8 kb and 5kb in Brachypodium and rice, respectively. Our result suggests that the complete colinearity of genes was found in Brachypodium as compared to the orthologous region in rice.

Topics: Biology, Biochemistry

Keywords: *Brachypodium sylvaticum*

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P/16 – Step-wise approaches to the *in silico* screening of large 3D-databases

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Based on a X-ray structure of a native Ligand in a receptor protein, we wished to identify ligands with high affinity.

For the screening of the virtual compound libraries, we used (1) a receptor-based pharmacophore model. The active site was scanned by probes like methane (hydrophobic), benzene (aromatic), ammonium (cation, H-bond donor), and methanolate (anion, H-bond acceptor) using simulated annealing molecular dynamics with flexible side chains of the proteins active site to identify preferred interaction sites with corresponding properties. The resulting low energy areas of the interactions of the probes with the proteins were translated to a pharmacophore using the corresponding tools of MOE. We used also (2) molecular docking into the receptor protein and (3) quantitative-structure-activity-relationships based on 2D molecular descriptors. The identification of potential hits suggests that this method can be a useful approach for lead finding by virtual screening.

Topics: Bioinformatics

Keywords: in silico screening, pharmacophore, docking, QSAR

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P/17 – Extended Molecular Analysis – Comparing Datasets at an Atomic Scale

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Natural products play a major role in modern drug development. Cheminformatic analysis of the frequency of selected structural elements occurring in natural products in comparison to compounds of synthetic origin should gain more insight in essential differences between both classes of compounds. For this purpose, eight datasets of various vendors, with three among them containing natural products only, were analysed to understand the diversities between compounds synthesised by nature and compounds synthesised by man at an atomic scale.

Topics: Chemoinformatics

Keywords: atom descriptors, cheminformatics, molecular dataset analysis, natural products

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P/18 – Establishment of human single-chain antibodies against the plant heterotrimeric G-protein

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G-proteins, short for guanine nucleotide binding proteins belong to the larger grouping of GTPases. "G-protein" usually refers to the membrane-associated heterotrimeric G-proteins.

The heterotrimeric G proteins are made from three subunits (α), (β) and (γ) subunits), with the G domain located on the largest one (the α unit); together with the two smaller subunits (β and γ units), they form a tightly associated protein complex. α and γ unit are associated with the membrane by lipid anchors. Heterotrimeric G proteins act as the specific reaction partners of G protein-coupled receptors. Only one $G\alpha$ gene has been found in the genomes of *Arabidopsis thaliana* (GPA1), with ~30-40% identity to non-plant $G\alpha$ subunits, as well as one $G\beta$ gene (AGB1), with ~40% sequence similarity to the mammalian $G\beta$ s. The two $G\gamma$ -subunit genes (AGG1 and AGG2) have only 12% overall identity to the human $G\gamma$ s.

G-proteins are perhaps the most important signal transducing molecules in cells. Previous works have shown G protein involvement in ion-channel regulation, control of seed germination, light responses, cell division and elongation, response to the phytohormones abscisic acid, gibberillic acid and auxin and plant interaction with symbiotic bacteria.

The aim of my work will be the purification of $G\alpha$, β and γ proteins, production of ScFv's and polyclonal antibodies against the plant G proteins, the identification of interaction partners with the help of IP, ELISA and other methods. The production of specific ScFv's against the G-protein subunits is an essential step of the work. In the phage display peptides or proteins (antibodies) are functionally presented on the surface of bacteriophages. From large recombinant phage display libraries the ideal binding partner for a specific G-protein subunit can be isolated and identified. The specific ScFv's can then be used in co-immunoprecipitation experiments and for epitope mapping purposes.

Topics: Biology, Biochemistry

Keywords: plant G-proteins

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P/19 – Population structure of *Arabidopsis thaliana*

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Recent studies provided evidence for a large-scale population structure of *Arabidopsis thaliana*. Since population structure cause artifacts in association and LD mapping, these methods need to account for population structure. Furthermore, knowledge of population structure and history is important for the identification of adaptive trait genes with a signature of positive selection.

My project is to investigate the population structure of *Arabidopsis thaliana* particularly in its glacial refugia in the Mediterranean area. We use a dataset of approximately 500 accessions which are genotyped at 115 genome-wide and unlinked SNP markers. In the context of this work several subquestions have to be answered. First the most likely number of subpopulations need to be identified. Subsequently, a Bayesian model based clustering approach implemented in the software STRUCTURE can be applied on the dataset which results in definable population clusters characterised by their specific allele frequency. Both points have to be statistically ensured. Using the degree of admixture of populations to each accession, conclusions about the origins, glacial retreats and ways of recolonisation of central Europe can be drawn. Since this approach tends only to infer the top-level population structure more fine grained analyses of subpopulations and with other methods are necessary. I will present preliminary analyses of my work.

Topics: Biology, Bioinformatics

Keywords: population structure, *Arabidopsis thaliana*, glacial refugia

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P/20 – Elimination of selectable marker genes via segregation of uncoupled T-DNAs in populations of doubled haploid barley

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Barley represents one of the economically most important and most widely distributed crops worldwide, and genetic engineering is expected to play a crucial role in its further improvement. Once transgenic plants are obtained, the selectable marker gene is not any more necessary or even unwanted.

Here we present a fast and efficient method to produce selectable marker-free, homozygous transgenic lines. Primary co-transgenic barley plants (T0), containing both the selectable marker gene (hygromycinphosphotransferase, in this case coupled with a green fluorescent protein gene) and a 'gene of interest' (β -glucuronidase, without selectable marker) were generated via *Agrobacterium*-mediated gene transfer to immature embryos using separate T-DNAs. The binary vectors were introduced into the *Agrobacterium* strains AGL1 and LBA4404. The efficiency of the method was optimised by comparing more than ten different protocols to co-transfer independent T-DNAs to immature embryos. Uncoupled T-DNAs present at hemizygous state in the primary transformants are randomly and independently distributed during male meiosis to the pollen grains. Homozygous selectable marker-free transgenic regenerants of such segregating androgenetic cultures can be efficiently produced and identified among doubled haploid individuals of the resultant T1. The method presented is not covered by complicated intellectual property rights as is the case in some alternative approaches. Moreover, only comparatively small populations are needed to find selectable marker-free, true-breeding transgenic T1-plants, and further segregation analyses are unnecessary.

Topics: Biology

Keywords: barley, *Agrobacterium*, selectable marker-free, doubled haploid

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P/21 – *Agrobacterium*-mediated transformation of maize

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Stable genetic transformation of maize is of vital importance to enable detailed functional analyses of genes. However, efficient maize transformation is still difficult to perform since only a few exotic genotypes are well amenable to this method and published protocols are only hardly reproducible in other labs. The goal of this work is to develop a reliable method of *Agrobacterium*-mediated gene transfer to highly embryogenic Hi II hybrid maize immature zygotic embryos. The induction of regenerable callus represents an essential step in the transformation process. Several media components were varied with the main focus on growth regulators. As a result, N6 basal medium supplemented with L-proline, casein hydrolysate and dicamba turned out to be most suitable for the efficient formation of somatic embryos at the callus surface. For the subsequent plant regeneration, a simple MS-based medium was utilised. Gene transfer was conducted using the *Agrobacterium tumefaciens* strain EHA105 harbouring a standard binary vector with the *bar* gene as selectable marker and the *gus*-intron reporter gene under the control of a doubled enhanced *CaMV35S* promoter. Several factors which had been presumed to influence gene transfer and survival of the target explants were examined including basal salt concentration, addition of antioxidant substances and carbohydrate source. The highest transient expression of the *gus* gene was observed using a low salt concentration along with an addition of the antioxidants L-cysteine and dithiothreitol. A similar effect was found when sucrose was substituted by maltose whereas maltose in combination with dithiothreitol did not result in an additive effect. Our first transformation experiments based on the newly developed N6-based medium revealed that stable integration of transgenes followed by formation of somatic embryos is possible. The analysis of numerous further factors is in the experimental pipeline.

Topics: Biology

Keywords: maize, *Agrobacterium*, transformation, regeneration

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P/22 – Novel transcription factors involved in the gametophyte-sporophyte transition

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Genetic analyses of the mode of reproduction in *Poa pratensis* identified five loci with differences in gene expressivity and penetrance to be required for the control of apomixis, including a Parthenogenesis initiator (Pit) and a Parthenogenesis preventer (Ppv) (Matzk et al., 2005). These findings challenge efforts to define these genetic loci in molecular terms. The wheat ‘Salmon’ system (Matzk and al., 1995) provides an excellent experimental model for molecular studies on genes which are involved in the control of the parthenogenetic initiation of embryogenesis as a key element of apomixis. cDNA-libraries from sexual and parthenogenetic egg cells have been generated (Kumlehn and al., 2001). Various subtractive approaches have been used to enrich for egg cell specific genes. One of them defines a member of a novel, mainly plant specific class of transcription factors. The wheat egg cell-derived clone shares sequence similarity to *Chlamydomonas* genes, known to be required for gametogenesis. RT-PCR experiments, in situ hybridisation and the use of reporter genes confirm the egg apparatus specific localisation of these nuclear factors both in wheat and *Arabidopsis*. We will present results from loss-of-function and gain-of-function analyses of these genes which suggest that they play a role in the gametophyte-embryo transition.

References:

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Topics: Biology

Keywords: apomixis, egg cell, wheat, *Arabidopsis*

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P/23 – The sinapoylglucose:choline sinapoyltransferase (SCT) from *Brassica napus*

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In species of the *Brassicaceae* family, the serine carboxypeptidase-like (SCPL) enzyme sinapoylglucose:choline sinapoyltransferase (SCT; EC 2.3.1.91) catalyzes the transacylation step from sinapoylglucose to sinapoylcholine (sinapine). As the related enzyme sinapoylglucose:malate sinapoyltransferase (SMT; EC 2.3.1.92), the SCT has been recruited from hydrolases during evolution to take over a glucose-ester dependent acyltransfer in plant secondary metabolism. This raises the questions for molecular mechanisms favouring acyltransfer over hydrolysis and substrate recognition of the positively charged acyl acceptor choline. To solve this questions, a comprehensive expression system is applied that allows systematic site-directed mutagenesis. In *B. napus*, SCT is controlled by a seed specific promoter directing SCT expression to the embryo and the aleuron layer. To unravel the molecular mechanism for seed specificity, we perform promoter deletion analysis to detect cis-acting elements and study the interaction with seed specific transcription factors.

Topics: Biology

Keywords: *Brassica napus*, SCT, secondary metabolism

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P/24 – Identification and Characterization of Transcription Factors involved in Salt and Osmotic Stress Response

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Transcription factors (TFs) are master-control proteins in all living cells. They often exhibit sequence-specific DNA binding and are capable of activating or repressing transcription of multiple target genes. In this way, they control or influence many biological processes, including cell cycle progression, metabolism, growth and development, and responses to the environment.

To identify TFs that have an essential regulatory impact in response to salt stress, we used a qRT-PCR based platform to screen almost all *Arabidopsis thaliana* TFs (Czechowski et al., 2004). *Arabidopsis thaliana* seedlings have been grown for nine days in a liquid culture system and subsequently stressed with 100 mM NaCl respectively 200mM Mannitol for three hours. Material from three biological replicates has been used to screen approximately 2000 TFs. Among them, twenty candidate genes displaying an altered gene expression with respect to salt stress or osmotic treatment were selected. To confirm the results additional experiments have been carried out including a hydroponic growth system and time course experiments. Six out of this 20 TFs showed a significant expression change combined with an interesting expression pattern. They have been selected for further analysis using a reverse genetic approach. Overexpressors were analyzed on a physiological and molecular level. The results from these experiments revealed potential regulators of early salt stress response whose impact on gene expression is now examined on a genome wide level.

References:

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Topics: Biology

Keywords: *Arabidopsis*, transcription factors, salt stress

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P/25 – Jasmonates in cut side shoots from *Petunia hybrida*

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The vegetative propagation of ornamental plants is economically important. A great part of such plants are produced vegetatively via so-called cuttings produced by cutting lateral side shoots from stock plants. By this procedure the plant is wounded and a wound response is caused in the harvested side shoots. Due to the fact that jasmonates are known as central mediator of the plant response against wounding, we analysed the jasmonate biosynthesis in cuttings of *P. hybrida*.

Jasmonates are products of the octadecanoid pathway. A key enzyme of this pathway is the allene oxide cyclase. A cDNA coding for AOC of *P. hybrida* was isolated and characterized. The cDNA consists of 946bp and codes for a protein of about 26 kDa, which contains a putative signal peptide cleavage site. Because of its high similarity to tomato AOC (LeAOC), we tested an antibody raised against LeAOC via immuno blot showing that the LeAOC antibody is able to recognize the AOC in *P. hybrida*. By the use of an immuno cytological approach it has been shown that the PhAOC is localized in plastids of vascular tissue. Immuno blot analyses demonstrated that the PhAOC levels are increased transiently in cuttings after harvesting. This increase was accompanied by increased transcript accumulation of the PhAOC as revealed by real-time RT-PCR. Moreover, the accumulation of jasmonic acid (JA) and the main intermediate of the JA biosynthesis, oxo-phytodienoic acid (OPDA), were monitored in different parts of cuttings up to 12 h after harvest. JA increased transiently in leaves and stems of cuttings, whereas OPDA increased transiently in stems of cuttings only, both with a maximum at 0.5 h after harvest. Furthermore, the basis of cuttings contained the highest levels of JA and OPDA. The putative function of jasmonates in the formation of adventitious roots as a prerequisite for vegetative production of plants will be discussed.

Topics: Biology, Biochemistry

Keywords: jasmonates, wounding, *Petunia hybrida*

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P/27 – The world of *Rhynchosporium secalis* looks rhomboid

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Rhynchosporium secalis causes the leaf scald disease of barley and rye. Restriction-mediated insertion mutagenesis resulted in the identification of several non-pathogenic fungal mutants, in which pathogenicity genes appeared to be inactivated. In one of these mutants the insertion had occurred in the promoter region (90 bp upstream of the translation start site) of a gene, *RsRho1*, whose product shows high homology to a family of integral membrane proteins, the rhomboid proteases. These enzymes are conserved in species ranging from bacteria to humans and regulate diverse biological processes by catalyzing the proteolysis of transmembrane substrate proteins. Recently, the crystal structure of the transmembrane core domain of GlpG, a rhomboid-like protease from *E. coli*, was solved. GlpG-based modeling of the *RsRho1* protein revealed high structural similarity to the bacterial enzyme in the six transmembrane helices, whereas the interhelical loop regions are more divergent.

In addition to the rhomboid proteases of *Drosophila melanogaster*, which are involved in regulating epidermal growth factor receptor signaling during fly development, only few rhomboid homologues have been functionally characterized. To study the function of the rhomboid-like protein of *R. secalis* a $\Delta RsRho1$ mutant was generated by targeted gene disruption. Comparative analysis of wild type and mutant phenotype on host plants along with gene expression and protein localization studies are carried out to answer the question as to which role the *RsRho1* gene may play in fungal development or pathogenicity.

Topics: Biology, Biochemistry

Keywords: *Rhynchosporium secalis*, *RsRho1*

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P/28 – The role of Jasmonates in metabolite accumulation of *Medicago truncatula* roots

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The colonization of plant roots by an arbuscular mycorrhizal fungus leads to elevated levels of the lipid-derived signaling compound jasmonic acid (JA), which is accompanied by the cell-specific expression of genes coding for enzymes of JA biosynthesis and of JA-induced proteins. To get insights into possible mechanisms of JA action during mycorrhization, roots of the model legume *Medicago truncatula* were transformed using *Agrobacterium rhizogenes* harboring the *35S::MtAOC1-RNAi* construct, the *35S::MtAOC1sense* construct or the empty DsRed vector leading to chimeric plants with wild-type shoots and transformed roots. Suppression of *MtAOC1* resulted in decreased JA-levels leading to a delayed mycorrhization as visualized by the amount of fungal rRNA and transcripts of the mycorrhizal-specific plant gene *MtPT4*. Analyses of metabolite patterns in transgenic roots can help to identify the processes that are mediated by jasmonates during mycorrhization. Therefore, methanol extracts of roots with enhanced (*AOCsense*), reduced (*AOC-RNAi*) and unaltered (empty DsRed vector) *AOC* expression, both non-mycorrhizal and mycorrhizal, were investigated. Strong changes in the pattern of secondary metabolites were detected in mycorrhizal *AOCsense*-roots and in non-mycorrhizal *AOC-RNAi*-roots in comparison to the empty vector control. Mycorrhizal *AOCsense*-roots revealed among others a significant decrease in the level of isoflavonoids while non-mycorrhizal *AOC-RNAi*-roots exhibited increased isoflavonoid levels. The isoflavonoid pattern of non-mycorrhizal *AOCsense*-roots remained unchanged. These data indicate that jasmonates can affect the isoflavonoid content of roots.

Topics: Biology

Keywords: *Medicago*, mycorrhiza, jasmonates, root transformation, flavonoids

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P/29 – Isoprenoid biosynthesis in tomato: About isogenes of 1-Deoxy-D-xylulose 5-phosphate synthase

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Isoprenoids are essential for plant life with diverse roles. Carotenoids as prominent examples help plants to protect against radicals and give colour to flowers and fruits. Another important role is attributed to volatiles (mainly mono- and sesquiterpenes) which are responsible for attracting insects. All isoprenoids derive from the common precursor isopentenyl diphosphate and its isomer dimethylallyl diphosphate, synthesized either via the cytosolic mevalonate or via the plastidial methylerythritol (MEP)-pathway. The first step of the MEP-pathway is catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (DXS) generating 1-deoxy-D-xylulose 5-phosphate from glyceraldehydes 3-phosphate and pyruvate. At least two isoenzymes of DXS were found in plants (DXS1 and DXS2; Walter et al., 2002). Both proteins share a sequence identity of 70 percent. In this work we focused on DXS1 and DXS2 from tomato. Only transcripts of DXS1 are found in ripening fruits. However, transcripts of both genes could be detected in leaves which might be due to the presence of trichomes, producing volatiles (mono- and sesquiterpenes). Trichome isolation experiments demonstrated that DXS2 transcripts accumulate to higher levels in these organs as compared to DXS1. To get more insights into differential occurrence of both isoenzymes, their localization is being investigated with specific antibodies. Antibodies against peptides from DXS1 and DXS2, raised in rat and rabbit, were tested against total protein extracts from different organs of tomato. Currently functional analysis and transgenic approaches to suppress DXS2 expression are in progress. In order to investigate the regulation of both genes promoter analyses will be performed.

References:

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Topics: Biology, Biochemistry

Keywords: tomato, terpenes, DXS

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P/30 – Pollen Embryogenesis in *Arabidopsis thaliana*

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Pollen embryogenesis has been successfully employed in many plant species to generate doubled haploid lines (DHLs) that are entirely homozygous. The wide application of this process in biotechnology and practical plant breeding is in strong contrast to the poor understanding of the underlying biological mechanisms. To gain more detailed information, we aim to establish a pollen embryogenesis system in the major experimental model plant *Arabidopsis thaliana*. To this end, immature pollen of different developmental stages will be isolated, exposed to diverse conditions that should disturb further gametophytic development, and cultivated under conditions supporting embryonic development. This will not only be conducted with a variety of *Arabidopsis* accessions, but also with transgenic lines that over-express regulators of early embryogenesis or stem cell homeostasis, or lines in which genes involved in pollen differentiation are down-regulated or knocked out. A method of embryo formation from *Arabidopsis* pollen will greatly facilitate a process-oriented in-depth transcriptome analysis along with a comprehensive, integrated approach to study the cellular mechanisms that govern the initiation of pollen embryogenesis. Furthermore, an extremely valuable tool to efficiently generate homozygous lines, recombinant inbred lines or near-isogenic lines will be available for the *Arabidopsis* research community. Eventually, the knowledge gained through these investigations may provide a valuable basis for application-oriented research on pollen embryogenesis in crop species.

Topics: Biology

Keywords: *Arabidopsis thaliana*, pollen embryogenesis, isolated microspore culture, doubled haploid

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P/31 – Identification of novel auxin response mutants in *Arabidopsis thaliana*

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The plant hormone auxin regulates numerous aspects of plant development including embryogenesis, vascular differentiation, organogenesis, tropic growth, and root and shoot architecture. The endogenous auxin indole-3-acetic acid (IAA) regulates transcription by promoting the proteolysis of a family of transcriptional repressors called Aux/IAA proteins. Genetic and biochemical studies have shown that this degradation is dependent on the ubiquitin proteasome system, which is a key regulator of many biological processes in eukaryotes. This mechanism employs several types of enzymes, with the ubiquitin E3 ligases catalysing the attachment of polyubiquitin chains to target proteins for their subsequent degradation by the 26S proteasome. The SCF-type E3 ubiquitin ligases, consist of a multi-subunit protein complex in which an F-box protein is conferring specificity by recruiting the target substrate. Strikingly, appr. 700 F-box proteins have been predicted in *Arabidopsis thaliana*, suggesting that plants have the capacity to assemble a multitude of SCF complexes, possibly controlling the stability of hundreds of substrates involved in a plethora of biological processes. IAA binds to a small family of F-box proteins (TIR1 + AFB1-3) and thereby facilitates the recruitment of Aux/IAA proteins, resulting in their ubiquitination and degradation which enables IAA-induced gene expression followed by the plant's response to the auxin stimulus. To identify novel components of auxin signalling, we conducted a second site mutant screen for suppressors of the auxin response defect in the *tir1-1* auxin receptor mutant. We have so far identified 10+ independent *tir1-1* suppressors (tis) that exhibit wild-type like responses to IAA and/or the synthetic auxin 2,4-D. Furthermore, several of them reconstitute auxin-induced expression of the BA3:GUS reporter construct, which is impaired in the original *tir1-1* mutant. Map-based cloning of the respective tis mutations should provide new insights into auxin signalling in *A. thaliana* and possibly SCF complex regulation in eukaryotes.

Topics: Biology

Keywords: auxin, SCF^{TIR1}, signal transduction, 2,4-D, *Arabidopsis*

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P/32 – Antisense-Inhibition of the Oxoglutarate/malate translocator in seeds of *Pisum sativum*

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Ammonia assimilation resulting from nitrate reduction and from photorespiration by the plastidic glutamine synthetase/glutamate synthase system requires 2-oxoglutarate as a carbon precursor. The precursor molecule is synthesized in the mitochondria by the citric acid cycle and the cytosol by cytosolic ICDH. It is imported into plastids by the 2-oxoglutarate/malate translocator (OMT). This transporter together with the glutamate/malate translocator are necessary components for photorespiratory nitrogen recycling and ammonia assimilation in plastids in general.

The role of OMT in developing seeds of *Pisum sativum* and its significance for ammonia assimilation is uncertain. Here, we study the importance of this translocator. Its gene expression was antisense-repressed in transgenic pea plants. Currently 5 independent homozygotic lines are available. The seeds show differences in albumine and globuline content as well as C/N-relation in comparison to the wildtype. In developing pea seeds the composition of amino acids was measured.

Topics: Biology, Biochemistry

Keywords: plastids, dicarboxylate transport, C/N metabolism, ammonia assimilation, amino acids

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P/33 – Establishment of broad-spectrum resistance against powdery mildew

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The recessive *mlo* genotype of barley (*Hordeum vulgare* L.) has been commonly applied to provide broad-spectrum resistance against the obligate biotrophic fungal pathogen barley powdery mildew (*Blumeria graminis* f.sp. *horde*) under field conditions. Respective resistance mechanisms were also found by transient MLO *knock-down* experiments in *Arabidopsis* and tomato. A stable recessive *mlo* genotype in the agro-economically important hexaploid wheat, which is thought to carry three independent *Mlo* homologues, is extremely unlikely to obtain, neither naturally nor by artificial breeding. Natural MLO-defective barley lines show premature leaf senescence and are slightly more sensitive to biotic and abiotic stresses. This represents a negative pleiotropic effect of MLO-dysfunction and renders such lines not fully competitive with wild type plants. Since the interaction of *B. graminis* with its host is confined to the epidermal cell layer, the pleiotropic effects of MLO-dysfunction may be circumvented by epidermis-specific expression of *Mlo*-RNAi constructs in *T. aestivum*. To provide a high quality of the transgenic plants generated, gene transfer will be mediated by *Agrobacterium tumefaciens*. Binary vectors carrying an *Mlo*-RNAi cassette under control of either the epidermis-specific *HvGSTA1*-promoter or the constitutive *ZmUbi1*-promoter have been created. Functional analysis of the vectors were performed by transient induced gene silencing (TIGS) as well as stable transformation of barley cv. 'Golden Promise'. Characterisation of the plants will include analyses of the transgene copy numbers, the spatial and temporal expression of the RNAi constructs and an assessment of the general resistance against *B. graminis* upon artificial infection. Establishment of wheat lines resistant against *B. graminis* through epidermis-specific expression of an appropriate *Mlo*-RNAi cassette would by itself constitute a substantial advance in plant breeding.

Topics: Biology

Keywords: wheat, *mlo*-resistance, powdery mildew

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P/34 – A putative apospory marker in *Hypericum perforatum*

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Some higher plants reproduce asexually by apomixis, a natural way of cloning through seeds. Apomictic plants produce progeny that is an exact genetic replica of the mother plant. Harnessing apomixes to stabilise hybrid effects is considered to be a major goal of plant reproduction research.

St. John's wort (*Hypericum perforatum*) contains compounds with antiviral and anticancer activity and has become an important medicinal crop plant. Breeding programs have been initiated to further improve these quality traits, however, the usual breeding schemes are not easily applicable because *Hypericum* is a facultative pseudogamous apomict (Matzk and al., 2001; 2003). Application of the flow cytometric seed screen has revealed a highly complex mode of reproduction and has identified eleven different mechanisms of seed formation, including obligate and facultative sexuals and apomicts, unreduced double fertilisation and reduced parthenogenesis, as well as combinations of these pathways. Apospory and parthenogenesis have been calculated to be unlinked (Matzk and al. 2001).

A DNA-AFLP approach has been used to isolate a marker fragment present in 10 apomictic accessions and 6 sexual accessions which, after sequencing, has been converted into a CAPS marker which co-segregates with apospory but not with parthenogenesis. The correlation between marker and apospory has been demonstrated for more than hundred plants from geographically distant populations, and for offspring of various directed crosses. A *H. perforatum* BAC library has been screened with the probe containing the marker, and positive clones have been sub-cloned and sequenced. We will report the characterisation of the cloned genomic region.

Topics: Biology

Keywords: apomixis, apospory, *Hypericum perforatum*, BAC library

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P/35 – Reverse engineering approach for investigation of urea cycle action in *Arabidopsis thaliana*.

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Modern technology is capable of providing a huge amount of information concerning nearly all levels of living system, including transcripts, metabolites, peptides and enzyme activities. However an important question is how we can use these data to reconstruct the underlying cellular system. We propose an experiment design suitable for such purpose and MCA[Metabolic Control Analysis]-based algorithm. We make use of possibility to introduce perturbations in gene expression by inducible gene silencing to infer the overall system properties from multiple perturbation states. We try to answer the question if transcripts, proteins, enzymatic activities and metabolic fluctuations, belonging to just one metabolic pathway (which can be regarded as a conventionally closed subsystem, or module), carry a full information about its actual structure. For this purpose we selected system with known stoichiometry and easily achievable perturbation states - in this case urea cycle with initial reactions leading to polyamines synthesis.

Topics: Biochemistry, Bioinformatics

Keywords: metabolic control analysis, urea cycle, reverse engineering

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P/36 – Analysis of Diphosphate Binding Sites in Proteins

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Isoprenoids form a very large and diverse group of natural products and are especially widespread in plants. The diversity of the enzymes involved in the biosynthesis of these compounds is obviously based on their structural variety, but also on the basis of their function. Up to now the evolutionary origin of prenyl enzymes is not well studied although almost all of them use substrates with a common activating group: diphosphate (pyrophosphate).

As a starting point of a more detailed analysis of the evolution of prenyl enzymes, diphosphate binding sites are analysed. For that purpose diphosphate binding proteins were isolated from the RCSB Protein Data Bank. Taking into account metal ions and water molecules, amino acids in proximity to diphosphate residues were identified by using the SYBYL programming language SPL. A classification of the binding sites based on these results should uncover typical binding modes for pyrophosphates. Unfortunately, up to now no satisfying classification could be obtained, although several approaches were used. At this point of research, an improvement of the recognition of neighbouring amino acids is implemented, that enhances the basis for classification and therefore should yield to the desired result.

Topics: Biochemistry, Bioinformatics

Keywords: diphosphate, prenyl enzymes, proteins, classification

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P/37 – Functional analysis of MAP kinase cascades in defence signalling in *Arabidopsis thaliana*

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Plants are able to dynamically respond to a variety of environmental influences such as pathogens, cold, drought and salt stress via rapid signal transduction networks. Mitogen-activated protein (MAP) kinase cascades are involved in the intracellular signal transduction events linking recognition and response to environmental impacts. With a set of 20 MAP kinases [1] the plant is able to respond specifically to either biotic or abiotic stresses. In mammalia and yeast, MAP kinases are part of signalling complexes which are structured by scaffolding or adaptor proteins [2,3]. To analyse protein interactions in plants the tandem affinity purification (TAP) method has already been established [4,5]. By two-step affinity chromatography, tagged kinase complexes can natively be isolated out of plant extracts. We generated transgenic plants for MAP kinases involved in the biotic stress response, namely MPK3, MPK4 and MPK6 [6,7]. We could show that the TAP tag does not influence MAP kinase activity since the protein MPK4-TAP could complement the *mpk4* mutant dwarf phenotype [7] and the activated proteins MPK3-TAP and MPK6-TAP could phosphorylate an artificial substrate *in vitro*. The purified complexes are then either separated on 1D PAGE followed by LC-MS or directly analysed with 1D-nanoLC-MS/MS. Thus identifying MAP kinase interacting proteins could reveal mechanisms of how signalling cascades specifically regulate stress responses.

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Topics: Biochemistry

Keywords: MAP kinase signalling, tandem affinity purification

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P/38 – Production of spider silk fusion proteins in tobacco seeds

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Spider silk proteins are interesting raw materials for different industrial applications. Elasticity and tensile strength are the obvious advantages of these versatile biomaterials, accompanied by high temperature tolerance and a very low immunogenicity, which could be interesting for medical applications (coating for implants). Unfortunately the direct production is not possible due to the strong territorial behaviour of spiders.

Therefore, the production *in planta* is a promising alternative. Various constructs are already expressed in peas, potatoes and tobacco leaves. But these systems suffer from storage instabilities. To circumvent this significant problem, the expression of spider silk fusion proteins in tobacco seeds became increasingly important. Also as a model system for the production in cereals the seed specific expression is of great use.

So the long-term stability and the optimised protein purification from tobacco seeds are crucial for the further usability of this production platform.

Topics: Biology

Keywords: spidroins, protein expression, purification

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P/39 – Wavelet based peak picking of LC/MS data

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Accurate peak picking of LC/MS data is still a major challenge. A two-phased approach based on centroid density clustering and wavelet analysis of extracted ion chromatograms is presented.

Topics: Chemoinformatics, Bioinformatics

Keywords: LC/MS, peak picking

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P/40 – Cloning of Chlorogenic Acid:Glucaric Acid Caffeoyltransferase from Tomato (*Lycopersicon esculentum*)

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Hydroxycinnamic acid conjugates occur in a vast array of primary and secondary compounds, but only a few of them have been investigated with regard to their enzymatic synthesis. HCA esters are either built up via transesterification with CoA thioesters or 1-*O*-acyl glucosides as acyl donor. In cotyledons of tomato a new acyltransferase, chlorogenic acid:glucaric acid caffeoyltransferase (LeCGT) was found, which accepts chlorogenic acid as acyl donor.¹ Although this enzyme has been described biochemically, its molecular characterization remains elusive.

Based on enzyme purification from cotyledons of tomato (*Lycopersicon esculentum*) peptide sequences of LeCGT were generated and used for full length cDNA cloning. Sequence analysis of the cloned cDNA reveals homology of the LeCGT to a lipase-like protein that belongs to the group of GDSL enzymes. This reveals evolutionary recruitment of this enzyme from lipases of the primary metabolism.

Current work deals with the heterologous expression of the LeCGT cDNA in *Nicotiana benthamiana* to prove by activity assay that the right gene was cloned. Future work will focus on kinetic analysis, localisation, overexpression and knock down of the LeCGT in tomato to study its biological role.

References:

¹ Strack, D. and Gross, W. (1989) Properties and Activity Changes of Chlorogenic Acid:Glucaric Acid Caffeoyltransferase From Tomato (*Lycopersicon esculentum*) Plant Physiol. 92, 41-47

Topics: Biology, Biochemistry

Keywords: CGT, HCA esters

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P/41 – Jasmonate signalling in *Solanum nigrum*: Fundament of a death trap?

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The hexaploid *Solanum nigrum* (*S. nigrum*) is a weedy species that is native to Europe, but occurs nearly all over the world. We are interested in induced responses of *S. nigrum* to herbivory. Microarray analysis of herbivore-infested plants revealed candidate genes which were selected to generate lines with decreased expression of these genes. Two of these genes are *LOX3* and *HPL*, genes that are part of oxylipin and C6 volatile biosynthesis respectively. Transgenic lines, silenced for *LOX3* and *HPL* were created, and with these lines the interaction of *S. nigrum* with its insect community will be studied. There is an interesting interaction between the Colorado Potato Beetle (CPB) and *S. nigrum*, as this plant seems to attract the female beetles for oviposition, but the newly hatched larvae are unable to use it as a host plant. The CPB's lethal love will be dissected using *lox* and *hpl* lines, as these are likely to have impaired insect resistance and altered volatile levels.

Topics: Biology

Keywords: jasmonates, herbivory, colorado potato beetle, volatiles, *Solanum nigrum*

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P/42 – Identification of plant Aurora regulators and substrates

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Aurora-like kinases are multifunctional highly conserved regulators of mitosis. *Arabidopsis thaliana* encodes three different types of Aurora-like kinases (AtAurora 1, 2 and 3). The challenge ahead is to define the precise function, physiological regulation and signaling networks of the plant-specific Aurora kinases.

The aim of presented investigation is to identify substrates and interacting partners of AtAuroras. Recently we isolated genes of AtAuroras 1, 2 and 3 from wild type *Arabidopsis* ecotype Columbia. Vectors, carrying the genes, fused with HASTrep-tag, were constructed for affinity purification of protein complexes, associated with AtAuroras, by insertion of AtAurora genes into pXCS-HASTrep vector. To increase stability of Strep-tagged AtAurora 1 in plants, destruction box was deleted from the gene.

N. benthamiana transient transformation with *A. tumefaciens*, carrying AtAuroras 1 gene, was repeatedly performed thrice. SDS-PAGE, followed by Western blotting and hybridizing with StrepTactin-HRP did not reveal tagged AtAuroras in transformed plants, whereas RT-PCR demonstrated presence of relevant mRNA.

Stable transformation of *A. thaliana* was performed with the HASTrep-tagged AtAurora 1, AtAurora 2, AtAurora 3 and AtAurora 1 with deletion of a part of D-box. Currently, we are selecting transformants.

Stable transformation of *A. thaliana* C-24 cell suspension culture will be performed to obtain cell material with a high number of dividing cells, which would provide us with higher level of proteins of interest.

Investigation of recombinant AtAurora1 kinase activity toward recombinant CENP-A is currently in a progress.

Topics: Biochemistry

Keywords: Aurora, affinity purification, destruction box, CENP-A, transformation

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P/43 – Differential expression of *Glomus intraradices* genes during the asymbiotic and symbiotic phase of the fungus

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Mycorrhizas are the most wide-spread symbioses on earth. Two major types of mycorrhizal symbioses can be differentiated: ectomycorrhizas and arbuscular mycorrhizas. *Glomus intraradices* belongs to the group of arbuscular mycorrhizal fungi (AM fungi), developing a symbiotic association with plant roots. These fungi are more widespread than ectomycorrhiza and under different conditions such as nutrient limitation or salt stress they form a symbiotic interaction with more than 80% of all higher plants.

Their history dates back to the Ordovician era. At least 450 Myr ago there was a co evolution of land plants and AM fungi. Fossil record indicates that the structure of the mycorrhiza has not changed over the course of time.

The aim of this work is to find genes expressed in different developmental stages of the fungus. RNA and DNA were extracted from germinated spores and extraradical hyphae out of an *in vitro* culture. *In vitro* culturing of AM fungi is difficult due to their obligate biotrophic nature, so for a monoxenic cultivation of AM fungi a root culture is essential. Here we used a Ri-T-DNA transformed carrot root. We established an *in vitro* culture with a commercial used *Glomus intraradices* strain. Two cDNA libraries of this *Glomus intraradices* strain were constructed, on the one hand from the extraradical hyphae in a symbiotic stage and on the other hand from germinated spores in an asymbiotic stage. Two-thirds of the ESTs indicated no similarities to known sequences from other organisms. Now we try to use a suppressive subtractive hybridization to find genes which are specific for one of the developmental stages.

Topics: Biology

Keywords: arbuscular mycorrhizal fungi, *Glomus intrardices*, Ri-T-DNA transformed carrot roots, EST, suppressive subtractive hybridization

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P/44 – YFP-CenH3/H2B-DsRed double transformants do not stably express both constructs simultaneously in *Arabidopsis* interphase nuclei

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Sister chromatid alignment mediated by cohesin complexes is a basic biological process important for nuclear division, gene expression, post-replicative recombination repair and meiotic recombination. A ring-shaped complex of cohesin proteins apparently mediates cohesion of newly replicated sister chromatids until complete bi-polar orientation is achieved during metaphase.

As a prerequisite to analyse the impact of cohesins on the chromosome organization in dividing and differentiated plant cells, T-DNA insertion mutants (for cohesin genes) and wildtype plants of *Arabidopsis thaliana* were transformed simultaneously with H2B-DsRed and EYFP-CenH3 to label in vivo nuclei/chromosomes and centromeres, respectively. 80 T1 double transformants were obtained. Only 14 out of these showed expression of both DsRed and EYFP in some nuclei of root and leaf tissue. Simultaneous expression of both constructs in one and the same nucleus occurred very seldom. Also the segregating T2 progeny originating from the best expressing T1 plants did not allow to select stably co-expressing transformants. In contrast, ~90% of the progeny originating from homozygous single H2B-EYFP transformants showed a stable and strong expression in nearly all root and leaf nuclei. The results suggest that the H2B-DsRed and EYFP-CenH3 constructs influence each other in a way that double transformants show the suppression of fluorescence expression in most nuclei and tissues. To trace nuclear divisions, only single transformations with fluorescence markers are useful.

Topics: Biology

Keywords: cohesion, nuclear division, transformation

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P/45 – Post translational modification of the lipid raft protein, Remorin during gene for gene interaction; a proteomic approach

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Plant defense mechanism plays an important role for its survival against pathogens. It involves a complex signal network which requires precise spatial and temporal regulation of signaling components. So far, transcriptomics has been the main "omic" platform for such studies. Proteomics, a systematic analysis of protein expression, may serve as an additional tool to investigate the regulation of proteins involved in the defense mechanism. Transgenic Arabidopsis harboring an avirulence bacterial gene (avrRpm1) under the control of a dexamethasone-inducible promoter was used as a model system in proteomics study. Genetic interaction between the avirulence gene and the corresponding resistance gene will activate defense responses, including a hypersensitive response, that eventually limit pathogen invasion[1,2]. Using 2D-PAGE and Mass Spectrometry Analysis, differential protein expression during gene-for-gene defense response was studied. To enhance the detection of low abundant proteins, different prefractionation approaches were applied. The use of Rubisco-depleted total and microsomal fractions enabled us to detect a membrane-associated protein which is post translationally modified during the defense response[3]. Remorin, a plant specific protein with unknown function, was phosphorylated during avrRpm1-Rpm1 interaction. Its presence in lipid raft suggests a possible role in signal transduction at the plasma membrane for avrRpm1-Rpm1 defense signaling. Using "over-expression" and "knock-down/out" plants, we try to elucidate the role of Remorin during defense activation. In summary, our proteomics analysis enabled us to identify a low-abundant candidate protein for "gene-for-gene" defense regulation which would, otherwise, elude classical transcriptomics methods.

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Topics: Biology, Biochemistry

Keywords: proteomics, 2D PAGE

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