Plant Bioinformatics, Systems and Synthetic Biology Summer School
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Poster Abstracts

1. Gianluca Abate
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Plants have evolved a variety of defense mechanisms to protect themselves against microbial attack. The plant cell wall serves as an initial barrier to microbial penetration. Should penetration occur, recognition leads to the release of a wide range of anti-microbial peptides (AMPs). AMPs represent an ancient component of the innate immune response of both Metazoa and plants. While defensins, a well-known class of such peptides, are found in almost all multicellular organisms, there are other interesting classes restricted to the plant kingdom. These include thionins, lipid-transfer proteins and cyclotide family of proteins. Almost all plant AMPs isolated so far are small peptides (less than 100 amino acid residues) containing an even number of cysteines (4, 6, or 8) that are pair-wise connected by disulfide bridges, thus providing high stability to the peptides. The high variability of antimicrobial cysteines-rich peptides in plants has been determined in the past trough molecular biology techniques where AMPs were purified from plant extracts, novel peptide sequence identified and their genes subsequently cloned. More recently, the availability of complete genome sequences has enabled new bioinformatics-based approaches that made possible automatic genome-wide analysis and detection. Using this approach, Silverstrein et al. (2007) assessed that individual plant genomes (Arabidopsis thaliana, Oryza sativa) contain a surprising abundance of genes coding for cysteines-rich peptides. Cysteine bridges must play a key role in the mode of action of membrane-active antimicrobial peptides; they affect the conformation and surface charge distribution of the AMP and are therefore responsible for the lipid-AMP interaction within the microbial membrane. The Structural Bioinformatics analysis we carried out on available 3D structures of antimicrobial peptides from plants allowed us to identify reasonable patterns of lipid membrane perturbation caused by cysteine-rich AMPs. If confirmed, these results may open up a new experimental window on the development of synthetic lipid-targeted peptides for the treatment of drug-resistant infections.

2. Fiammetta Alagna

Olive Fruit Transcriptome Analysis Through 454 pyrosequencing

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Olive is the sixth most important oil crop in the world, presently spreading from native Mediterranean to new production areas due to the beneficial nutritional properties and the high economic value of olive oil. Despite its primary economic importance, genomics information on olive tree is still lacking and few EST data are available in bioinformatic databases.This work is aimed at defining the transcriptome of olive drupes and at identifying those ESTs involved in phenolic and lipid metabolism during fruit development. Four different cDNA libraries were prepared applying the SMART technology (Clontech)

and sequenced using pyro-sequencing technology (454 Life Sciences Corporation). Drupes from two cultivars have been used, characterized by a very high oleuropein (main terpene secoiridoid) content and an oleuropein-lacking natural variant, respectively, at two developmental stages: just after fruit set and at mesocarp development, thus representing a diverse set of genes expressed in the olive fruit.Between 50,000 and 77,000 reads were obtained for each sample, for a total of 260,000 reads and an output of about 58 Mb. The average read lengths were between 217 and 224 bp. Quality filtered sequences from whole shotgun sequencing were de novo assembled, obtaining 26,563 contigs and 75,570 singletons, for a total of about 102,000 ESTs. Row sequence data were processed using a four step pipeline procedure and data were stored in a relational database (MYSQL) with a web interface. EST assemblies were annotated and classified according to their biological functions. Comparative transcript profiling allowed the identification of differentially expressed genes with potential relevance in regulating the fruit metabolism during ripening, with a particular focus on phenolic compounds. The massive EST characterization described here can be considered an initial platform for the functional genomics of *Olea europaea* and will be a starting point for the establishment of molecular tools for improving the major quality traits in this tree crop species.

3. Vadivel Arumugam

Mining of expressed sequence tags
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4. Mirza Saqib Baig

Homology modeling and docking studies of Comomonas testosterone B-356 biphenyl-2, 3-dioxygenase involved in degradation of polychlorinated biphenyls
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Biphenyl dioxygenase is a microbial enzyme which catalyzes the stereospecific dioxygenation of aromatic rings of biphenyl congeners leading to their degradation. Hence, it has attracted the attention of researchers due to its ability to oxidize chlorinated biphenyls, which are one of the serious environmental contaminants. In the present study, the three-dimensional model of α-subunit of Biphenyl dioxygenase (BphA) from Comamonas testosteroni B-356 has been constructed. The resulting model was further validated and used for docking studies with a class of chlorinated biphenyls where the kinetic parameters of these biphenyl compounds were well matched with the docking results in terms of conformational and distance constraints. The binding properties of these biphenyl compounds along with identification of critical active site residues could be used for further site-directed mutagenesis experiments in order to identify their role in activity and substrate specificity, ultimately leading to improved mutants for degradation of these toxic compounds.

5. Clemence Bonnot

Chemical genetics dissection of the adaptation of Arabidopsis to a phosphate deficient medium
Arnaud Carole[1], Bonnot Clémence[1], Clement Mathilde[1], Desnos Thierry, Nussaume Laurent.
[1]These three authors contributed equally to this work.
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Mineral starvation is though to reduce plant growth by reducing metabolic activity, however this might be an oversimplified view. Our laboratory results provide evidences for the existence of a low phosphate signalling pathway (a major QTL involved in primary root growth arrest in phosphate starvation condition and root tip contact with low phosphate medium necessity to root growth arrest).In order to dissect this signaling we have started a chemical genetics strategy to find small organic compounds that interfere with the phosphate signaling. We found several drugs acting on this signaling pathway in *Arabidopsis thaliana*. Some of these drugs seem to inhibit phosphate starvation signal whereas other mimic the low phosphate symptoms (root architecture and low phosphate markers expression etc).

6. Diana Elena Coman

Regulation Of Isoprenoid Pathways In Arabidopsis: integration of GGPP synthase isozymes into the isoprenoid metabolic network

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Isoprenoids represent the largest group of biologically active secondary metabolites in plants, with at least 30.000 chemical compounds whose functions in physiological and biochemical processes such as photosynthesis, pathogen defense, hormonal signaling, are essential. Many of them have significant commercial, pharmacological and agricultural value (essential oils, flavours, food colorants, taxol-anticancer drug, artemisinin-antimalarial drug, etc.). Understanding the regulation of isoprenoid biosynthesis pathway and its integration into cellular metabolic network is therefore of imminent scientific and commercial interest. In addition to the novel scientific insight, this topic has significant implications for biotechnology approaches designed to use plants as bioreactors for increased production of specific metabolites. One of the most important regulatory points in the isoprenoid metabolic pathway is synthesis of GGPP (geranyl-geranyl-PP, C20). GGPP pool is at the early metabolic branch point in the isoprenoid pathway and most of plant isoprenoids are derived from GGPP. In the model plant Arabidopsis thaliana, 12 GGPP synthase (GGPPS) isozymes are predicted in silico to be responsible for the synthesis of GGPP. They are thought to have acquired specificity by differential organelle, and tissue-specific expression. In addition, temporal control of their activity can assign them to be part of distinct metabolic fluxes. The goal of the presented work was to explore temporal and spatial regulation of GGPPS gene expression and to use this information to predict integration of GGPPS isozymes into the isoprenoid metabolic network. We have used Arabidopsis thaliana as model plant, together with functional genomics approaches and bioinformatics. Bioinformatics tools play an important role in development and progress of each part of this project; so far I have used *in silico* screening of publicly available databases, nucleic acid structure prediction software, alignment and BLAST algorithms, gene expression profiling experiments integrating microarray data with those obtain by real-time PCR and gene co expression analysis.

7. Esteban Czwan

Hypothesis-driven cancer survival analysis from gene expression data: non-uniform distribution of p-values under the null hypothesis

German Cancer Research Center

In gene expression microarray studies, the distribution of p-values under the null hypothesis is often not uniform. This can jeopardize conclusions as it renders traditional significance thresholds meaningless (Storey and Tibshirani, 2003). Previous studies based

on biologically defined gene sets, where the gene expression signature of a biological process is tested as a prognostic estimator, have failed to acknowledge the possible non-uniform distribution of null p-values. In these particular studies this false assumption may lead to the assertion that the biology of a gene set is associated with cancer prognosis when such a claim is actually not possible to confirm. Our study investigates such distribution and its impacts by developing an automated hypothesis-driven cancer survival methodology. This methodology empirically approximates the distribution of p-values under the null hypothesis using a permutation approach based on 100,000 random sets of unrelated genes, and tests whether predefined sets of biologically-related genes are associated with prognosis using common-practice methods (i.e. hierarchical clustering and log-rank test). The results show that null p-values are indeed not uniformly distributed and suggest that this non-uniformity can lead to wrong conclusions. The results were also compared to those of a standard hypothesis-driven approach, which assumes uniformly distributed null p-values (Chang et al, 2004). These comparative results also show that null p-values are not uniformly distributed and that overlooking this problem can cause overestimation or underestimation of significance.

8. Maksym Danchenko

Comparative Proteomics of Soybean Seeds Grown in Chernobyl Environment

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Explosion on the Chernobyl Nuclear Power Plant is the worst environmental nuclear disaster in human history. Huge amount of radioactive materials were released, and even presently nearby lands remain significantly contaminated with long-living isotopes. Decades of comprehensive investigations delivered general agreement that, plant adaptation to chronic irradiation might involve: genetic mutations, as well as epigenetic changes for genome stabilization and differential gene expression. However, molecular mechanisms behind plant ability to survive and successfully reproduce in harmful environment remain controversial. The aims of the project are the following: 1) perform first wide-scale protein profiling of developing seeds under chronic ionizing radiation; 2) create system-wide overview of biochemical pathways, potentially connected with the adaptability of plants, toward unfavorable conditions. Newly established soybean, Glycine max variety Soniachna, plantations were grown in polluted (163 times and 244 times more contaminated with 137Cs and 90Sr respectively) and control plots. Proteins from first generation mature seeds were separated by two-dimensional electrophoresis. Computer-assisted analysis of gels quantified 698 statistically reliable spots, significant differences in abundance showed 9.2% of them. Altogether 26 proteins were identified by tandem mass spectrometry. All identified proteins fit into the following main functional categories: metabolism, energy, transporters, disease/defense, cell growth/division, protein destination/storage. Based on acquired data, we propose a model for plant adaptation toward chronic irradiation, which includes: a) general stress adaptation component (cysteine synthase and dehydrins), b) specific for protection against ionizing radiation damage component (betaine aldehyde dehydrogenase), c) complex response of seed storage proteins might further confirm their role in plant defense [Danchenko 2009].Currently comparative analysis of second generation developing soybean seeds is ongoing. Two-dimensional gel reference maps of 2, 4, 5 weeks and mature developmental stages have been created. But also we started to utilize complementary gel-free approach, based on direct reverse-phase UPLC peptide profiling, on-line coupled to MS/MS. Data to be obtained, then bioinformatically processed, should clarify, give more details and

improve our radiation adaptation model. The project has received funding from FP7 of the European Union (MIRG-CT-2007-200165). This abstract reflects only the authors' views.

M. Danchenko, L. Skultety, N. Rashydov, V. Berezhna, L. Matel, T. Salaj, A. Pretova, M. Hajduch 2009 Proteomic analysis of mature soybean seeds from the Chernobyl area suggests plant adaptation to the contaminated environment. *Journal of Proteome Research* V. 8 p. 2915-2922

9. Cristian Dranca

Drug delivery systems based on hydrotalcites- like anionic clays with magnetic properties

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Layered double hydroxides (LDHs) with magnetic properties are proposed as novel supports for drug delivery systems to targeted specific locations in the body. The magnetic LDHs were prepared by loading iron oxides on iron partially substituted hydrotalcite- like materials. Further, hybrid nanostructures, such as chloramphenicol- magnetic LDHs were synthesized using the reconstruction method. The XRD and FTIR analysis reveal that the drug is detected in the clay interlayer. TEM analysis shows that drug coexist with iron oxide nanoparticles.

10. Carlo Fasano

Design of a high density DNA microarray to study gene espression profiles on potato polyploids

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Polyploids are very common within angiosperms. Polyploidy may cause the appearance of novel variability, thus assuming important significance from several standpoints. Little information exists for polysomic polyploids (autopolyploids) useful to understand the events underlying polyploid formation, to study the plasticity of plant genomes and to interpret metabolomic and proteomic changes associated to polyploidy. In light of this, we are trying to analyze and identify differences in trascriptome and metabolome of tetraploids of the model diploid (2n=2x=24) species *Solanum commersonii* obtained through somatic polyploidization induced by oryzalin. Therefore, comparisons are made between the diploid species and its artificially induced tetraploids. In particular, whole genome analysis, through microarray and real-time PCR are being carried out to fully visualize transcriptome. Bioinformatic approaches will be then used to analyze data obtained. To design a CustomArray 90K Combimatrix which allows to analyze up to 30000 gene sequences, we are using the SolEST database from where about 25000 known tentative consensus (TC) potato sequences have been downloaded. Differences in secondary metabolites in leaves are also being analyzed through HPLC. The aim of these analyses is to find genes or also classes of genes whose expression variation could be linked to a single molecular model that may help to clarify the polyploidization process.

11. Thorsten Forster

Meta-analysis of microarray gene expression studies of macrophages treated with interferon gamma

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12. Hoe Han Goh

Tapping into the mechanical link of expansins in leaf morphogenesis
University of Sheffield

Plant development relies largely on cell expansion as compared to animal counterpart. Expansins are conserved family of non-enzymatic proteins involved in cell wall-loosening with widespread role in development having to be pre-requisite for cell expansion. Induced expression of expansin at meristem and leaf flank respectively resulted in out-of-order leaf initiation and aberrant leaf shape formation. However, its actual mechanism is still unclear plus expansin activity has only been demonstrated in vitro.=20My research focuses on functional analysis of expansins involved in leaf morphogenesis. Atomic force microscopy has been employed to characterise cell wall mechanical properties during early leaf growth in an attempt for in vivo quantification of expansin activity. For the first time, I show that there is a distinct difference in cell wall extensibility between leaf abaxial and adaxial side and along proximo-distal axis. Next step is to test the function of expansin through inducible amiRNA silencing approach and investigate the cellular behaviour when expansin function is abolished as to how leaf development is affected using a viscoelastic leaf growth model.

13. Kai Gräber (joint poster with **Ada Linkies**)

Comparative Seed Biology - A Reverse Genetics Approach To Study Endosperm Weakening By Using Brassicaceae Relatives
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Lepidium sativum (garden cress) is a close relative of *Arabidopsis thaliana*. The mature seeds of both species are highly similar in physiology and structure, both have a thin endosperm layer between embryo and testa, but cress seeds are 10 times bigger facilitating tissue specific analysis. We use a combined approach, benefiting from the advantages of both Arabidopsis and Lepidium to investigate the mechanisms of seed germination and the role of the endosperm therein. We carried out heterologous transcriptome analyses on Arabidopsis full-genome microarrays. Therefore we hybridised specific Lepidium seed tissues, micropylar endosperm and radicle, harvested at different time points during germination in the presence and absence of abscisic acid (ABA), respectively. We showed that genetic transformation of Lepidium is possible, which provides the possibility to study the function of candidate genes from the microarray analysis in cress. Furthermore we are able to quantify endosperm weakening as part of the germination process in these transgenic cress plants biomechanically. Comparative Brassicaceae investigations provide a promising phylogeny-based approach to unravel conserved mechanisms in seed germination.

14. Samiullah Khan

Modification of Glycosylated Antioxidants by the Thermostable Glucosidase Bgl1A
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The aim of the proposed research is to make an impact in recovery of high-value compounds from agricultural byproducts/waste materials using environmentally sustainable techniques. Polyphenolic antioxidative molecules in onion skin, are examples

of such compounds. Thermostable enzymes are used in high-temperature/pressure processes for extraction and conversion into deglycosylated form in order to facilitate analytical determination, increase the yield, and obtain higher antioxidizing power[(1)]. Thermostable b-glucosidase from Thermotoga neapolitana (Bgl1A of Glycoside hydrolase family 1 (GH1)), is utilized in, and developed for, deglycosylation reactions of quercetin-glycosides from onion waste using an environmental friendly process with subcritical water. Quercetin is a powerful antioxidant of the flavonoid type. The sub-critical water extraction procedure requires high temperatures, so use of a thermostable enzyme is necessary. The selectivity of TnBgl1A was studied and the results show higher selectivity for quercetin-4' glycoside than quercetin-3' glycoside. To influence activity/selectivity, mutants of TnBgl1A were designed. Residues for mutation were selected based on alignment of enzymes within GH1, of different specificities, including the primary sequence of TnBgl1A. All mutants were expressed in E. coli and purified with immobilized metal ion affinity chromatography, utilizing the C-terminal His-tag included in the cloning design. Results from this work will be displayed. Our next goal is immobilization of selected enzymes for use in an online process. Different immobilization supports and methods are tried and data from these experiments will be shown.

1. Turner, C., Turner, P., Jacobson, G., Waldebäck, M., Sjöberg, P., Nordberg Karlsson, E., and Markides, K. (2006) Green Chemistry 8, 949-959.

15. Julien Lavenus

Julien Lavenus, Bert De Rybel, Pierre Hilson and Tom Beeckman
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Research in plant development has mainly focused on the role of phytohormones in controlling the various aspects of embryonic and post-embryonic development. However an increasing number of recent works show that small secreted peptides may also have major developmental functions, particularly in cell-to-cell communication processes, like in animals. These peptides are thought to work in pairs with Leucin-Rich-Regions Receptor-like Kinases (LRR-RLK). From the common characteristics of the currently known plants secreted peptides encoding genes, bioinformatic tools have enabled researchers to find new families of these type of genes as well as RLK encoding genes in the genome of the model plant *Arabidopsis thaliana*. For instance RLK encoding genes have been found to account for up to 2% of the coding sequences (Shiu et al., 2001). Thus it is highly probable that at least some of theses genes are involved in lateral roots development. All the more that De Smet et al. have recently demonstrated that the receptor-like kinase ACR4 notably promotes formative division in the pericycle during the first stages of lateral root primordium formation in *A. thaliana*. Thereafter Stahl et al. demonstrated that the ligand of ACR4 in the primary root meristem belongs to the CLE/CLV3 family and that the ACR4/CLE40 system controls the balance between proliferation and differentiation of root meristematic cells (Stahl et al., 2009).Using the publicly available datasets on tissue specific expression (AREX database, Birnbaum et al., 2003 ; Brady et al., 2007), several families of peptides were identified with a possible role in lateral root development. In order to study the organization of lateral organs and primordia along the primary root of plants treated with the corresponding synthetic peptides, tools were developed in R and scilab that enable easier and deeper analysis of the data from primordia staging experiments (an organization index can be calculated that gives information about the order/randomness of the sequence of emerged and non-emerged primordia; basic periodicity analysis can be carried out). Detailed phenotypical analysis has identified several novel classes of peptides that cause lateral root phenotypes, such as a reduced initiation, when exogenously applied on wild-type plants. One family of peptides has been studied into

more detail by analysing the promoter expression pattern and by analysing overexpression (35S and tissue specific) and knock-out (RNAi, amiRNA, SAIL) lines have been analysed and confirm the involvement in lateral root development. Next, a model in scilab was then developed to test hypotheses about the possible mechanisms that could explain the evolution of the expression profile of a particular peptide over time.

16. Chiara Lezzi

Fungal laccases phylogenetic analysis: a tool to drive a large scale heterologous expression program

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Laccases are multicopper oxidoreductases that have been identified in higher plants, fungi, insects and bacteria. Laccase natural roles embraces lignin biosynthesis in plants, melanin biosynthesis in bacterial spores, lignin degradation and pathogenesis in fungi, and esclerotisation in insects. Fungal laccases have enormous potential for various environmental and industrial applications. Several reports described the laccase purification directly from natural producers, such as numerous species of white rot fungi, but, in order to achieve production of great quantities of pure proteins, the expression of recombinant laccase genes has been recently carried out in heterologous hosts. Here we proposed a simplified approach to select laccase isoforms with peculiar physico-chemical and catalytic characteristics from different fungi, as genetic source. We performed a bioinformatic analysis, both by stochastic and parsimony methods, on all available laccases belonging to two different genera of Basidiomycetes (Pleurotus and Trametes) and those belonging to the genera of Ascomycetes fungi named Aspergillus. Each cluster deriving from the phylogenetic analysis will group laccases according to their sequence similarity, thus indicating enzymes which are likely to have similar biochemical features. This analysis we will allow the identification of laccase isoforms with peculiar biological properties thus driving the selective gene expression in a heterologous system. The phylogenetic analysis, performed separately on each different genus, revealed that the isoenzymes of Trametes laccases were assigned to the four alpha, beta, gamma and delta groups and that Pleurotus laccases were unambiguously clustered in different phylogenetic groups. Laccases deriving from several Aspergillus species, i.e. *A. flavus, A. clavatus, A. fischeri, A. fumigatus, A. nidulans, A. oryzae and A. terreus*, clustered in at least eight main groups.

17. Marie Maitrejean

Synthesis and trafficking of the tonoplast potassium channel AtTPK1

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Sorting signals of tonoplast proteins and the pathway they follow through the endomembrane system are still poorly characterized. To date, most studies have focused on tonoplast intrinsic proteins (TIPs) and indicated that these proteins are delivered from the endoplasmic reticulum to their destination by a Golgi-independent pathway. Is this a general pathway for tonoplast delivery: To address this question, we are studying the tandem-pore potassium channel AtTPK1. This channel has been shown to be located at the tonoplast in transient heterologous expression systems. We confirmed this localisation in planta, by over-expressing a TPK1-GFP fusion in transgenic Arabidopsis. Treatment with Brefeldin A, an inhibitor of Golgi-mediated secretory protein traffic, leads to mislocalization of TPK1-GFP to the endoplasmic reticulum. This indicates that the Golgi-independent pathway is not the only route for tonoplast delivery. Pulse chase analysis shows that TPK1-GFP is quite stable, with a half life of at least 24 h, and undergoes slow

removal of the C-terminal, cytosolic GFP. This processing is not affected by brefeldin A, indicating that mislocalisation does not alter the stability of the fusion protein. To further investigate the role of the Golgi apparatus, we wanted to take advantage of its glycan modification properties. However, treatment with the N-glycosylation inhibitor tunicamycin indicates that the potential TPK1 N-glycosylation site at Asn131 is not used in vivo. We finally showed that TPK1-GFP forms homodimers. We are now investigating the relationship between TPK1 dimerisation and its sorting to the tonoplast. We generated several chimeras between TPK1 and TPK4, the homologous channels located at the tonoplast and the plasma membrane respectively. Study supported by the EU Marie Curie Research Training Network Vacuolar Transport Equipment for Growth Regulation in Plants (MRTN-CT-2006=96035833).

18. Colette Meyer

University of Edinburgh

GnRH analogues have been shown to have antiproliferative effects in some breast cancers in vivo in mouse models and in clinical trials. However, the response is highly variable and the factors determining a favourable response remain elusive. We are using high-throughput transcriptomic and proteomic arrays with both in vitro and in vivo (mouse xenograft) samples. By combining these data we will generate hypotheses about the signalling downstream of the GnRH receptor, which can then be validated. We expect to use a Bayesian modelling approach to build a model of this signalling. These approaches will develop analytical techniques for handling data from novel antibody microarrays, as well as time-course gene expression microarrays within the field of cancer research.

19. Domenica Nigro

Functional Markers For Glutamine Synthetase And Correlation With Grain Protein Content In Durum Wheat

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Durum wheat (Triticum turgidum L. var. durum) is one of the most important cereal crops grown world-wide and provides most of the proteins in human diet, especially in the less developed countries. Seed storage proteins are directly related to the nutritional and technological value of the derived products. Several studies have attested the key-role of the glutamine synthetase enzyme in plant nitrogen metabolism. Glutamine synthetase gene encodes for an enzyme responsible of the first step of ammonium assimilation and transformation into glutamine and glutamate, essential compounds in amino acid-biosynthetic pathway. High protein content is a very important quantitative trait controlled by several genes located on wheat chromosomes. Glutamine synthetase genes are located on the homeologous chromosomes 2A, 2B, and 2D where several authors reported major QTL for protein content. The goal of the present study was to assess the linkage between GS gene and the QTL for protein content. For this purpose, the nucleotide sequence of glutamine synthetase gene acc. DQ124214 was aligned to all the wheat ESTs available in public data bases by means of BLAST tool (http://www.wheat.pw.usda.gov/GG2/blast.shtml.). The bioinformatic analysis allowed to find 40 sequences with a similarity > 94% to the GS2 gene, of which three covered the whole gene sequence (DQ124213, DQ124212 and CJ705909). For each of these sequences we designed two or three primer pairs identifying a total of 7 functional markers that were screened among the parents of three segregant populations. Mapping analysis performed by Join Map software allowed to localize the amplified polymorphic fragments

and to identify 4 loci: Gs-A2, Gs-B2, Gs-A4, Gs-B4, respectively mapped on chromosome 2A, 2B, 4A and 4B. The QTL analysis for protein content was carried out in a RIL population obtained from the crossing the two durum wheat cultivars Ciccio and Svevo. Two major QTLs were identified through Composite Interval Mapping (CIM) performed by the Q-Gene software: one QTL was identified by the functional marker Gs-B2 located on chromosome 2B, and the other one was identified by the functional marker Gs-A4 located on chromosome 4A. These data were confirmed by a linkage disequilibrium analysis carried on a collection of 75 different wheat genotypes. The present study represents the first step for the identification and sequencing of GS2 gene, which could be employed in breeding programs aimed to increase grain protein content commercial cultivars. Moreover, Gs-B2 and Gs-A4 represents functional markers that could be also efficiently used in marker assisted selection (MAS) programs and map-based cloning.

20. Elad Noor

Weizmann Institute of Science

Central metabolism uses a complex series of enzymatic steps to convert sugars into metabolic precursors. These precursors are then used to generate the entire biomass of the cell. Are there simplifying principles that can explain the structure of such metabolic networks: We address this by studying central metabolism in E. coli and defining a game which takes into account the detailed biochemical structure of each compound. For this purpose, we use all known classes of enzymes that work on carbohydrates to generate rules for converting compounds and for generating possible paths between compounds. We find that central metabolism is built as a minimal walk between the twelve precursor metabolites that form the basis for biomass: Every pair of consecutive precursors in the network is connected by the minimal number of enzymatic steps. Similarly, input sugars are converted into precursors by the shortest possible enzymatic paths. This suggests an optimality principle for the structure of central metabolism. The present approach may be used to study other metabolic networks and to design new optimal pathways.

21. Beata Orman

Dissecting the genetic regulation of barley (*Hordeum vulgare*) root architecture

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Little is known about the genetic basis of root system formation and architecture in cereals. Based on *Arabidopsis thaliana* data, effective auxin uptake by the AtAUX1 influx carrier is an important process underlying the patterning of root architecture. Sequences similar to auxin influx carriers have been found in *Oryza sativa*, yet its existence in barley has not been confirmed. From *in silico* search for barley ESTs matching the AtAUX1 sequence, we have assembled a putative HvAUX1 and we have confirmed its expression in root tissues of the Optic barley variety. Sequence and phylogenetic analyses of the amino acid/ auxin permease (AAAP) protein family, which includes AtAUX1, revealed that putative HvAUX1 protein is the most similar (90% of similarity) to Os01g63770 (Os Like AUX1) protein sequence and shares 81% similarity with AtAUX1. This *O. sativa* protein sequence is also the most similar to AtAUX1 (82% of similarity). Taken together, we have identified a putative HvAUX1, which is a potential ortholog of *A. thaliana* AUX1. Functional analysis is underway to check this hypothesis.

22. Marko Petek

Omics-Based Systems Approach To Potato-Pvy Interaction

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In nature plants encounter various factors which influence their growth and development and consequently affect plant product quantity and quality. One of agronomically extremely important biotic factors are plant viruses. Plants responses to viral infection and disease development are different and much less explored in comparison to the bacterial or fungal infection. There are no chemical means for virus control available (such as fungicides for the control of fungi), and therefore the knowledge of plant-virus interactions is even more important as it provides basis for development of new molecular diagnostic tests, faster progress of agronomic expertise and alternative ways of virus spread control. Plant responses to viruses are complex and show a broad spectrum of physiological and histological changes. Studying single components of the interaction can lead to limited conclusions or results, which fail to take into account the complexity of interactions between the different pathways of the response. Omics approaches are a major step forward in understanding plant - pathogen interactions as they offer a more holistic view of the processes involved. Potato virus Y (PVY) is of extreme economic importance as it is responsible for yearly losses in production of crops from family Solanaceae in Europe, and thus the subject of investigation in many research groups all over the world. The tuber necrotic strain variety of Potato virus Y (PVYNTN) causes potato tuber necrotic ringspot disease in sensitive potato cultivars. In our studies, gene expression in the disease response of the susceptible, tolerant and resistant potato (*Solanum tuberosum* L.) cultivars to PVY infection was investigated at different times after infection, using transcriptomics approaches, among them subtractive hybridization, cDNA microarrays and real-time PCR. In parallel with the biological experiments, we have explored and developed several aspects of microarray data analysis and visualization. The expression of several genes involved in various metabolic pathways, including those involved in photosynthesis, sugar and starch metabolism, cell wall processes and secondary metabolism, was changed shortly after viral inoculation, which suggests their important role in the potato-PVY interaction. Functional analysis of a selection of those genes is currently being performed, and their role in the interaction will be confirmed by silencing or over-expression. Moreover, a potato leaf proteome analysis platform, combining 2D-electrophoresis analysis with identification by LC-MS-MS, was recently established and was used for further investigation of the interaction. Finally, all the data obtained will be integrated with pre-existing literature data to construct a structural model of potato-PVY interaction.

23. Eleftherios Pilalis

A compartmented *in silico* model of Rapeseed central metabolism

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Systems level modeling of cellular metabolism has proven to be indispensable for the design of rational genetic modification strategies for the redistribution of the metabolic flux network towards desired end-products. The exploitation of whole-genome pathway databases in combination with the appropriate mathematical techniques and modern high-throughput measurement methods, gives new perspectives in terms of understanding and

controlling the pleiotropic functionality of complex biological reaction networks. In this work a large-scale *in silico* model is constructed for the simulation of the central metabolism of rapeseed (*Brassica napus*) embryos. Rapeseed is an organism of particular interest in oil industry and the *in silico* reconstruction of its metabolism can help our understanding regarding the regulation of lipid biosynthesis. The model comprises 300 reactions extracted from the Aracyc and KEGG databases. In order to validate this model, we performed constraint-based Flux Balance Analysis, through application of linear optimization methods, and by incorporating relevant experimental data from literature. The model successfully predicted flux distributions close to those experimentally reported. Further exploiting the derived model, an in-silico gene deletion analysis is performed in order to evaluate and comprehend the plasticity of the real network and infer conclusions regarding its robustness as well as predict target for oil biomass overproduction.

24. Roman Pleskot

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Phosphatidic acid, sphingosine 1-phosphate and ceramide 1-phosphate are important signalling lipids for the eukaryotic cell. These molecules are produced by related enzymes, members of lipid kinase family. However, no comprehensive study about origin and evolution of lipid kinase gene family has been done yet. We therefore performed phylogenetic and motif analysis of this family in 52 eukaryotic genomes with special interest to plants. To obtain a broader insight into the origin of lipid kinase family we also searched for lipid kinases in eubacteria and archaebacteria. The main signature of this family is diacylglycerol kinase catalytic motif (Pfam00781, Smart DAGKc). Diacylglycerol kinases (DGKs) contain so-called diacylglycerol kinase accesory domain (Pfam00609, Smart DAGKa), but at least weak homology to this domain can be found in the other members of lipid kinase family. Apart from these signatures, some DGKs, particularly in spermatophyta and unikonta group further have extra regulatory domains, e.g. C1 domain, EF-hand. On the basis of our phylogenetic analysis, we speculate that the last common ancestor of eukaryotes already had separated DGK and sphingosine/ceramide kinase subfamily. Many duplications and secondary losses occured during the evolution of the lipid kinase family, e.g. fungi lost DGK subfamily and replaced it by the enzyme with CTP transferase domain. Spermatophyta and *Trichomonas vaginalis* have specific lipid kinases, which resemble bacterial enzymes. Our analysis suggests that the presence of this groups is probably result of two independent horizontal gene transfers. We also constructed model of the catalytic core of the members of different plant lipid kinase subfamilies in an effort to uncover similarities in the reaction mechanism and the membrane docking with their bacterial homologs with known 3D structures. Financial support from grants IAA601110916 and LC06034 is acknowledged.

25. Patrik Sahlin

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Large-scale pattern formation is a frequently occurring phenomenon in biological organisms. One example is plant phyllotaxis which has gained extra attention due to patterns that are otherwise found in mathematics and physics. Terms from the Fibonacci series can be found in spiral patterns and the golden angle can often be measured between the radial direction of two consecutive elements. It has been shown that Fibonacci phyllotaxis can be achieved from a combination of a growing apex and a suitable spacing mechanism for primordium initialization. Here we present and analyze a model based on polarized auxin transport as a candidate for such a spacing mechanism.

26. Uzma Saqib

Central Drug Research Institute

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) has attracted attention during the last few years due to its potential as a target for the treatment of metabolic syndrome and type 2 diabetes. It plays a central role in regulating intracellular concentrations of glucocorticoids by converting inactive cortisone to the metabolically active hormone cortisol. The latter has been shown to promote gluconeogenesis, adipogenesis, lipolysis and release of free fatty acids leading to metabolic syndrome and type2 diabetes. We report here, the molecular docking and CoMFA (Comparative Molecular Field Analysis) based de novo ligand design using LeapFrog (LF) studies on highly potent piperidine amide inhibitors of 11β-HSD1. Molecular docking reproduced conformations favorable for inhibitor binding and revealed various hydrophobic interactions involved in ligand binding. CoMFA studies were performed and a robust model was developed which produced statistically significant results with cross-validated and conventional correlation coefficients of 0.650 and 0.989 respectively. The contours of CoMFA model was used to serve as a pharmacophoric model to generate hypothetical cavity for LeapFrog calculations. We have designed 7 new Piperidine amides analogues which showed better predicted activity with respect to the reported compounds, suggesting that newly proposed molecules in this series of compounds may be more potent and selective towards 11β-HSD1 inhibition.

27. Marta Sawczak

Comparison and evaluation of rank-based methods for generating differentially expressed gene lists from microarray data

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Identification of genes whose expression is changed under different experimental conditions is an important task in many microarray studies. This goal is especially difficult in case of experiments with low number of repetition. The main object of this study is to evaluate performance of rank-based methods on selection of differentially expressed genes in comparison to popular t-test based method. In the present study we tested two existing methods (Rank Product and Rank Sum) and three new proposed by us (Differential Rank Product, Rank Distance, Reverse Rank Distance) along with fold change and four t-test methods (ANOVA, Welsh t-test, Empirical Bayes T-Statistic, Significance Analysis of Microarrays). We split 9 large two-classes datasets on training and test set. Four different classifiers (Between group analysis, Naive Bayes Classifier, K-nearest neighbors, Support vector machine) were learnt based on gene list produce by each method. Subsequently, trained classifiers classified previously unseen sample (test set). Prediction success of gene list were measured by Relative Classifier Information Metrics (RCI) When the number of samples in train data sets were high Rank Sum, Empirical Bayes T-statistic, Reverse Rank Distance and Differential Rank Product performed nearly equally good. Differences in performance of selection methods were more significant when the number of samples in training set decreased. When the training consisted of 5 samples the best prediction power had the list of genes produced by Differential Rank Product followed by Rank Product. Our result confirmed that rank-based method are valuable alternative for t-test based method and are especially recommended for datasets with small number of samples.

28. Shahid Masood Siddique

Institute of Plant Protection, BOKU, Vienna, Austria

In plants, UDP-glucuronic acid is synthesized by the oxidation of UDP-glucose by UDP-glucose dehydrogenase. However, a second pathway has been described and involves the oxygenation of free myo-inositol by myo-inositol oxygenase (MIOX). In Arabidopsis, myo-inositol oxygenase is encoded by four genes (MIOX1, MIOX2, MIOX4, MIOX5), MIOX3 being a pseudogene. Transcriptome analysis of syncytia induced by the cyst nematode *Heterodera schachtii* in Arabidopsis roots revealed MIOX genes are among the most strongly upregulated genes in syncytia. We have used GUS analysis, in situ RT-PCR, and real-time RT-PCR to study the expression of all 4 MIOX genes in syncytia induced by H. schachtii in Arabidopsis roots. All these methods showed that MIOX genes are strongly induced in syncytia. GeneChip data were analysed for the expression of genes related to the MIOX pathway (MapMan). Two complementary double mutants were used to study the importance of MIOX genes. Results of the infection assay with double mutants in two combinations (Δmiox1+2, Δmiox4+5) showed a significant reduction ($p < 0.05$) in the number of females per plant when compared with the wild-type. Furthermore, syncytia in double mutants were significantly smaller than in wild-type plants.

29. Damien Simon

Role and function of DNA-binding proteins in Arabidopsis root cell elongation.

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Cell elongation in roots occurs between the division and differentiation processes and is highly anisotropic. Specific changes in the water up-take, the cell wall structure and the DNA content (endoreduplication) are involved. In order to identify different actors in this complex physiological process, a micro-array analysis has been performed on roots of the model plant Arabidopsis thaliana in control situations and after treatment with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC). This treatment results in the inhibition of cellular elongation. Analysis of the micro array results lead to the identification of a putative DNA binding protein which is down-regulated after the ACC treatment. Transgenic plants were generated to study the expression pattern of the gene and the subcellular localisation of the resulting protein. The knock-out line, despite showing any root length phenotype, is showing an unidirectional curvature of the root. This peculiar fenomenon can, surprisingly, be as well observed in the over-expression lines. All the results point towards the fact that this putative DNA binding protein is a transcription factor with a certain importance in gravitropism perception or its signal transduction. Further investigations are ongoing to verify this hypothesis. Furthermore, several close homologues of this gene are currently under detailed study. Their roles in cell elongation and gravitropism will be discussed as well.

30. Vasantika Singh

Cross-species transcriptomics to study trait evolution in Arabidopsis

University of Heidelberg

The aim of our study was to examine the genomic changes and mechanisms especially the role of gene deletions and copy number variations in the evolution of species specific traits in model plant Arabidopsis thaliana and two of its closely related species *Arabidopsis halleri* and *A. lyrata*. Cross-species comparative genome hybridizations (CGH) were performed on ATH1 chip of Affymetrix followed by mRNA hybridizations. This CGH data was analysed computationally to identify the genomic variations in related species and

subsequently to further enhance the evaluation of transcriptomic hybridizations. The genes thus identified to be truly differentially expressed in *A. halleri* for the model trait of metal hyperaccumulation and tolerance are being verified experimentally.

31. Hui Sun

Comparative analysis of response of Conifer tissues to necrotrophic capability in *Phlebiopsis gigantea* and *Heterobasidion annosum*

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Root and butt rot caused by *Heterobasidion* species is one of the most destructive diseases of conifers in the northern temperate regions of the world. *Phlebiopsis gigantea* (Fr.) Jül., is currently used as biocontrol agent against *Heterobasidion* infection. A major problem is that although the effectiveness of *P. gigantea* in biocontrol has empirically been shown, the long term biological effect of this fungus on conifer trees as well as other soil flora has not been empirically proven. Equally, mechanism for its biological and antagonistic activity is still unknown. The most probable mechanism for the biocontrol ability of *P.gigantea* against *H. annosum* could be due to its induced resistance as it causes more lignified cells in conifer tissues. The aims of the present study are to assess the potential risk for the long term use of P.gigantea as biocontrol agent in forestry in terms of developing nectrotrophic capability and other impact on the environment. To investigate this, 8 - 10 year old spruce and pine seedlings were inoculated with either *P. gigantea* or *H. annosum*. Samples were taken at defined time intervals and examined for morphological changes (lesion size, cell death in necrosis). Comparative analyses of molecular responses of the conifer host to the two fungi were also documented with aid of Pyrosequencing and qPCR methods. Initial result indicated that *P.gigantea* induced necrosis both in the phloem and xylem on *P. sylvestris* like *H.annosum*, but there was significant difference ($p<0.001$) in the lesion size. However, at prolonged incubation, no further increase in lesion size was observed for trees inoculated with *P. gigantea*. Also there were more necrotic related dead cells in the fungal treated samples than in the control. This indicated that P.g has very limited necrothophic ability in conifer trees in future. Additional separate studies on induced resistance and transcript profiling by pyrosequencing were conducted. The results will be presented and discussed.

32. Peter Thorpe

Bioinformatic Analysis of Palindromes in Bacterial Genome Sequences

Leeds University and SCRI (Dundee)

Palindromes are thought to have a major role in the expression of genes in up and downstream operons by forming hairpin structures that interact with transcription factors. Computer programs were developed to enable Bioinformatic research on the genomes of *Nanoarchaeum equitans*, *Escherichia coli*, *Pectobacterium atrosepticum* and *Pseudomonas syringae* to determine if palindromes are present, their distribution (intergenic or intragenic) and if palindrome clustering is observed. The data in this study shows there is statistically significant evidence supporting the observation that palindromes are selected for and may clustered within intergenic regions in the bacterial genome, compared to 30 randomly generated genomes derived from the corresponding sequenced genome. The intergenic distribution of palindromes is consistent with the theory describing their involvement with the regulation of up and down stream gene expression. This evidence suggests that palindromes may have a significant role in the composition of genome sequences, which may have been selected for by evolution, hence

their presence in both prokaryotes (identified in this study) and eukaryotes (Katz et al., 2003; Chartrand et al., 1999).

33. Dorian Urbanski

Establishing of artificial microRNA-mediated gene silencing for *Lotus japonicus*

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Center for Carbohydrate Recognition and Signalling Laboratory of gene expression, Department of Molecular Biology, University of Aarhus.

Nodulation research using lotus has mostly been based on the forward genetics approach, where a mutant phenotype is linked to a genetic mutation through map-based cloning. On the other hand, reverse genetics, where the activity of a gene of interest is modified to discover its mutant phenotype, is still not well developed in lotus. This is mainly due to laborious transformation methods and lack of a reliable technique for gene silencing. One of the recently introduced approaches in plant reverse genetics is the use of artificial microRNAs (amiRNA) for gene-specific silencing. amiRNA silencing has already been used successfully in Arabidopsis and rice. Our aim is to introduce amiRNA-mediated silencing in lotus to fill the gap in revered genetics, caused by the lack of a specific and reliable gene-silencing method. In addition, the flexibility and specificity of the amiRNA method will greatly facilitate studies of lotus gene families.

34. Sascha Waidmann

Functional analysis of stress response in *Arabidopsis thaliana*

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In many parts of the world, agriculture is limited by abiotic stresses, including drought, temperature extremes and high soil salinity, singly and in combination. Plants as sessile organisms have developed different mechanisms to respond including complex systems in signal perception and transduction. Phosphorylation events play an important role in stress signalling networks.The glycogen synthase kinase 3 (GSK3)/Shaggy family of serine/threonine protein kinases is involved in several biological processes in animals, plants and yeast. The GSK3 family in Arabidopsis thaliana includes ten known members that can be grouped into four classes on the basis of their sequence homology. Although the biological functions of most of these proteins are still unknown, they play a role in hormone signalling, development and stress response. We are interested in the role of Arabidopsis GSK3/Shaggy-like kinase ASK7 in stress signalling. It has been shown the expression levels of this kinase are altered under different stress conditions. In order to study the involvement of ASK7 in stress signalling, ASK7 over-expressor and knock out lines were obtained. ASK7 activity mutants showed a modified stress tolerance when they were exposed to drought and high salinity. Consistent with this, ASK7 protein kinase activity was rapidly changed under stress conditions. A yeast-two-hybrid screen was performed to identify putative interaction partners of ASK7. The identified partners are currently under investigation. To identify genes controlled by ASK7-based signalling, we will perform a microarray experiment with ASK7 activity mutants and compare the expression levels under different types of stress. Furthermore we are interested to investigate the metabolomic response in these activity mutants under high salt conditions and drought. In an integrative multiparallel approach we want to correlate the physiological phenotype of ASK7 activity mutants with transcriptome and metabolome data, study the

dynamic responses of Arabidopsis thaliana under abiotic stress and position ASK7 within the stress signal transduction network.

35. Charlotte Worthy

Plymouth Marine Laboratory, Rothamsted Research & University of Nottingham

Emiliania huxleyi is a representative of the coccolithophores, a group of calcifying unicellular alga found throughout the world's oceans. Significant genetic variation in coccolithophore populations enables *E. huxleyi* to be a successful species (Inglesias-Rodriguez et al 2006), and greatly impact on marine ecosystems and in particular, on the global carbon and sulphur cycles (Charlson et al 1987). Frequent blooming (rapid increases in population size over vast areas) occurs in the upper stratified oligotrophic waters of the ocean, and can easily be viewed by satellite given that they are characterised by high light backscatter caused by free floating coccoliths; extracellular calcium carbonate scales (Holligan et al 1993). Crashing blooms cause sudden and substantial shifts of calcite to the seafloor and fluxes of cloud-forming dimethyl sulphide to the atmosphere (van Rijssel & Gieskes 2002). Phosphate availability can impact the formation and development of *E. huxleyi* blooms (Lessard et al 2005), as can the presence of coccolithovirus; giant dsDNA viruses from the monophyletic group within the Phycodnaviridae (Wilson et al 2002)Historically *E. huxleyi* has produced a plethora of information relevant to geological history, climate change and evolution. In more recent times the determination of the full genome sequence of the coccolithovirus (Wilson et al 2005) and the draft sequence of *E. huxleyi* has allowed the development of a significant molecular toolbox armoury placing *E. huxleyi* in a strong position to become the marine world's equivalent of model laboratory strains such as *E. coli*. Our current research involves the characterisation of lipid profiles from over one hundred and twenty *E. huxleyi* strains originating from regions such as the Sargasso Sea, North and South Atlantic, North Sea and Norwegian Fjords with a view to identify commercially relevant strains and lipids. In addition, over 500 samples of total biomass were collected from a mesocosm experiment conducted in Norway, June 2008, and distributed amongst three research teams; JN/MA/DW/CW investigating fatty acid profiles, Bidle/Vardi/Van Mooy representing Rutgers USA investigating sphingo- and glycerol lipid diversity, and finally SR/MA/CW investigating long chain alkenones. The objective of the study is to assess the role of phosphate availability on coccolithovirus/ *E. huxleyi* dynamics and lipid production in a natural system. An additional aim of this project is the identification of *E. huxleyi* genes involved in the synthesis of omega-3 long chain polyunsaturates, with the ultimate goal of expressing these activities in transgenic plants.

36. Kunal Saini

Investigation of role of Rab GTPases in fruit ripening in tomato

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Tomato (Solanum lycopersicum L.) is one of the most important vegetable crops cultivated worldwide. Fruit softening is a complex trait and involves many gene products. Rab GTPases are involved in processes of intracellular membrane traffic, protein secretion and signal responses for targeting of molecules for secretion into the cell wall. The aim of the project is to investigate the role of Rabs and other elements of the trafficking machinery in secretion of cell wall modifying factors associated with softening and thereby manipulating texture without affecting flavour and colour of fruits.

The genes encoding Rab GTPases in tomato were identified by in silico analysis. Using the HMM for Rabs, Sixty genes were found by screening the TIGR Tomato Gene Index

database (Release 12) by using PSi-Blast and Blastx. The sequence alignments revealed the conserved Rab Family (RabF1-F5) and Sub-family motifs (RabSF1-SF5). Further these genes were grouped into 8 different functional classes ranging from Rab1, 2, 5, 6, 7, 8, 11 and Rab18. A phylogenetic tree was constructed which indicated all the genes were falling into 8 different cluster belonging to each class. These results were further validated by constructing another phylogenetic tree by using the 57 Rab genes from Arabidopsis as the reference from TAIR database. The protein structure and function prediction was done, 3Dstructure of LeRab11a was done by homology modelling using swiss modeller and I-TASSER. The micro-array expression data from TED was used for the hierarchical and K-means clustering to get the relative expression profiles of the genes. The candidate genes were identified which were up-regulated during peak period of ripening. Primers were designed for these genes to get the real time expression by qRT-PCR at 8 different developmental stages of ripening. The textural analysis to characterise the pattern of fruit softening of the wild type and transgenic plants is in progress and the preliminary results show differences in softening and a sequential pattern of delayed softening in the transgenic lines.
Future work will include silencing of the candidate genes by RNAi. Characterization of fruit softening by textural analysis and enzyme assays followed by co-localization of Rab proteins and vesicle cargoes by GFP-protein fusions. The last step is to develop gene regulatory network model and pathway profiling by profiling the protein–protein interaction with Arf and SNARES and. This will help to conclude the role of each Rab in intracellular vesicle trafficking during tomato fruit ripening.

37. Syed Murtuza Baker

A review of Kinetic Parameter Estimation Methods
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Metabolic networks are extremely complex and highly interconnected systems which regulate vital cellular processes for all living organisms. To understand this complex behavior it is necessary to translate the metabolic network into a dynamical model with rate laws for each enzymatic reaction. These rate laws are defined as mathematical expressions which heavily depend on the underlying mechanism of the enzymatic reactions and can become quite complex with a large quantity of parameters. Therefore system-level computational approaches are required to model and understand this mechanism. To model the system as accurately as possible, it is very much important to have a complete and accurate set of parameters which characterize the system. This poster is an attempt to review different parameter estimation methods and categorize themselves according to different functionalities.

38. Muhammad Nadeem Zafar
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Cellobiose dehydrogenase (CDH) is an extracellular fungal enzyme with two domains, one containing flavin adenine dinucleotide (FAD) and one containing heme. CDH belongs to the restricted group of redox enzymes that show efficient direct electron transfer (DET) with electrodes. However, with the use of artificial mediators the current density in the presence of the enzyme substrate will be increased (mediated electron transfer, MET). In this study, we used CDH from the ascomycete *Corynascus thermophilus*. The DET and MET properties of CtCDH were compared. In this study CtCDH was wired with a low potential Os2+/3+ redox polymer acting as a very efficient mediator. To increase the current density further for both DET and MET, different types of single walled (SWCNTs) and multi walled (MWCNTs) carbon nanotubes were used. Highest catalytic currents for

glucose and lactose as substrates were obtained when CtCDH was wired with the Os redox polymer in the presence of SWCNTs followed by MWCNTs and fractionated SWCNTs from pH 6 to 8.5. For DET using glucose, the linear range was from 0.75 mM to 30 mM and the lower limit of detection was 125 µM. The linear range and the lower limit of detection can be improved with MET reaching at least down to µM level.