

# EPSO-Conference 2002

## Session

## Basic Biological Processes Poster 1.1 – 1.55

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## EPSO-Conference 2002 - Poster 1.1

Session	Basic Biological Processes
Title	The presence of vector backbone sequences, short inverted repeats and DNA methylation is not sufficient or necessary for the induction of transgene silencing in Arabidopsis
Author(s)	Trine J. Meza, Biljana Stangeland, Inderjit S. Mercy, Magne Skårn, Dag A. Nymoen and Reidunn B.Aalen  Division of Molecular Biology Department of Biology University of Oslo P.O.Box 1031 Blindern N-0557 Oslo, Norway  Author e-mail: <a href="mailto:reidunn.aalen@bio.uio.no">reidunn.aalen@bio.uio.no</a>
<b>Abstract</b>  In genetically transformed plants, transgene silencing has been correlated to multiple and complex insertions of foreign DNA, e.g. T-DNA and vector backbone sequences. Occasionally, single-copy transgenes also suffer transgene silencing. We have compared integration patterns and T-DNA/plant DNA junctions in a collection of 37 single-copy T-DNA transformed Arabidopsis lines, of which 13 displays silencing. Vector sequences were found integrated in five lines, but only one of these displays silencing. Truncated T-DNA copies, positioned in inverse orientation to an intact T-DNA copy, were discovered in three lines. The whole nptII gene with pnos promoter was present in the truncated copy of one such line in which heavy silencing has been observed. In the two other lines no silencing has been observed over five generations. Thus, vector sequences and short additional T-DNA sequences are not sufficient or necessary to induce transgene silencing. DNA methylation of selected restriction endonuclease sites could not be correlated to silencing. Our collection of T-DNA/plant DNA junctions has also been used to evaluate current models of T-DNA integration. Data for some of our lines are compatible with T-DNA integration in double-stranded breaks, while for others initial invasion of plant DNA by the left or by the right T-DNA end seem important.	

## EPSO-Conference 2002 - Poster 1.2

Session	Basic Biological Processes
Title	DNA Repair in Plants: What can we Measure in Individual Cells?
Author(s)	Karel J. Angelis  Institute of Experimental Botany CAS Na Karlovce 1 160 00 Praha 6, Czech Republic  Author email: angelis@ueb.cas.cz
<p><b>Abstract:</b></p> <p>We used seedlings and root culture of standard <i>Arabidopsis thaliana</i> Col0 and mim- mutant that is deficient in homologous recombination, to study repair of DNA breaks and of alkylation damage. Protocols designed to detect double strand breaks (N/N), true DNA breaks (A/N) and breaks plus Alkali Labile Sites (A/A) were adopted for <i>Arabidopsis</i>.</p> <p>We have found adaptive phenomenon (less DNA damage) when pretreatment by low, conditioning dose precedes high, challenge, dose of MNU or MMS by A/N assay. When A/A assay is used, the effect of conditioning is additive to challenge treatment, indicating conversion of ALS into breaks that masks adaptive repair of fewer true breaks. Majority of breaks detected in A/N assay most likely represent intermediates of DNA excision repair.</p> <p>Bleomycin was used to induce dsb and ssb to see the difference in their repair in standard Col0 and mim- <i>Arabidopsis</i> plants. Inactivation of mim gene coding chromosome maintenance protein leads to extreme sensitivity of plants to various mutagens and to loss of homologous recombination. Surprisingly there is no measurable difference between mutant and standard plant in the repair of dsb measured by N/N assay or of DNA breaks measured by A/N assay. Most likely this is because MIM is utilized only during short period of late G2 and mitosis. Moreover nuclei from dividing cells in plant meristems represent only minor fraction of already differentiated cells and this is why the effect is hardly detectable in the nuclei isolated from whole seedlings. On the other hand comet assay shows rapid dsb and break joining in all cells. This indicates that break joining is likely the most prominent DNA repair pathway in plants.</p> <p>This work was supported by Grant Agency of the Czech Republic contract 521/01/1418 and Grant Agency of Czech Academy of Sciences A 6038201.</p>	

## EPSO-Conference 2002 - Poster 1.3

Session	Basic Biological Processes
Title	Novel plant specific SR proteins in Arabidopsis
Author(s)	Andrea Barta, Maria Kalyna; Sergiy Lopato, Christine Forstner, Julia Hilscher, and Zdravko Lorkovic  Institut fuer Medizinische Biochemie Vienna Biocenter Universitaet Wien Dr. Bohrgasse 9/3, A-1030 Vienna, Austria  Author email: Andrea@bch.univie.ac.at
<p><b>Abstract:</b></p> <p>A new plant specific RS- rich splicing factor, atRSZ33, with an RNA recognition motif (RRM) and two Zn finger domains has been characterized. AtRSZ33 interacted only with splicing factors in a yeast two-hybrid screen: atSRp34/SR1, an ortholog of SF2/ASF, atSRZp21 and atRSZp22 which are similar to human 9G8, four SC35-like splicing factors termed atSCL28, atSCL30, SCL30a, and atSCL33/SR33 and the orthologue of mammalian SC35, atSC35. These specific interactions, the results of expression analysis and the fact that atRSZ33 is a phosphoprotein with many features of an SR protein suggest that its main activity occurs during spliceosome assembly. Furthermore, overexpression of atRSZ33 in Arabidopsis thaliana plants showed that it changes splicing pattern of specific genes including its own mRNA. Overexpression resulted in a severe phenotype caused by increased cell expansion and changed polarization of cell elongation and division, affecting cell shape and fate. Analyses of transgenic plants showed that the observed phenotype can be partially explained by altered auxin levels.</p> <p>The four SC35-like proteins, atSCL28, atSCL30, SCL30a, and atSCL33/SR33, also seem to be specific to plants. The analysis of their expression pattern and their activities in a heterologous in vitro splicing extract will be discussed in detail.</p> <p>Furthermore, we have characterized three SR-cyclophilins, one of which contains an RRM domain, a feature not detected in animal proteins. Yeast-two hybrid results indicate that one of the cyclophilins is associated with U1 snRNP. Studies of nuclear localizations showed that the SR-cyclophilin redistributes and co-localizes with its respective binding partner.</p>	

## EPSO-Conference 2002 - Poster 1.4

Session	Basic Biological Processes
Title	The fer gene, a bHLH protein, regulates iron uptake responses in tomato
Author(s)	Petra Bauer, Zsolt Berczky, Hongyu Wang, Hong-Qing Ling, Beat Keller, Martin Ganai  Institute of Plant Genetics and Crop Plant Research (IPK) Corrensstr. 3 D-06466 Gatersleben Germany  Author email: bauer@ipk-gatersleben.de
<p>Abstract:</p> <p>Iron deficiency is among the most common nutritional disorders in plants. To cope with low iron supply, plants with the exception of the Gramineae increase the solubility and uptake of iron by inducing physiological and developmental alterations including iron reduction, soil acidification, Fe II transport and root hair proliferation (strategy I). The chlorotic tomato fer mutant fails to activate the strategy I. Here, we present a detailed characterization of the fer mutant. fer plants exhibit root developmental phenotypes upon low and sufficient iron nutrition indicating that FER acts irrespective of iron supply. Mutant fer roots are devoid of iron reductase activity and display altered iron transporter gene expression compared to wild type roots. We isolated the fer gene by map-based cloning and demonstrate that it encodes a protein containing a bHLH domain. fer is expressed in a cell-specific pattern at the root tip independently from iron supply. Our results suggest that FER may control root physiology and development at a transcriptional level in response to iron supply and thus may be the first identified plant regulator gene controlling multiple aspects of nutrient uptake.</p>	

## EPSO-Conference 2002 - Poster 1.5

Session	Basic Biological Processes
Title	New hoemotic barley mutant
Author(s)	A.Bieliuniene, D.Svegziene, V.Rancelis Department Botany and Genetics, Faculty Nature Sciences, Ciurlionio 21, Vilnius University, Vilnius 2009, Lithuania
<p>Abstract</p> <p>The investigation of flower structure in the new homeotic barley mutant</p> <p>An original collection of barley (<i>Hordeum vulgare</i>) mutants is cultivated in the Botanical Garden of Vilnius University. Some of the barley mutants in our collection are homeotic. In the flowers of tweeky spike (<i>tw</i>) mutant lodicules are ectopically converted to carpels or stamens. That peculiarity of <i>tw</i> is not strongly fixed. The flowers of mutant <i>tw</i> could be with: 1) normal lodicules; 2) one or both lodicules partly converted to stamen; 3) one or two lodicules replaced with carpels; 4) mixed conversion to both, carpel and stamen. Degree of the conversion is different in various flowers and ears of the same barley plant also. The addition stamens were smaller than normal and the number of locules in additional stamens is altered too. The second peculiarity is that in the flowers of <i>tw</i> mutants there are flowers with various numbers of flowers parts. Conversion of flowers is increasing in direction to upper part of ear. The most upper spikelets of ear become multiflorous. Phenotypical expression mutants of <i>tw</i> locus (polymorphism and phenotypical gradient in ear structure, homeosis, and other properties) let us think, that the <i>tw</i> is a novel type of the homeotic mutants with alterations of ear/ spikelet/ flowers development.</p>	

## EPSO-Conference 2002 - Poster 1.6

Session	Basic Biological Processes
Title	The investigation of flowers structure in the homeotic barley mutant tweaky spike
Author(s)	A.Bieliuniene, D.Zvingila, D.Svegziene, V.Rancelis  Department Botany and Genetics, Faculty Nature Science, Ciurlionio 21, Vilnius University, Vilnius 2009, Lithuania
<p>Abstract:</p> <p>The barley mutant tweaky spike (tw) is a new homeotic barley mutant in which flowers lodicules converted to generative organs (stamens and/or carpels). This peculiarity in tw mutant is not strongly fixed - about 30% of investigated flowers have the normal lodicules, 26.4% flowers have lodicules, which both ectopically converted to stamens, 3.8% of flowers have two carpels in additional, which were formed from lodicules. Homeosis phenomenon is accompanying by alteration number of flower organs also. Only 76.7% mutant flowers have three stamens, two lodicules and one carpel. In the other flowers the number of stamens varied from 0 to 4, the number of lodicules varied from 0 to 3, the number of carpels varied from 0 to 2. It was mark out the group (17%) of flowers, in which were only 2 stamens. Mutant tw will contribute to characterization of the floral development in barley and other monocots.</p>	

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## EPSO-Conference 2002 - Poster 1.7

Session	Basic Biological Processes
Title	Evidence for a peroxidase-dependent apoplastic oxidative burst in Arabidopsis
Author(s)	<p>Laurence V Bindschedler, Kris A Blee*, Chris Gerrish, Dewi R Davies, Frederick M Ausubel**, G Paul Bolwell</p> <p>School of Biological Sciences Royal Holloway University of London Egham, TW20 0EX U.K.</p> <p>*Dept of Biological Sciences, California State University, Chico, CA, USA. **Department of Molecular Biology, Harvard Medical School, Boston, MA 02114, USA</p> <p>Author-email: laurence.bindschedler@rhul.ac.uk</p>
<p>Abstract:</p> <p>The oxidative burst is an early response to pathogen attack leading to production of reactive oxygen species (ROS). One mechanism proposed is a three-component system involving extracellular alkalinisation, release of low molecular weight reductant(s) and cell wall peroxidase. The extent in which the apoplastic oxidative burst involving peroxidase may be responsible for H<sub>2</sub>O<sub>2</sub> generation has been investigated in Arabidopsis. In this work three systems were utilised, namely a fungal elicitation system in cell suspension cultures and leaf disks, and Arabidopsis plants transformed with a cDNA encoding the oxidative burst peroxidase from French bean in the antisense orientation. An elicitor derived from <i>Fusarium</i> induced an oxidative burst that was sensitive to peroxidase inhibitors and relatively insensitive to DPI. Reduced H<sub>2</sub>O<sub>2</sub> was detected in leaf disks from transformed plants compared to col-0 plants when treated with this elicitor. Using diaminobenzidine staining, also less H<sub>2</sub>O<sub>2</sub> was detected in antisense lines than in col-0 plants when challenged with avirulent bacteria. This was correlated with the decreased level of an anionic cell wall bound peroxidase of antisense lines, as observed on IEF activity gels. Transformed plants were much more susceptible than col-0 plants when challenged with virulent and avirulent strains of <i>Pseudomonas syringae</i> and the fungal pathogens <i>Botrytis cinerea</i> and <i>Fusarium oxysporum</i> but more resistant to the <i>Fusarium</i> toxin, fumonisin. The transgenic lines therefore resemble enhanced disease susceptibility mutants (eds) in their response. The exact contribution of apoplastic peroxidase to the oxidative burst still requires determination by identifying the endogenous peroxidase but there appears to be a significant possibility of a major contribution in addition to the NADPH oxidase proposed in other studies.</p>	

## EPSO-Conference 2002 - Poster 1.8

Session	Basic Biological Processes
Title	Transgenerational Effects in Plant Gene Silencing
Author(s)	Todd Blevins and Fred Meins  Friedrich Miescher Institute WRO-1066.1.64 Maulbeerstrasse 66 CH-4058 Basel  Author email: todd.blevins@fmi.ch
<p>Abstract:</p> <p>Post-transcriptional gene silencing (PTGS) and transcriptional gene silencing (TGS) have different implications for ontogeny (short-term development) and phylogeny (long-term evolution). If PTGS affects or induces TGS, then this could provide a mechanism for transmitting specific patterns of gene expression over many generations. We are using the monogenic <i>Arabidopsis thaliana</i> ecotype Columbia transformed line 5, which is homozygous for a green-fluorescent protein transgene (35S2-GFP), to study transgenerational effects on silencing that increase slowly with successive sexual generations. In the S3 generation (i.e., the third selfed generation of the primary transformant), 30% of mature plants showed a completely silent phenotype, which was associated with reduced GFP mRNA levels as compared to a GFP high-expressing control line. From S3 to S6 the incidence of silent plants increased gradually to 100%. Nuclear run-on transcription studies showed that transcription of 35S2-GFP decreased from S3 to S5 suggesting that TGS increases with generation. During this time, methylation of symmetric Hpa II sites (CCGG) in the transgene increased and was correlated with the increase in TGS. Treatment with 5-Azacytidine, an agent shown to cause demethylation of cytosines in DNA, inhibited silencing in later generations, reactivating GFP expression. Our results are consistent with the hypothesis that PTGS can induce transgenerational increases in transgene methylation and hence progressive TGS.</p>	

## EPSO-Conference 2002 - Poster 1.9

Session	Basic Biological Processes
Title	Abstract Ethanol-inducible expression of inverted-repeat constructs efficiently triggers conditional gene silencing in plants
Author(s)	Shuai Chen, Daniel Hofius, Uwe Sonnewald and Frederik Börnke Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK) Corrensstr. 3 06466 Gatersleben Germany
<p>Abstract:</p> <p>Down-regulation of endogenous genes via post-transcriptional gene-silencing (PTGS) is a crucial tool to assess gene function in plants. Commonly used methods to achieve repression of target genes are the use of antisense or co-suppression constructs. However, these approaches can be cumbersome since they lack to yield consistent and predictable results. Recently, considerable progress in effectively triggering PTGS has been made by using constructs designed to express dsRNA, usually in the form of self-complementary hairpin RNAs (hpRNA), which are extremely effective triggers of PTGS. However, due to the high degree of silencing which is achieved by this approach, functional analysis of genes essential for plant development is limited since viable plant might not be recovered. Moreover, constitutive gene silencing often leads to pleiotropic effects which make it difficult to directly link phenotype to gene function. To overcome this problem a system for transient silencing of candidate genes would be desirable. A number of virus-based transient gene silencing systems have been established, however, their use in physiological studies is limited since the silencing phenotype is often accompanied by mild disease symptoms and also dependent on the host plant. To circumvent these limitations we took advantage of the ethanol-inducible expression system based on the <i>alc</i> regulon from <i>A. nidulans</i> which exhibits negligible basal expression and rapid induction upon application of a non-toxic dose of the inducer ethanol. In order to determine the practicability of the system to achieve transient PTGS in transgenic plants, inverted-repeat fragments of the <i>chl</i> gene, encoding a subunit of the magnesium chelatase complex, and of the <i>gsa</i> gene, involved in chlorophyll synthesis, and of the <i>fnr</i> gene, involved in photosynthetic electron transport, were linked to the ethanol-inducible promoter. Prior to the application of ethanol transgenic tobacco plants containing the <i>alc</i> expression constructs displayed no visible phenotype as compared to the control. However, root drenching with 0.1 % ethanol resulted in development of the characteristic phenotypes within 48 – 72 h, depending on the construct. In all cases the phenotype was first visible in the youngest leaves and persisted 7 to 10 days before the phenotype was reversed in the new growth. Taken together, the ethanol-inducible system promises to be a powerful tool to dissect primary and secondary effects of gene-silencing.</p>	

## EPSO-Conference 2002 - Poster 1.10

Session	Basic Biological Processes
Title	Analysis of plant intron splicing signals and proteins using the potato invertase mini-exon system
Author(s)	John W.S. Brown, Graham Thow, Gillian Clark, Jenny Watters, Nikki Jennings, and Craig G. Simpson  Scottish Crop Research Institute Invergowrie Dundee, DD2 5DA U.K.  Author email: <a href="mailto:jbrown@scri.sari.ac.uk">jbrown@scri.sari.ac.uk</a>
<p><b>Abstract</b></p> <p>Splicing follows a two-step reaction involving recognition of internal splicing signals. In vertebrate introns, 3' splice site selection identifies a branchpoint sequence and associated, downstream polypyrimidine (pY) tract. Through analysis of plant intron mutants only two plant branchpoints have been mapped, and the branchpoint adenosine was shown to be important in splicing efficiency. Plant introns generally do not contain a pronounced pY tract but often have a U-rich sequence between a putative branchpoint and the 3' splice site. Due to the requirement for UA-richness of plant introns for efficient splicing, it has been difficult to discriminate the function of UA elements from pY tracts. The potato invertase mini-exon (9nt) is constitutively included in invertase transcripts and is dependent on strong splicing signals. The key signals that determine mini-exon inclusion are a branchpoint adenosine, an adjacent downstream U-rich region and the distance between these signals and the 5' splice site flanking the mini-exon. These strong splicing signals make this system extremely sensitive to sequence changes and have allowed the first systematic mutational analysis of plant branchpoint and pY tracts. The mini-exon signals are also able to enhance splicing of poorly spliced introns. In addition, we have recently shown that, dependent on intron sequence context, the same U-rich sequence can have dual functionality, either as a pY tract or as a U-rich intronic element.</p> <p>The key difference between plant intron splicing and that of vertebrates and yeast is the UA-richness of introns, and putative proteins which recognise these sequences. Genes encoding RNA-binding proteins with affinity for oligo U in vitro have been isolated in the Filipowicz lab. To examine the relative properties of these proteins and their possible function in splicing, we have co-transfected plasmids expressing the proteins with mini-exon mutant constructs. The over-expression of different proteins had differential effects on inclusion of the mini-exon. This assay, therefore, can distinguish between the activities of some of the UA-binding proteins suggesting that the proteins have different affinities for U-rich sequences</p>	

## EPSO-Conference 2002 - Poster 1.11

Session	Basic Biological Processes
Title	Plant small nucleolar RNA (snoRNA) gene organisation, expression and evolution
Author(s)	John W. S. Brown Scottish Crop Research Institute Invergowrie Dundee, DD2 5DA U.K.  Author email: <a href="mailto:jbrown@scri.sari.ac.uk">jbrown@scri.sari.ac.uk</a>
<p>Abstract:</p> <p>SnoRNAs are a large family of non-coding RNAs involved in RNA metabolism and gene expression in eukaryotes. They are involved in precursor ribosomal RNA (pre-rRNA) processing and RNA modification (2'-O-ribose methylation and pseudouridylation). A small number of snoRNAs are essential for rRNA cleavage reactions, but the majority act as guide RNAs to determine sites of modification. The majority determine modifications of rRNAs, but more recently novel snoRNAs have been shown to guide modification of spliceosomal snRNAs, tRNAs (in Archae) and even mRNAs (in human).</p> <p>There are two main classes of snoRNAs: box C/D snoRNAs (2'-O-ribose methylation) and box H/ACA snoRNAs (pseudouridylation). The snoRNAs associate with sets of core proteins and function as snoRNPs. The core proteins, including the methylase and pseudouridine synthase, are conserved in plants. The modifications in rRNAs are required for ribosome biogenesis and function, contributing to global stability of rRNA structure, to structural and functional interactions between ribosomal subunits, and to binding of tRNA and mRNA, thus fine tuning the translational activity of the ribosome. In this respect, plant rRNAs contain more modifications than other higher eukaryotes, which may reflect the variation in conditions under which plant ribosomes must operate. Finally, snoRNAs may also have a chaperone function to aid correct folding of the nascent rRNA transcript or stabilise tertiary structure of the rRNA.</p> <p>Plant snoRNA genes differ from those of yeast and human in a number of aspects: 1) &gt;50% of Arabidopsis genes have 2-4 gene variants in; 2) the most common gene organisation is polycistronic with up to 10 different genes, 3) entirely novel intronic polycistrons have been found in Arabidopsis and more frequently in rice, and 4) processing of pre-mRNA transcripts containing intronic snoRNAs, both polycistronic and single gene, are splicing-independent (not the case in human or yeast) pointing to different processing machinery or organisation. To date, over 450 snoRNA gene sequences (<a href="http://www.scri.sari.ac.uk/plant_snoRNA/">http://www.scri.sari.ac.uk/plant_snoRNA/</a>) from a range of plant species provide information for investigation of mechanisms of evolution of snoRNA genes and their organisation. The characterisation of snoRNAs gene organisation, expression and processing may inform analyses of other non-coding RNA families.</p>	

## EPSO-Conference 2002 - Poster 1.12

Session	Basic Biological Processes
Title	Complexity in plant signalling: The Phytochrome A network
Author(s)	Jorge José Casal IFEVA, Faculty of Agronomy University of Buenos Aires Av. San Martín 4453, 417-Buenos Aires Argentina.  Author-email: <a href="mailto:casal@ifeva.edu.ar">casal@ifeva.edu.ar</a>
<p>Abstract:</p> <p>Phytochrome A plays a key role in the perception of specific light signals modulating plant growth and development. Phytochrome A signalling can be initiated by very low fluences of light or by prolonged exposures to far-red rich light. These discrete phases of sensitivity are developmentally regulated. To search for genes that fine tune phytochrome A signalling we used both a forward genetic approach taking advantage of natural variation in Arabidopsis accessions and a reverse genetics approach based on the characterisation of Arabidopsis T-DNA insertion lines in <i>pk1</i> and <i>pk2</i> and transgenic lines overexpressing <i>PKS1</i> or <i>PKS2</i>. <i>PKS1</i> is a phytochrome kinase substrate and <i>PKS2</i> is its closest homologue.</p> <p>Recombinant inbred lines derived from the Ler x Cvi cross and <i>pk1</i>, <i>pk2</i> and <i>pk1 pk2</i> mutants were exposed to brief hourly pulses of far-red light. Several quantitative trait loci affecting hypocotyls growth and/or cotyledon unfolding were identified. One of these loci, mapping to the top chromosome 1 was assigned to previously identified natural variation at the <i>cryptochrome 2</i> gene by using near-isogenic and transgenic lines. Noteworthy, these allele-specific effects of <i>cryptochrome 2</i> were observed under pulses of far-red; i.e. a condition where <i>cryptochrome 2</i> is not predicted to be activated by light. Compared to the wild-type, <i>pk1</i> and <i>pk2</i> mutants and the overexpressors showed enhanced phytochrome A-mediated responses. However, the <i>pk1 pk2</i> double mutant showed wild-type responses. A model is proposed where normal <i>phyA</i> signalling depends on the balance between <i>PKS1</i> and <i>PKS2</i> activity, which could be mutually regulated. The advances in the test of this regulatory loop will be presented. Neither the <i>cryptochrome 2</i> allele nor <i>PKS1</i> or <i>PKS2</i> affected the less sensitive phase of <i>phyA</i> signalling requiring sustained excitation with far-red light.</p> <p>These observations will be placed in the context of recent findings showing the involvement of different domains of the phytochrome A molecule, brassinosteroids and various genetic loci in the regulation of the relationship between signal input and output for the phytochrome A network.</p>	

## EPSO-Conference 2002 - Poster 1.13

Session	Basic Biological Processes
Title	The action of enod40, a sORF-containing RNA gene, may reveal novel regulatory mechanisms in plant development
Author(s)	<p>Martin Crespi  Institut des Sciences du Vegetal, CNRS, 91198, Gif sur Yvette, France</p> <p>Anna Campalans, Arnaud Complainville, Colette Breda, Lysiane Brocard, Luc Negroni*, Adam Kondorosi and M. Crespi</p> <p>Institut des Sciences du Vegetal, CNRS, 91198, Gif sur Yvette, France and  *Proteomic Platform IFR:"Sciences du Végétal", Station de Génétique Végétale, INRA/UPS/INA, Ferme du Moulon, 91190 Gif sur Yvette, France.</p> <p>crespi@isv.cnrs-gif.fr</p>
<p><b>Abstract:</b></p> <p>mRNAs that do not contain a long open reading frame (longer than 100 amino acids; sORF-RNAs) are generally not detected by usual sequence analysis and a large number of them likely remain to be discovered. Their functions may involve the RNA molecule itself (non-codingRNAs), and/or the short ORF-encoded oligopeptides. In eukaryotes, expression studies have revealed a striking diversity of these mRNAs in many cell types from various organisms, which are induced at specific stages of development.</p> <p>The early nodulin gene enod40, coding for a 0.7 kb sORF-RNA, is expressed in the nodule primordium developing in the root cortex of leguminous plants after infection by symbiotic bacteria. Ballistic microtargeting of this gene into Medicago roots induced division of cortical cells. Translation of two sORFs (I and II of 13 and 27 amino acids; respectively) present in the conserved 5' and 3' regions of enod40 was required for this biological activity. An immunolocalization signal was obtained on root apex and nodule samples with affinity-purified antibodies against the sORF I-encoded peptide. In parallel, we have developed methodologies for the extraction of small peptides (ranging from 1.5 to 5 kDa) from plant tissues since classical methods did not reveal peptides smaller than 5 kDa. The identity of the isolated peptides is being explored by using HPLC and mass spectral analyses.</p> <p>Deletion of a Mtenod40 region between the sORFs, spanning a predicted RNA structure, resulted in significantly decreased biological activity, suggesting a role for the RNA in enod40 function. A yeast three-hybrid screening has identified putative partners of the enod40 RNA from a nodule cDNA library, which seems to interact in vivo with the enod40 RNA after co-expression in plant cells. We plan to develop various approaches to identify plant sORF-RNAs since they may reveal novel ways of gene regulation mediated by sORF-encoded peptides and RNA-protein interactions in development.</p>	

## EPSO-Conference 2002 - Poster 1.14

Session	Basic Biological Processes
Title	Determination of the influence of the genomic context on the expression of single copy b-glucuronidase genes in different transgenic plants
Author(s)	Sylvie De Buck and Ann Depicker  Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University K.L. Ledeganckstraat 35 B-9000 Gent, Belgium  Author email: sylvie.debuck@gengenp.rug.ac.be
<p><b>Abstract:</b></p> <p>In the past years, it became clear that although a gene is stably expressed especially when no repeated genes are present, repressive states can also be imposed on single copy genes. Two explanations have been proposed for the inactivation of single-copy transgenes. First, these single-copy transgenes can be recognized as non-plant sequences. Second, not mutually exclusive, single copy genes are silenced due to position effects on the basis of chromatin spreading (De Buck and Depicker, 2002, and references therein; Müller and Wassenegger, 2002).</p> <p>In order to analyze the effect of the genomic context on gene expression, the T-DNA integration position and transgene expression of single copy transgenes was determined in transgenic Arabidopsis plants. The plants were obtained after root transformation with an Agrobacterium containing a T-DNA harbouring the b-glucuronidase (<i>gus</i>) gene under control of the 35S promoter. A population of 34 transgenic plants was screened by Southern blot analysis and T-DNA fingerprinting (Theuns et al., 2002) and 11 transgenic plants containing a single T-DNA were retained for further analysis. The T-DNA/plant junctions were sequenced (Windels et al., 2001) to determine the T-DNA integration position. Gus expression was determined in 5 seedlings per transformant at two timepoints during development in homozygous and hemizygous plants. The obtained results will be discussed.</p> <p><b>References</b></p> <p>De Buck, S., and Depicker, A. (2002). Gene expression and level of expression. In Handbook of plant biotechnology. Klee, H and Christou, P. (Ed.). In press.</p> <p>Theuns, I., Windels, P., De Buck, S., Depicker, A., Van Bockstaele, E., and De Loose, M. (2002). Identification and characterisation of T-DNA inserts by T-DNA fingerprinting. <i>Euphytica</i> 123: 75-84.</p> <p>Müller, A., E., and Wassenegger, M. (2002). Control and silencing of transgene expression. In Handbook of plant biotechnology. Klee, H and Christou, P. (Ed.). In press.</p> <p>Windels, P., Taverniers, I., Depicker, A., Van Bockstaele, E., and De Loose, M. (2001). Characterization of the Roundup Ready soybean insert. <i>Eur. Food Technol</i> 213: 107-112.</p>	

## EPSO-Conference 2002 - Poster 1.15

Session	Basic Biological Processes
Title	The role of the secretory pathway in plant-pathogen interactions
Author(s)	Jurgen Denecke Centre for Plant Sciences University of Leeds Leeds LS2 9JT U.K.  Author email: <a href="mailto:j.denecke@leeds.ac.uk">j.denecke@leeds.ac.uk</a>
<p>Abstract:</p> <p>The secretory pathway of plants shares many features with the endomembrane system of yeasts and mammals, but also exhibits unique characteristics, particularly with respect to the role of the endoplasmic reticulum in protein storage, the divergent export pathways from the ER and the complexity of vacuolar compartmentalisation in plants. Recent findings from my laboratory have opened up prospects for applied biology, which are based on advanced forms of pathway engineering. The important role of the ER during stress responses in plants will be discussed and experimental results will be shown to illustrate how up-regulation of endoplasmic reticulum chaperones precedes that of the well known defense responses. This early response is required to prepare the secretory pathway for increased protein synthesis and delivery of defense-related proteins to the vacuoles or the extracellular matrix. PR protein synthesis will only commence when BiP levels have reached a threshold level adequate for the handling of the de-novo synthesized transcripts. Salicylic acid-mediated induction of BiP occurs via a signal transduction pathway that is independent of the route that mediates PR1 induction. Moreover, it is shown that overexpression of the chaperone BiP accelerates the salicylic acid mediated defense-response, because it exhibits an accelerated PR1 induction and also reduces in planta proliferation of the plant pathogenic bacterium <i>Erwinia carotovora</i>.</p> <p>Secretory pathway engineering may thus provide an environmentally benign crop protection strategy which does not rely on the use of chemicals in the field, relies on the acceleration of a natural defense response, and may provide a good example to promote public acceptance of plant biotechnology.</p>	

## EPSO-Conference 2002 - Poster 1.16

Session	Basic Biological Processes
Title	The Arp2/3 complex is required for cell differentiation and cell elongation in <i>Physcomitrella patens</i>
Author(s)	Andrija Finka, Jean-Pierre Zrýd and Didier G. Schaefer  Laboratory of Plant Cell Genetics Institute of Ecology University of Lausanne Lausanne, Switzerland  Author email: afinka@ie-pc.unil.ch
<p>Abstract:</p> <p>In eukaryotic cells, cell polarity is considered as a primary event determining cell function and developmental fate. The actin cytoskeleton plays an essential role in the establishment of cell polarity, being involved both in the local reinforcement of the spatial information and in the final reorganization of the cell. The ARP2/3 complex is directly involved in these processes: it choreographs the formation of branched actin networks by regulating actin polymerisation and nucleation. In plants, genes and ESTs for the seven subunits of the ARP2/3 complex have been identified but the role of the complex in plant morphogenesis and cell differentiation is still poorly understood.</p> <p>We have isolated and characterized the genomic sequence of the Arp3 gene from the moss <i>Physcomitrella patens</i>. It encodes a predicted 425 amino acid peptide that shares 76% and 56% amino acid identities with the <i>Arabidopsis</i> and <i>S. pombe</i> homologues, respectively. Arp3 knock-out moss strains were generated by gene targeting. These strains display a complex developmental phenotype: the filamentous protonema is composed exclusively of chloronemal cells that display a length to width ratio of 1:1 whereas this ratio is 7:1 in WT cells. Caulonemal cells are absent from the protonema: consequently buds directly differentiate from chloronemata to form stunted gametophores with shorter internodes but carrying normally differentiated leaves. Rhizoids, which differentiate from the basis and the stem of the gametophore in WT, are absent in Arp3 knock-out strains. Furthermore, caulonema and rhizoid differentiation could not be induced by auxin, suggesting that the Arp3 gene is essential for the differentiation of this cell type. Finally, tropic responses are apparently not impaired in Arp3 knock-out since protonemal cells display normal photo- and polarotropic responses whereas negative gravitropism can be observed in gametophores grown in darkness. That this phenotype is associated with alteration of the actin cytoskeleton is further supported by the analysis of the Arp3 knockout mutation in GFP-talin moss strains in which the actin network is labelled in vivo with GFP.</p> <p>This is the first report of an Arp3 knockout in plant. Our data support the essential role of the actin network in plant cell differentiation and growth and suggest that this function could be cell specific. Further studies will certainly clarify the relation between actin and plant morphogenesis in <i>Physcomitrella</i>.</p>	

## EPSO-Conference 2002 - Poster 1.17

Session	Basic Biological Processes
Title	Novel plant protein phosphatases interacting with SNF1-like kinases, regulators of carbon metabolism
Author(s)	Tony Fordham-Skelton CLRC, Daresbury Laboratory Keckwick Lane Warrington Cheshire WA4 4AD, U.K.
<p><b>Abstract</b></p> <p>The completion of the Arabidopsis genome project has revealed numerous novel proteins implicated in controlling signal transduction events. Several of these proteins with unique domain organisations have been targeted for structure - function studies on the assumption that they may be involved in processes specific to plants. One of these proteins, AtPTPKIS1, is a dual-specificity phosphatase belonging to the protein tyrosine phosphatase (PTP) superfamily. This phosphatase also contains a KIS domain (kinase interaction sequence) and is predicted to interact with SNF1-like kinases, conserved regulators of plant responses to metabolic, hormonal and stress signals. These kinases are believed to be complexes in vivo with at least two other regulatory subunits which have been conserved from yeast and animals to plants. AtPTPKIS1 has a unique domain organisation and interacts with the Arabidopsis SNF1-like kinase AKIN11 in the yeast two hybrid system and in vitro in a GST "pull-down" assay (Plant Journal [2002] 29: 705 - 715). Publicly available microarray data indicates that AtPTPKIS1 expression is reciprocally regulated during the photoperiod when compared with the expression of AKINb1; a "canonical" regulatory KIS domain protein which also forms complexes with SNF1-like kinases. Taken together these data suggest a role for AtPTPKIS1 in signalling pathways involving SNF1-like kinases through the ability to form "alternate" complexes other than those possible in either yeast or animals. Arabidopsis (and other plants) also contains further related KIS domain proteins, absent in other eukaryotes, which could contribute to specificity in SNF1-like kinase signalling. Progress towards determining the structures of these novel proteins will also be described.</p>	

## EPSO-Conference 2002 - Poster 1.18

Session	Basic Biological Processes
Title	Physcomitrella patens - a new model plant to analyse the abiotic stress response
Author(s)	<p>Kerstin Kröger, Hauke Holtorf, Ralf Reski, Wolfgang Frank</p> <p>Lehrstuhl für Pflanzenbiotechnologie Albert-Ludwigs-Universität Freiburg Schänzlestr. 1 79104 Freiburg Germany</p> <p>Author's email: wolfgang.frank@biologie.uni-freiburg.de</p>
<p>Abstract:</p> <p>Some representatives of the mosses are well known to tolerate a wide range of abiotic stresses. We have chosen the moss <i>Physcomitrella patens</i> which has recently become a model system to study gene function by reverse genetics achieved by a highly efficient mechanism of homologous recombination to analyse genes involved in the adaptation to abiotic stress. The identification of such genes in combination with the generation of targeted knockout plants will make <i>Physcomitrella</i> a suitable system to analyse the molecular events during the abiotic stress response. To obtain information about the capability of <i>Physcomitrella</i> to cope with different abiotic stresses the moss has been subjected to various abiotic stress treatments. The degree of tolerance against osmotic, salt and dehydration stress is remarkably high compared to other model plants like <i>Arabidopsis</i>. The characterisation of the underlying molecular mechanisms is approached by different strategies. To identify molecular markers of the stress response we make use of a comprehensive EST database. We have performed macroarray experiments with a set of 50 cDNAs and were able to identify genes with putative regulatory as well as protective functions which were specifically induced by dehydration. Among these we found genes displaying homology to stress-related genes from other species indicating that some of the adaptive mechanisms have been evolutionarily conserved. Furthermore, we will focus on the expression profiling of cDNAs which could not be annotated and may present novel genes. We have also started a differential display approach to clone stress-related genes from <i>Physcomitrella</i> which may not be represented in the EST database. The functional analyses of the identified genes by the generation of targeted knockout plants will focus on those genes which may encode proteins of the signal transduction machinery. The results of the expression profiling experiments and the analyses of the knockout plants will be discussed.</p> <p>Acknowledgements: The cDNA libraries and the EST database were created in a joint project with BASF Plant Science GmbH.</p>	

## EPSO-Conference 2002 - Poster 1.19

Session	Basic Biological Processes
Title	The WEX gene encoding an RNAase D-like protein is required for PTGS in Arabidopsis
Author(s)	E. Glazov*, J. Z. Levin**, F. Meins Jr.*  *Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland. **Syngenta Biotechnology, Inc, 3054 Cornwallis Rd., Research Triangle Park, NC 27709.  Author email: glazov@fmi.ch
<p>Abstract:</p> <p>Posttranscriptional gene silencing (PTGS) or the related phenomenon RNA interference, have been described in many organisms including plants, fungi, <i>C. elegans</i>, <i>Drosophila</i> and mammals. Comparative studies of genetic mutants suggest that some proteins required for PTGS have been highly conserved in evolution. We identified an Arabidopsis Werner Syndrome-like exonuclease (WEX) gene, which is related to mut-7 required for PTGS in <i>C. elegans</i> and encodes an RNase D domain most similar to that in human Werner Syndrome protein. The T-DNA mutant of this gene, <i>wex-1</i>, showed greatly reduced expression of WEX mRNA. This mutant was crossed with the indicator line 8z2 carrying a green fluorescent protein (GFP) reporter gene driven by the cauliflower mosaic virus 35S RNA promoter (P35S), which consistently exhibited PTGS. F3 progeny homozygous for both <i>wex-1</i> and P35S-GFP did not exhibit PTGS. PTGS was restored in homozygous <i>wex-1</i> plants ectopically expressing a P35S-WEX transgene. These results together with expression studies of WEX mRNA in mutant and wild-type plants strongly suggest that WEX is required for PTGS in Arabidopsis. Although catalytic activity of WEX has not as yet been confirmed biochemically, we speculate that WEX helps degrade the products of endonucleolytic cleavage by mechanisms that could involve exosomes.</p>	

## EPSO-Conference 2002 - Poster 1.20

Session	Basic Biological Processes
Title	A role of TRH1 potassium transporter in plant mineral nutrition.
Author(s)	Alexander Grabov Department of Agricultural Sciences Imperial College Wye Campus Wye Ashford Kent TN25 5AH UK  Author email: a.grabov@ic.ac.uk
<p><b>Abstract:</b></p> <p>Potassium is a key mineral nutrient which affects many aspects of crop physiology. Balanced potassium supply is essential for utilization of other mineral nutrients and improvement of plant resistance to adverse environmental factors, pests and diseases. As potassium is continuously removed from soil solution due to the uptake by plants and leaching it must be replenished through the soil fertilisation. Intensive use of fertilisers, however, has a highly negative impact on the environment and increase cost of agricultural production. Development of crop with improved ability to uptake potassium from soils with a low K<sup>+</sup> status, would, therefore benefit sustainable development of agricultural systems. This goal can not be achieved without identification of molecular mechanisms of high affinity K<sup>+</sup> uptake system in plants.</p> <p>To date it is well documented that there are two types of K<sup>+</sup> uptake systems in plant root. While low affinity transport mediates K<sup>+</sup> uptake in soils with high contents of K<sup>+</sup>, the high affinity transporters are important for plant growth at conditions of mineral deficiency.</p> <p>Uptake of potassium from soils with low concentration of this nutrient can also be facilitated by improved root architecture and, particularly, root hairs are known to be of extreme importance for mineral acquisition. Development of this tubular outgrowths enables plants to exploit new undepleted soil layers with a net result similar to activation of a high affinity uptake system.</p> <p>It has been recently found that tiny root hair 1 mutation blocks transition between initiation and elongation of root hairs in Arabidopsis indicating that TRH1 gene product is a key regulator of root hair development. Interestingly, TRH1 gene encodes a potassium transporter belonging to KT/KUP/HAK gene family. We found that in Arabidopsis seedlings, TRH1 activity is accountable for approx.40% of total potassium accumulation when plants are exposed to the condition of potassium starvation. Genes of KT/KUP/HAK family are presented in all plants studied so far. Given the high impact of TRH1 gene product on K<sup>+</sup> accumulation and root architecture we suggest that activities of some members of KT/KUP/HAK in agriculturally important plants are crucial for adaptation to low level of mineral nutrition. Transport properties and mechanisms of TRH1 regulation will be discussed in a context of development of crop less dependent on fertilisers.</p>	

## EPSO-Conference 2002 - Poster 1.21

Session	Basic Biological Processes
Title	Understanding Pathogenicity, Susceptibility, and Resistance in Important Bacterial-Plant Pathogen Interactions
Author(s)	M. Arlat, C. Balagué, C. Boucher, S. Génin, N. H. Grimsley, T. Kroj, Y. Marco, D. Roby, A. Trigalet, and J. Vasse.  LBM RPM, CNRS-INRA, B.P. 27 Auzeville, 31326 Castanet-Tolosan FRANCE  Author email: <a href="mailto:grimsley@toulouse.inra.fr">grimsley@toulouse.inra.fr</a>
<p><b>Abstract:</b></p> <p>We are building up integrated and detailed knowledge about the molecular mechanisms of pathogenesis of two wide host range plant pathogens which have different infection strategies, <i>Ralstonia solanacearum</i>, a root pathogen, and <i>Xanthomonas campestris</i>, a foliar pathogen, by studying the functions of both the bacterial (analysis of the diversity and fluidity of completely sequenced genomes, host specificity, regulatory networks, pathogenicity effectors) and plant partners (pathogen perception, hypersensitive response, resistance, susceptibility, disease). The genome of <i>R. solanacearum</i> was sequenced and predicted to encode 5120 proteins (Salanoubat, M., et al. <i>Nature</i> 415, 497-502 [2002]). The two replicons that constitute the genome, a chromosome and a megaplasmid of 3.7 and 2.1 Mb, respectively, have a mosaic structure with 7 % of sequences probably originating from horizontal gene transfer. Certain genomic rearrangements in the pathogen may be provoked during the interaction with plants. Genome analyses predict that about 200 genes encode candidate pathogenicity functions, including cell attachment determinants, hydrolytic enzymes, plant hormone metabolism components, toxins, resistance to oxidative stress and over 40 effector proteins predicted to be translocated through the type III-protein secretion pathway encoded by <i>hrp</i> genes. The functions of these candidate genes are currently being assessed by classical and molecular approaches. Preliminary results suggest that there may be functional redundancy between different effectors and we are now developing new approaches to study effectors by their transient expression in plant cells. Plant proteins that may be targeted by such effectors are currently being identified using the yeast 2-hybrid system.</p> <p>Genetic, molecular and genomic approaches show that plant resistance in our pathosystems is controlled by both polygenic (Mangin, B., et al. <i>Genetics</i> 151, 1165-1172 [1999]) and single-gene traits, depending on bacterial strains and host plants, and in <i>Arabidopsis</i> these studies have resulted in the identification of key genes such as <i>RRS1</i>, governing resistance to <i>R. solanacearum</i>, (Deslandes, L., et al. <i>P.N.A.S. USA</i> 99, 2404-2409 [2002]) or <i>AtMYB30</i>, a plant regulatory protein involved in response to <i>X. campestris</i> pv. <i>campestris</i> and other important plant pathogens (Vaillau, F., et al. <i>P.N.A.S. USA</i> 99, 10179-10184 [2002]).</p>	

## EPSO-Conference 2002 - Poster 1.23

Session	Basic Biological Processes
Title	RNA transport in plants
Author(s)	Manfred Heinlein Friedrich Miescher Institute for Biomedical Research Maulbeerstrasse 66 CH-4058 Basel Switzerland  Author Email: heinlein@fmi.ch
<p>Abstract:</p> <p>Studies in higher plants have revealed the existence of RNA species that travel cell-to-cell and through the vasculature to serve as signaling molecules in plant development and gene silencing. RNA viruses interact with the RNA transport machinery to spread their genomes from cell-to-cell. This interaction is dependent on virus-encoded movement proteins (MP). We use Tobacco mosaic virus (TMV) and its MP to study the cellular mechanism by which RNA molecules are targeted to plasmodesmata (Pd) for transport into adjacent cells. Microtubules (MT) appear to play a key role, although the particular function of MP-interacting MTs remains to be shown. Despite that MP:GFP accumulates on microtubules during later stages of infection, the functional MP:MT complex must occur early, in cells at the leading front of infection. Microscopy of these cells revealed the presence of MP:GFP-containing granules moving along almost invisible, MP-associated tracks. We propose that the granules are vRNPs that are transported along microtubules. Our biochemical approaches indicate that MP binds to microtubules through direct interactions with tubulin. E.coli-purified MP(His6) binds tubulin, microtubules and RNA in vitro and is now used for affinity isolation of novel MP-interacting host factors.</p>	

## EPSO-Conference 2002 - Poster 1.24

Session	Basic Biological Processes
Title	Towards the characterisation of heavy metal tolerance in <i>Thlaspi caerulescens</i>
Author(s)	<p>Pierre Czernic<sup>1*</sup>, Stéphane Mari<sup>1*</sup>, Katia Pianelli<sup>1*</sup>, Véronique Vacchina<sup>2</sup>, Laurence Marquès<sup>1</sup>, Ryszard Lobinski<sup>2</sup> and Michel Lebrun<sup>1§</sup></p> <p><sup>1</sup>: BPMP, UMR5004, Université Montpellier2, Place E. Bataillon, 34095-Montpellier cedex5, France</p> <p><sup>2</sup>: LCABIE, UMR5034, Hélioparc, 2 Av. Prof. Angot, 64053-Pau, France</p> <p>*these authors contributed equally to this work</p> <p>corresponding author: <a href="mailto:lebrun@univ-montp2.fr">lebrun@univ-montp2.fr</a></p>
<p>Abstract:</p> <p>Metal hyperaccumulator plant species constitute ideal model systems to study metal accumulation and tolerance mechanisms. Except few examples concerning metal uptake systems and synthesis of metal chelators, the molecular basis of metal homeostasis is still under documented.</p> <p>Strategies based on yeast complementation by plant cDNA libraries to identify genes involved in resistance to various abiotic stresses have already been described. The probability to find a particular gene is influenced, however, by the starting material, e.g. : desiccated organs to search for osmotic stress tolerance. We decided, therefore, to rely on biodiversity by choosing a plant naturally adapted to metalliferous soils which belongs to the <i>Brassicaceae</i> family and is phylogenetically close to <i>Arabidopsis thaliana</i>: <i>Thlaspi caerulescens</i>. Indeed, this plant is not only able to grow on toxic soils but also to accumulate high levels of metals in its tissues. These findings suggest the development of resistance mechanisms at the cellular level within these tissues.</p> <p>Our results indicate that <i>T. caerulescens</i> is not only able to accumulate nickel to high level in its aerial part when grown hydroponically but also exhibits nickel resistance at the cellular level as demonstrated by protoplast survey assays.</p> <p>A cDNA library from <i>T. caerulescens</i> leaves was constructed in an expression vector and screened for its ability to render yeast cells tolerant against toxic nickel concentration. Isolated cDNAs will be presented and the involvement of their product in metal tolerance will be discussed.</p>	

## EPSO-Conference 2002 - Poster 1.25

Session	Basic Biological Processes
Title	Alterations of the gene expression patterns in plants infected with the flavum strain of tobacco mosaic virus
Author(s)	Kirsi Lehto, Jean-Baptiste Hiriart, Mikko Tikkanen and Eva-Mari Aro  Department of Plant Physiology and Molecular Biology University of Turku FIN-20014 Turku Finland  Author email: klehto@utu.fi
<p>Abstract:</p> <p>The Flavum strain of Tobacco mosaic virus (TMV) has been initially characterized as a strikingly severe, temperature sensitive strain of the wild type TMV. In the fully expanded leaves this strain causes severe yellowing, and in the systemically infected apical leaves it causes mosaic of dark green, and of very severely chlorotic light tissues. These different tissue types also show distinct cytological alterations: The cell nuclei in the dark green apical tissues are strongly enlarged and granular, and the cellular membranes are malformed. The cells of the chlorotic apical tissues contain only very few albeit normal looking chloroplasts, suggesting that in these tissues the chloroplast biogenesis has been prevented by the infection. Chloroplasts of the fully expanded, chlorotic leaves show very severe malformation, lack of thylakoid membranes and accumulations of unidentified granular matter. Analysis of the protein composition of the thylakoid membranes indicated that the P<sub>I</sub> SII reaction centres and the 33 kDa protein of the OEC are specifically depleted in the mature tissues infected by the virus. No major changes were seen in the thylakoid protein composition of the systemically infected apical leaves. A number of the different photosynthesis- or stress response related gene products were analysed also on the mRNA level. Particularly, the transcripts for the LhcB1, LhcB2, CES, ChlD, ChlI, ChlH, PorB, and RbcS were strongly reduced in the mature chlorotic leaves, while AlaD, CS, and Fche were increased and GSA remained constant, as compared to the healthy control leaves. These results indicate that the expression of different genes is very differently affected by the infection-related stress, or by the break-down of the chloroplast ultrastructure, and inhibition of normal PSII turnover.</p>	

## EPSO-Conference 2002 - Poster 1.26

Session	Basic Biological Processes
Title	Towards the positional cloning of <i>DMI3</i> , a gene of <i>Medicago truncatula</i> involved in Nod factor transduction, nodulation, and mycorrhization
Author(s)	Julien Lévy <sup>1</sup> , Cécile Bres <sup>1</sup> , Frédéric Debelle <sup>1</sup> , Olga Kulikova <sup>2</sup> , Françoise de Billy <sup>1</sup> , Charles Rosenberg <sup>1</sup> , Ton Bisseling <sup>2</sup> , and Jean Dénarié <sup>1</sup> <sup>1</sup> Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, CNRS-INRA UMR215, BP27, 31326 Castanet-Tolosan Cedex, France; <sup>2</sup> Laboratory of Molecular Biology, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands.
<p>Abstract:</p> <p>Rhizobia are soil bacteria which elicit on their specific leguminous hosts the formation of new organs, called nodules, in which they fix nitrogen. The genetic analysis of the bacterial partner showed that the establishment of this symbiosis implies the synthesis by <i>Rhizobium</i> of extracellular signals, the Nod factors. These Nod factors induce on the host-plant many of the response elicited by the bacterial symbionts.</p> <p>We have performed a genetic analysis to dissect the mechanisms controlling Nod factor perception and signal transduction in the model legume <i>Medicago truncatula</i>. Three genes (<i>DMI1</i>, <i>DMI2</i>, <i>DMI3</i>) have been identified, which are involved both in the early steps of Nod factor signal transduction pathway and in the establishment of the endomycorrhizal symbiosis (Catoira et al, Plant Cell 12:1647-1666). Contrary to <i>DMI1</i> and <i>DMI2</i>, <i>DMI3</i> is not required for the induction of calcium spiking in root hairs, which is a very early response to Nod factors. <i>DMI3</i> is therefore likely to act downstream of <i>DMI1</i> and <i>DMI2</i> in the Nod factor signal transduction pathway (Wais et al. PNAS, 97:13407-13402).</p> <p>In order to better understand its function, we have started the positional cloning of <i>DMI3</i>. AFLP markers closely linked to <i>DMI3</i> have been identified by bulk segregant analysis, using a F2 mapping population. By testing some of these markers on the population used to construct the <i>M. truncatula</i> genetic map (Thoquet et al, BMC Plant Biology 2:1), we showed that <i>DMI3</i> is located on chromosome 8. Fine mapping experiments indicated that the two closest markers to <i>DMI3</i> are located at 1 cM on one side and 2 cM on the other side. In order to estimate the physical distance between these markers, the corresponding BAC clones were isolated and used as probes in high resolution FISH experiments performed on pachytene chromosomes (Ané et al, MPMI accepted for publication). A contig of BACs spanning this region is currently being constructed. A fine genetic analysis will allow a more precise localization of the <i>DMI3</i> locus, and the identification of candidate genes.</p> <p>Grafting experiments have been performed which showed that the nodulation phenotype of the three <i>dmi</i> mutants is determined at the root level. Thus it will be possible to use the rapid transformation system of <i>M. truncatula</i> by <i>Agrobacterium rhizogenes</i> to test the ability of different candidate genes to restore nodulation in the <i>dmi3</i> mutant.</p>	

## EPSO-Conference 2002 – Poster 1.27

Session	Basic Biological Processes
Title	Expression profiling analysis on plant phosphatidylinositol signaling pathway via cDNA chips
Author(s)	Wen-Hui Lin, Re Ye, Hui Ma, Zhi-Hong Xu and Hong-Wei Xue  State Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and Partner Group of Max-Planck-Institute of Molecular Plant Physiology on "Plant Molecular Plant Physiology and Signal Transduction", Shanghai 200032, P. R. China  Author email: lin@mpimp-golm.mpg.de; hwxue@iris.sipp.ac.cn
<p>Abstract:</p> <p>Phosphatidylinositol (PI) metabolic pathway was shown involved in many plant developmental processes including gene expression, cell response to environmental factors, function of hormones and so on. Previous studies have revealed the presence of multigene families. Through the detailed search on arabidopsis genome, around 76 mRNA clones encoding the isoforms involved in PI signaling pathway were detected, which belonged to different gene families including PI synthase, PI-phosphate kinase, phospholipase, inositol polyphosphate phosphatase and so on. Expression profiles of the relevant genes were then analyzed via cDNA chips. Results showed that PI pathway, especially different isoform of proteins, involved in multiple developmental procedures and regulated on different level. The results will provide informative hints for the function of relevant proteins.</p>	

## EPSO-Conference 2002 - Poster 1.28

Session	Basic Biological Processes
Title	Phosphoinositide specific – phospholipase C activity in tobacco cell culture and its role in signal transduction
Author(s)	I. Machackova, S. Zdarova, K. Nokhrina, O. Valentova, J. Martinec Institute of Experimental Botany, Academy of Science of the Czech Republic, Rozvojova 135, 165 02 Praha 6, Czech Republic Author mail: machackova@ueb.cas.cz
<p>Abstract:</p> <p>Phosphoinositide specific phospholipase C (PI-PLC) is one of the key molecules in signal transduction pathways in animals as well as in plants. It operates as a part of the phosphoinositide signalling system that transduces signals from the extracellular space to the cell. The enzyme catalyses hydrolysis of lipid phosphatidylinositol-4,5-bisphosphate into inositol-1,4,5-trisphosphate and diacylglycerol, both well-known second messengers.</p> <p>The subject of the presented work was biochemical characterization of PI-PLC of tobacco cell culture and study of the possible role of PI-PLC in biotic and abiotic stresses. The effect of the plant hormones auxins and cytokinines on PI-PLC was also investigated.</p> <p>The highly purified plasma membrane was prepared by phase partitioning in an aqueous PEG/dextran polymer two-phase system from tobacco suspension culture VBI-0. PI-PLC activity was determined using radiolabelled PI(4,5)P<sub>2</sub> as substrate. Products of enzymatic reaction were analyzed by HPLC. The enzyme was activated by micromolar Ca<sup>2+</sup> concentrations and its pH optimum was in the range from 6.4 to 6.9. These values slightly differ from the values determined for another tobacco cell culture BY-2. We did not find any influence of auxins (IAA, NAA) and cytokinin (iP) on PI-PLC activity in vitro.</p> <p>As an alternative approach we used fluorescently labelled PI(4,5)P<sub>2</sub> as substrate and whole cell set-up. First we showed that both radiolabelled and fluorescent substrate give comparable results in experiments in vitro with PI-PLC specific inhibitor U73122. Then we studied incorporation of fluorescent PI(4,5)P<sub>2</sub> to cell membranes and found that within 5 min the substrate is preferentially incorporated into plasma membrane. The fluorescent method was further verified in an experiment with osmotic stress. The results were in agreement with previously published data. Using this fluorescent method, the influence of salicylic acid, benzothiadiazol, NAA and iP on tobacco PI-PLC was investigated.</p> <p>This work was supported by the grant no. 522/00/1332 of GACR and project no. LN00A081 of MSMT CR.</p>	

## EPSO-Conference 2002 - Poster 1.29

Session	Basic Biological Processes
Title	Polar Localization of Potyviral Genome Linked Protein VPg in Virions and Its Phosphorylation with Host Kinases
Author(s)	<p>Pietri Puustinen<sup>1</sup>, Minna-Liisa Rajamäki<sup>2,3</sup>, Konstantin Ivanov<sup>1</sup>, Jari P.T. Valkonen<sup>2,3</sup>, and Kristiina Mäkinen<sup>1*</sup></p> <p><sup>1</sup>Institute of Biotechnology, Viikki biocenter, PO Box 56, FIN-00014 University of Helsinki, Finland; <sup>2</sup>Department of Plant Biology, Genetics Centre, SLU, PO Box 7080, S-750 07 Uppsala, Sweden; <sup>3</sup>Department of Applied Biology, PO Box 27, FIN-00014 University of Helsinki, Finland * Presenting author</p> <p>Author email: kristiina.makinen@helsinki.fi</p>
<p>Abstract:</p> <p>The multifunctional genome-linked protein (VPg) of Potato virus A (PVA; genus Potyvirus) was phosphorylated in vitro as a part of the virus particle by a cellular kinase activity from tobacco. Immunoprecipitation, immunolabeling and immunoelectron microscopy experiments showed that VPg is exposed at one end of the virion and thus it is accessible to protein-protein interactions. Substitution Ser185Leu at the C-proximal part of VPg reduces accumulation of PVA in inoculated leaves of the wild potato species <i>Solanum commersonii</i> and delays systemic infection. This is not observed in tobacco plants. Our data show that kinase(s) of <i>S. commersonii</i> can differentially recognize the VPg containing either Ser or Leu at position 185, whereas both forms of VPg are similarly recognized by tobacco kinase(s). In conclusion, our data implicate that the virion-bound VPg may interact with host proteins and that the VPg-mediated functions may be regulated by phosphorylation during the infection cycle of potyviruses.</p>	

## EPSO-Conference 2002 - Poster 1.30

Session	Basic Biological Processes
Title	The buckwheat metallothionein-like protein-structure of the gene and proposed function
Author(s)	J.M. Brkljacic, J.T. Samardžic, D. Majic, M.M. Konstantinovic and V.R. Maksimovic Laboratory for Plant Molecular Biology Institute of Molecular Genetic and Genetic Engineering Vojvode Stepe 444a, P.O Box 446 11 000 Belgrade, Yugoslavia
<p><b>Abstract</b></p> <p>Metallothioneins (MTs) are low molecular weight, cysteine-rich proteins with high metal-binding capacity. Broad distribution among animals, eukaryotic microorganisms, certain prokaryotes and plants suggests common global physiological role in metal homeostasis. However, specific functions in different developmental and stress conditions are still unclear and remain to be elucidated. Although a number of cDNA clones coding for MT-like proteins were isolated from different plant species, a small number of corresponding genomic clones as well as purified proteins are available.</p> <p>In our previous work, we isolated cDNA clone coding for metallothionein-like protein from developing buckwheat seed. It was also shown that Zn and Cu ions could regulate expression of buckwheat MT gene. In order to isolate buckwheat MT genomic clone and to analyze corresponding 5' regulatory region, specialized subgenomic library was constructed, using PCR approach.. Sequence of the isolated buckwheat genomic clone was completely concordant with cDNA sequence, but interrupted with two introns and contained 655bp long 5' regulatory region. Computer analysis of that region, revealed 4 putative TATA boxes, 4 putative MREs, I-box, ABRE, as well as elements for binding Myb-like protein, Arabidopsis homeobox protein 1 and Dof1 transcription factor. Functional analysis of the promoter region will allow us to conclude more about regulation of buckwheat MT gene expression, and finally about protein function in development and stress conditions.</p> <p>The proposed function of MT protein in buckwheat seed could be discussed regarding the stability of storage proteins in developing and dormant seed. The mechanism of storage protein hydrolysis at the onset of germination is double step process, which starts with metalloprotease present as a complex with inhibitor in protein bodies. The divalent cations compete with Zn in the enzyme molecule for binding to the active site of the inhibitor and thus destabilize the enzyme-inhibitor complex, causing its dissociation, and/or prevention of its formation. In that sense, MT with its high metal binding capacity could be involved in regulation of cation concentration in developing buckwheat seed.</p>	

## EPSO-Conference 2002 - Poster 1.31

Session	Basic Biological Processes
Title	New components involved in homologous recombination
Author(s)	J. Molinier, E.J. Oakeley, O. Fritsch, D. Schürmann, I. Kovalchuk and B. Hohn  Friedrich Miescher-Institute Maulbeerstrasse 66 Basel CH-4002 Switzerland.
<p>Abstract:</p> <p>Homologous recombination is an important process in meiosis and during somatic development. This process is involved in both genome flexibility, which is important for evolution, and genome stability by participating in DNA repair processes.</p> <p>Although it was clearly shown that a variety of environmental factors, such as UV, pathogens, heavy metals, affect the frequency of somatic recombination events, little is known about the regulatory cascades involved and their connections leading to the recombination process. To address this question, we combined genetic and profiling approaches.</p> <p>Using homologous recombination as a marker for genome dynamics, a mutant collection of <i>Arabidopsis thaliana</i> was established and screened for altered recombination phenotypes in the absence of external stresses. Somatic homologous recombination was monitored by using a reporter line mutagenised by T-DNA activation tagging. Dominant recombination-up phenotypes were isolated and characterised.</p> <p>In a complementary approach global changes in gene expression of wild type plants under particular stress conditions known to induce HR were investigated. The influence of DNA-damaging agents (UV-C and bleomycin [BLM]) or pathogen attack (xylanase) on cellular metabolism and DNA integrity was studied using <i>Arabidopsis</i> microarrays. A comparison between each of the treatments allowed us to define genes specifically involved in response to a particular treatment and the genes that are commonly regulated in abiotic and biotic stress. In addition the expression profile of several of the recombination-up mutants was compared to the previously established database.</p> <p>Using and combining these two approaches we identified new plant components involved in the regulatory pathways related to homologous recombination and in the recombination process itself.</p>	

## EPSO-Conference 2002 - Poster 1.32

Session	Basic Biological Processes
Title	Ppi1, a novel interaction partner for the plasma membrane H <sup>+</sup> -ATPase of <i>Arabidopsis thaliana</i>
Author(s)	<p>Piero Morandini</p> <p>Dipartimento di Biologia "L. Gorini" Sezione di Fisiologia e Biochimica delle Piante, Via Celoria 26, 20133 Milan, Italy</p> <p>Author email: <a href="mailto:piero.morandini@unimi.it">piero.morandini@unimi.it</a></p>
<p>Abstract:</p> <p>Using the two-hybrid technique we identified a novel protein whose N-terminal 88 amino acids (aa) interact with the C-terminal regulatory domain of the plasma membrane (PM) H<sup>+</sup>-ATPase from <i>Arabidopsis thaliana</i> (aa 847-949 of isoform AHA1). The corresponding gene has been named Ppi1 for Proton pump interactor 1. The encoded protein is 612 aa long and rich in charged and polar residues except for the extreme C-terminus, where it presents a hydrophobic stretch of 24 aa. Several genes in the <i>A. thaliana</i> genome and many ESTs from different plant species share significant similarity (50-70% at the aa level over stretches of 200-600 aa) to Ppi1. The PPI1 N-terminus, expressed in bacteria as a fusion protein with either GST or a His-tag, binds the PM H<sup>+</sup>-ATPase in overlay experiments. The same fusion proteins as well as the entire coding region fused to GST stimulate H<sup>+</sup>-ATPase activity. The effect of the His-tagged peptide is synergistic with that of fusicoccin (FC) and of tryptic removal of a C-terminal 10 kDa fragment. The His-tagged peptide binds also the trypsinized H<sup>+</sup>-ATPase. Altogether these results indicate that PPI1 N-terminus is able to modulate the PM H<sup>+</sup>-ATPase activity by binding to a site different from the 14-3-3 binding site and located upstream of the trypsin cleavage site. We are isolating knock out plants for both Ppi1 and 2 genes in order to identify their physiological role.</p> <p>Morandini et al (2000) <i>Plant J.</i> 31(4), 487-497.</p>	

## EPSO-Conference 2002 - Poster 1.33

Session	Basic Biological Processes
Title	Interactions between FIE and different SET domain polycomb proteins, mediate reproductive and vegetative developmental programs in Arabidopsis
Author(s)	Nir Ohad, Aviva Katz, Moran Oliva, Assaf Mosquna, Naomi Ravid and Ofir Chakim. Department of Plant Sciences Tel-Aviv University Tel Aviv 69978 Israel
<p>Abstract:</p> <p>A lesion in the FERTILIZATION-INDEPENDENT ENDOSPERM (FIE) gene induces the replication of the central cell, located within the female gametophyte, leading to the formation of endosperm and activation of fruit development without fertilization. FIE encodes a WD40 polycomb (PcG) protein. In insects and mammals PcG protein complexes control gene transcription via the modulation of chromatin structure thus mediating specific developmental programs. Previously we have shown that FIE interacts in-vitro with MEDEA (MEA), an additional SET domain PcG protein, which display autonomous endosperm development when mutated. This evidence supports our hypothesis that FIE may form a complex with PcG proteins within the central cell. Prior to fertilization such complexes prevent the expression of particular genes. In wild type ovules, fertilization leads to de-repression of target genes, which allow endosperm to develop. In addition, FIE may participate in the regulation of embryo development after fertilization by controlling the expression of different transcription factors. So far, homozygous <i>fie</i> mutant adult plants were not available due to the abortion of embryos bearing the maternal <i>fie</i> mutant allele. We have generated transgenic plants, in which FIE expression was silenced, and used them to explore FIE function in adult mutant plants. The characterization of mutant plants will be presented. In brief <i>fie</i> silenced mutant plants displayed abnormal development of both vegetative and reproductive organs as well as shortening of flowering time. These abnormalities correlate with the up-regulation of specific target genes expression. In light of phenotypic similarities between mutant plants of <i>fie</i> and Curly leaf (<i>clf</i>), a member of the SET domain PcG proteins, we hypothesized that both proteins may interact. Results of in-vitro experiments support this model. The role of FIE in the development of both the vegetative and reproductive phases will be discussed.</p>	

## EPSO-Conference 2002 - Poster 1.34

Session	Basic Biological Processes
Title	Maize endosperm development; aleurone organization
Author(s)	Al R, Lid SE, Krekling T, Opsahl-Ferstad H-G & Olsen O-A  Dept. of Chemistry & Biotechnology, Agricultural University of Norway P.O.Box 5040 1432 Ås, Norway  Author email: hilde-gunn.opsahl-ferstad@ikb.nlh.no
<p>Abstract:</p> <p>Following double fertilization, the triploid primary endosperm nucleus starts dividing in the absence of cytokinesis, leading to a multinucleate syncytium that occupies the periphery of the central cell. Subsequent cellularization results in formation of the four major endosperm cell types; starchy endosperm, aleurone, transfer cells and embryo surrounding region. At first, anticlinal cell walls form between nuclei, forming alveoli with their open ends towards the central vacuole. The nuclei within alveoli then divide synchronously in the periclinal plane, resulting in the formation of cell walls between the daughter cells, parallel to the outer wall of the central cell. This results in an outer layer of cells, and an inner layer of alveoli. The peripheral cells become aleurone cells, while the alveoli continue proliferation, cellularization and become the starchy endosperm. Subsequent reorientation of cell division planes in the peripheral layer to predominantly anticlinal is essential for the surface area of the aleurone to expand, while the strict cell division plane is lost in starchy endosperm cells. Anticlinal cell division of the peripheral cells results in a single layer of cuboidal aleurone cells similar in size and shape. In short, strict control of the division plane is essential for the characteristic and functional organization of the aleurone layer. In order to identify genes involved in cell division control and/or (re)differentiation of cereal endosperm cells, we have performed a microscopy based mutant screen of the Pioneer TUSC-collection, which consists of 43.000 maize lines with active transposable elements belonging to the Mutator family. From this screen we have identified several lines showing abnormal aleurone development. These include mutant lines in which the cell division plane control is perturbed, leading to unorganized aleurone layers. Two such mutants are defectal and disorgal1. Co-segregation analysis using Mu-probes has identified a band that may represent the disorgal1 gene. Efforts are underway to identify this gene.</p>	

## EPSO-Conference 2002 - Poster 1.35

Session	Basic Biological Processes
Title	Maize endosperm development; cloning and characterization of Dek1
Author(s)	<p>Stein Erik Lid<sup>1</sup>, Fred Gruis<sup>2</sup>, Rudolf Jung, Jennifer A. Lorentzen<sup>2</sup>, Mark Chamberlin<sup>2</sup>, Xiaomu Niu<sup>2</sup>, Bob Meeley<sup>2</sup>, Scott Nichols<sup>3</sup> and Odd-Arne Olsen<sup>1,2</sup></p> <p>Hilde-Gunn Opsahl-Ferstad            Dept Chemistry &amp; Biotechnology            Agricultural Univ. of Norway            P.O.BOX 5040            N-1432 Aas            Norway</p> <p>Author email: hilde-gunn.opsahl-ferstad@ikb.nlh.no</p>
<p><b>Abstract:</b></p> <p>Following double fertilization, the triploid primary endosperm nucleus starts dividing in the absence of cytokinesis, leading to a multinucleate syncytium that occupies the cytoplasm of the central cell. Subsequent cellularization results in formation of the four major endosperm cell types; starchy endosperm, aleurone, transfer cells and embryo surrounding region. In order to identify genes controlling cell fate specification in the cereal endosperm, we have performed a microscopy based mutant screen of the Pioneer TUSC-collection, which consists of 43.000 maize lines with active transposable elements belonging to the Mutator family. From this screen we have identified several lines showing abnormal aleurone development. These include two lines carrying novel alleles of dek1 (defective kernel 1) that fail to develop aleurone and severely affects embryonic shoot meristem formation. In an effort to clone Dek1, co-segregation analyses using various Mutator elements as probes have been carried out. Using this strategy, we have cloned Dek1, a 24,000 bp gene encoding a protein of 2159 amino acid residues, which is a member of the calpain gene super-family not previously reported from plants. Conventional calpains, found in multiple variants in animals are cytosolic Ca<sup>2+</sup> activated cysteine proteinases. DEK1 however contains, in addition to the cytosolic proteinase domain a series of transmembrane spanning regions as well as an extracytoplasmic domain, suggesting that DEK1 interacts with factors on the endosperm surface. The Arabidopsis thaliana genome also contains a Dek1 orthologue from which both the genomic structure and protein sequence is highly conserved. No other calpain-like sequences have been found in the Arabidopsis genome, nor in other available databases, strongly indicating that the DEK1-type of calpains is the only calpain type found in plants. These results suggest that DEK1 plays a conserved role in plant signal transduction.</p>	

## EPSO-Conference 2002 - Poster 1.36

Session	Basic Biological Processes
Title	Different plant responses to the infection by turnip mosaic potyvirus are determined by different viral genomic regions
Author(s)	Ponz, F., Jenner, C., Wang, X., Walsh, J., Sánchez, F. INIA. Dpt. Biotecnología. Autopista A6, km 7. 28040 Madrid. Spain HRI. Wellesbourne. Warwick. CV35 9EF, United Kingdom. Author email: fponz@inia.es
<p>Abstract:</p> <p>Turnip mosaic virus is a potyvirus with a remarkably wide host range. Responses to the infection by this virus are also quite diverse depending on plant species, virus strain, and a variety of other factors. Taking advantage of the availability of an infectious clone of TuMV, we have been investigating the involvement of different viral genomic regions in triggering different responses to infection, through the creation of several chimeric viruses and induced mutations. Results will be presented and discussed relative to avirulence determinants to resistance genes in Brassica and to symptom induction in systemically infected Arabidopsis.</p>	

## EPSO-Conference 2002 - Poster 1.37

Session	Basic Biological Processes
Title	RNAi as a tool to cure geminivirus infections.
Author(s)	Mikhail Pooggin, Shiva Prasad, K. Veluthambi and Thomas Hohn  Friedrich Miescher Institut Maulbeerstr. 66 4058 Basel Switzerland
<p>Abstract:</p> <p>Vigna mungo yellow mosaic (geminivirus) (VMYMV) is a major constraint in cultivation of mungbean and blackgram (Vigna mungo) in Asia. We established a new system to test the capacity of the RNA interference (RNAi) strategy to interfere with VMYMV infection: Vigna mungo plants are inoculated with the virus followed later by particle bombardment-mediated delivery of an interfering DNA construct. This construct is designed to express, under the control of a strong promoter, the dsRNA corresponding either to transcribed or non-transcribed (promoter) regions of the viral genome. Constructs that express dsRNA corresponding to the viral promoter interfere efficiently with the virus infection. Most effective is a construct equipped with an intron between the two arms of the dsRNA (as opposed to a spacer of the same length), which resulted in up to 90% recovery from infection. Since the promoter of a geminivirus normally does not exist in a transcribed form, the interference we observed most likely occurs in the nucleus and is transcriptional. Nevertheless, given that the total plant rather than its small (bombarded) regions were recovering from infection, there must be a silencing signal spreading systemically like in the case of post-transcriptional gene silencing. The requirement for the intron is consistent with involvement of a post-transcriptional component in the spread of the interference. Characterization of the molecular mechanism of the observed recovery, determination of an optimal timing between the infection onset and the interfering construct delivery and testing interfering constructs targeting other regions of the viral genome are currently under way.</p>	

## EPSO-Conference 2002 - Poster 1.38

Session	Basic Biological Processes
Title	Evaluation of the redox reactions composing the superoxide dismutase-ascorbate-glutathione pathway by computer-assisted metabolic modeling
Author(s)	Vitalija Povilaityte <sup>1</sup> , Andrea Polle <sup>2</sup> <sup>1</sup> Department of Food technology, Kaunas University of Technology, Radvilenu pl. 19, Kaunas, Lithuania <sup>2</sup> Forstbotanisches Institut, Universität Göttingen, Büsgenweg 2, 37077 Göttingen, email Germany: apolle@gwdg.de
<p>Abstract:</p> <p>Plants have both non-enzymatic and enzymatic antioxidant systems to prevent or alleviate the damage from reactive oxygen species (ROS). Several enzymes can efficiently detoxify ROS. Superoxide radicals are detoxified by superoxide dismutase (SOD) and hydrogen peroxide is destroyed by catalase and different kinds of peroxidases. A major hydrogen peroxide-detoxifying system in plants is the ascorbate-glutathione cycle that includes ascorbate peroxidase and glutathione reductase. The lipid and water-soluble antioxidants (ascorbate, glutathione, flavonoids, carotenoids and other phenolic compounds), which are important metabolites in plants, can be a part of a complex mechanism of protection from ROS. ROS act on cellular signaling and control of gene expression. An unrestricted accumulation of activated oxygen does not only disturb the cellular redox balance resulting in unspecific gene activation or repression (e.g. accelerated aging), but will also cause oxidative injury to DNA, proteins, and membranes. A strict control of the concentrations of reactive oxygen is required to maintain the delicate balance of systems involved in their destruction and their generation. By simple analysis of the concentrations of antioxidants, gene expression and activities of defense enzymes, it will not be possible to understand the regulation in the network of interacting redox reactions. For the theoretical and quantitative understanding of the functioning cellular redox regulation, knowledge about the fluxes of oxidants and antioxidants is required. To date, such information is not readily available. As a step towards the quantification of oxidant and reductant fluxes, computer-assisted simulations of the metabolic redox network comprising the superoxide dismutase-ascorbate-glutathione-related pathway have been developed. The models have been applied to simulate interactions between ascorbate and glutathione-related redox systems and to predict how changes in individual components will affect cellular oxidant levels.</p>	

## EPSO-Conference 2002 - Poster 1.39

Session	Basic Biological Processes
Title	Transformation and characterization of transgenic flax plants - expression of transgenes and their variability
Author(s)	<p>Hricova A./1/, Nap, J.P./2/., Pretova, A./1/ Mlynarova, L./1,2/</p> <p>Institute of Plant Genetics and Biotechnology, SAS, Akademicka 2, 950 07 Nitra, Slovak Republic /1/</p> <p>Plant Research International, Wageningen University and Research Centre, NL-6700, Wageningen, The Netherlands. /2/</p> <p>Author email: <a href="mailto:anna.pretova@savba.sk">anna.pretova@savba.sk</a></p>
Abstract:	<p>Gene transformation of flax (<i>Linum usitatissimum</i> L) was achieved using a <i>Agrobacterium tumefaciens</i> strain carrying a disarmed Ti-plasmid vector containing GUS gene driven by the Lhca3.St.1 light regulated promoter. The GUS gene was modified to contain flanking MAR elements around the gene and served as the reporter in the histochemical assay. The NPT II gene provided the selectable marker. Particular attention in the experiments was given to putative role of plant MARs in stabilizing GUS gene expression in transformed flax. Comparison of GUS gene expression between the flax populations containing MARs and those lacking MARs in T-DNA, showed a positive and beneficial effect of these short DNA sequences on expression. We observed a stable GUS expression in tested offspring generations, as well.</p>

## EPSO-Conference 2002 - Poster 1.40

Session	Basic Biological Processes
Title	Yeast Model of Geminiviral IMYMV-Bg genome replication
Author(s)	Vineetha Raghavan, Nirupam.R.Chauhury, Sunil.K.Mukherjee International centre for Genetic Engineering an Biotechnology Aruna Asaf Ali Marg New Delhi-110067 INDIA
<p><b>Abstract</b></p> <p>Geminiviruses are plant pathogens that cause significant yield losses in crop plants in tropical and subtropical belts of the globe. They are characterized by the geminate morphology of the virion particle and genetically by the genome consisting of one or two circular single stranded molecules, which replicates by rolling circle replication. Indian Mungbean Yellow Mosaic Virus (IMYMV) is a strain of geminivirus that poses a serious threat to legume cultivation in India. To understand the mechanisms of geminiviral pathogenesis, it is imperative to understand the biochemical steps of viral DNA replication within the nucleus of the infected plant. To facilitate such study, we used the genetically tractable yeast <i>Saccharomyces cerevisiae</i> as a model system for IMYMV-Bg replication. A yeast shuttle plasmid Ycp50 containing Ura3 marker was rendered inactive in its ARS activity. The DNA-A component of IMYMV-Bg containing the RCR origin of replication as well as the ORFs coding for replication and transcription functions was cloned in the ARS-deficient plasmid at a site distal to the centromeric region. The Ura auxotroph yeast strain W303a, when transformed with the Gemini-recombinant plasmid was able to grow on Ura- selection media, thus showing that geminiviral origin of replication was able to complement ARS function of the plasmid. One copy of DNA-A on transformation yielded miniature colonies, which did not grow in liquid selection medium. However, with two copies of DNA-A, the yeast growth was normal and the efficiency of ARS activity was restored to about 50%. Transforming the common region containing only the RCR origin of replication did not produce colonies indicating that other viral factors are essential to maintain replication in yeast. Presence of viral DNA was confirmed by Southern blot analysis of the yeast-derived plasmids. Different ORFs in the viral genome were subjected to site-directed mutagenesis to determine the viral factors important for viral DNA replication in yeast.</p>	

## EPSO-Conference 2002 - Poster 1.41

Session	Basic Biological Processes
Title	A novel, protochlorophyllide-containing protein import site into chloroplasts
Author(s)	Steffen Reinbothe <sup>1</sup> & Christiane Reinbothe <sup>2</sup>  1Université Joseph Fourier, Grenoble 1, UMR5575 "PLastes et Differentiation cellulaire", CERMO, BP53, F-38041 Grenoble cedex 9, France, 2Lehrstuhl für Pflanzenphysiologie, Universität Bayreuth, Universitätsstr. 30, D-95447 Bayreuth, Germany  Author email: <a href="mailto:steffen.reinbothe@ujf-grenoble.fr">steffen.reinbothe@ujf-grenoble.fr</a>
<p>Abstract:</p> <p>Chloroplasts are semi-autonomous cell organelles which must import roughly 2000 different proteins from the cytosol. Previous findings that protein import may be governed by a unique protein import machinery, which is composed of translocon of the outer (TOC) and translocon of the inner (TIC) envelope membrane complexes<sup>1</sup>, have recently been contradicted by the discovery of distinctive, though related, TOC receptor proteins<sup>2</sup>. This suggests the operation of distinct import sites. We were able to demonstrate that the cytosolic precursor of the NADPH:protochlorophyllide (Pchl) oxidoreductase A (pPORA)<sup>3</sup> does not embark the general import site<sup>4</sup>. Recent work has led to the discovery that there is a second, substrate-dependent protein import complex in the plastid envelope through which the pPORA is imported. Using various crosslinking approaches, combined with protein purification and mass spectrometry techniques, some of the components operating in the novel import site have recently been identified. It is the aim of this presentation to discuss the structure and function of the various identified components of the substrate-dependent protein import complex.</p> <ol style="list-style-type: none"><li>1. Keegstra, K. &amp; Cline, K. <i>Plant Cell</i> 11, 557-570 (1999).</li><li>2. Bauer, J., Chen, K., Hiltbrunner, A., Wehrli, E., Eugster, M., Schnell, D. &amp; Kessler, F. <i>Nature</i> 403, 203-207 (2000).</li><li>3. Reinbothe, S., Runge, S., Reinbothe, C., van Cleve, B. &amp; Apel, K. <i>Plant Cell</i> 7, 161-172 (1995).</li><li>4. Reinbothe, S., Mache, R. &amp; Reinbothe, C. <i>Proc. Natl. Acad. Sci. USA</i> 97, 9795-9800 (2000).</li><li>5. Jarvis, P., Chen, L.J., Li, H.-m., Peto, C.A., Fankhauser, C. &amp; Chory, J. <i>Science</i> 282, 100-103 (1998).</li></ol>	

## EPSO-Conference 2002 - Poster 1.42

Session	Basic Biological Processes
Title	RNA silencing in rice: screening of knockout mutant lines using the nucleotide sequences of QDE-3 and SDE1/SGS2
Author(s)	<p>Partha P. Samadder<sup>1</sup>, Y. Tanaka<sup>2</sup>, H. Okuizumi<sup>1</sup>, A. Mochizuki<sup>1</sup>, A. Miyao<sup>1</sup>, H. Hirochika<sup>1</sup> and M. Nishiguchi<sup>3</sup></p> <p><sup>1</sup>National Institute of Agrobiological Sciences, 2-1-2 Kan-nondai, Tsukuba Ibaraki 305-8602, Japan; <sup>2</sup>Wakasa Energ. Res. Center, 64-52-1 Hase, Tsuruga 914-0192, Japan; <sup>3</sup>Fac. of Agriculture, Ehime Univ., 3-5-7 Tarumi, Matsuyama 790-8566, Japan</p> <p>Author email: partha@nias.affrc.go.jp</p>
Abstract:	<p>Post-transcriptional gene silencing (PTGS) is a newly discovered mechanism of gene regulation based on sequence-specific targeting and degradation of homologous RNA transcribed from transgenes as well as endogenous genes. Recent research shows that this mechanism is conserved in a wide variety of organisms, including plants, animals and fungi, thus referred to as RNA silencing. This is a part of a sophisticated network of interconnected pathways for cellular defense which protects eukaryotes against viruses and transposons. To study PTGS using rice (<i>Oryza sativa</i>) homologs (OsQDE-3 and OsSDE1/SGS2) of QDE-3 (RNA helicase family, required for quelling or PTGS) and SDE1/SGS2 (RNA-dependent RNA polymerase, RdRp family) in <i>Neurospora crassa</i> and <i>Arabidopsis thaliana</i>, respectively, we have screened several mutant lines of rice where OsQDE-3 or OsSDE1/SGS2 is disrupted by insertion of retrotransposon (Tos17). These <i>Neurospora</i> and <i>Arabidopsis</i> genes have been found to be involved in RNA silencing. The homozygous individuals from F1 and F2 generation of each mutant line were screened by Southern blot analyses, which might be impaired in PTGS. Several GUS and GFP plasmids with inverted repeats were constructed to induce PTGS in rice calli by particle bombardment. Currently, we are investigating the effect of PTGS inducing plasmids on knockout mutant lines. On the other hand, we have cloned the partial fragment of OsQDE-3 and OsSDE1/SGS3 genes for further confirmation of knockout mutant lines.</p>

## EPSO-Conference 2002 - Poster 1.43

Session	Basic Biological Processes
Title	Transgene transcript level surpassing a gene-specific threshold rather than position effects trigger silencing in T-DNA transformants
Author(s)	Renate Schmidt Max-Planck-Institute of Molecular Plant Physiology 14424 Potsdam Germany  Author email: <a href="mailto:rschmidt@mpimp-golm.mpg.de">rschmidt@mpimp-golm.mpg.de</a>
<p>Abstract:</p> <p>For a comprehensive study of transgene expression in <i>A. thaliana</i>, we established single-copy T-DNA lines harbouring chimaeric reporter genes of various kind and number. The different reporter genes, the <math>\beta</math>-glucuronidase gene, the streptomycin-phosphotransferase gene and the gene for the green fluorescent protein, were placed under the control of the CaMV 35S promoter which confers strong expression.</p> <p>Below a certain number of identical transgenes we observed a positive correlation between copy number and reporter gene expression. Transgene expression was high, stable over all generations analysed and comparable between independent lines harbouring the same copy number of a particular transgene. Each copy contributed equally to total expression, even when the transgenes were present in a repetitive arrangement.</p> <p>Most notably, two T-DNA insertions that mapped to a heterochromatic domain located in the pericentromeric region of chromosome I showed high and stable reporter gene expression. In none of the 125 independent single-copy T-DNA transformants analysed silencing due to the site of T-DNA integration within the genome was observed.</p> <p>As soon as a certain copy number of a particular transgene driven by the CaMV 35S promoter was expressed in a transgenic plant silencing was inevitably triggered. Three copies of the GUS transgene were sufficient for the onset of silencing. Silencing was triggered regardless whether the copies were present in a repetitive arrangement at a single locus or at multiple loci. Silencing of the GFP and SPT reporter genes was observed in plants harbouring 5 and 9 copies, respectively. In contrast, promoterless or weakly expressed copies of a transgene did not trigger silencing. These data indicate that the transcript level of a particular transgene has to surpass a gene-specific threshold to trigger silencing.</p> <p>Hallmarks of RNA silencing were observed in silenced lines: meiotic reversibility, small interfering RNAs and methylation of the transcribed transgene region. All data taken together suggest that excessive transcription of transgenes causes RNA silencing in T-DNA transformants. In contrast, the position of T-DNA integration in the genome has only a negligible influence on the expression level of a transgene under the control of a strong promoter.</p>	

## EPSO-Conference 2002 - Poster 1.44

Session	Basic Biological Processes
Title	Characterization of small RNAs in <i>Nicotiana tabacum</i>
Author(s)	Corinne Schmitt, Hanspeter Schöb, Andreas Gisel and Frederick Meins, Jr.  Friedrich Miescher Institute Maulbeerstrasse 66 4058 Basel Switzerland  Author email: <a href="mailto:corinne.schmitt@fmi.ch">corinne.schmitt@fmi.ch</a>
<p>Abstract:</p> <p>Tiny RNAs in animals have been found associated with two regulation processes. One takes place at the translational level: micro RNAs (miRNA) originating from intergenic regions show partial homology to sequences within the 3'UTR of targeted genes and block their translation. The second process involves the formation of small interfering RNAs (siRNA) capable of promoting degradation of perfectly homologous messenger RNAs.</p> <p>To determine whether small RNAs are involved in the regulation of endogenous genes in plants, we cloned and sequenced small RNAs from wild type <i>Nicotiana tabacum</i> cv. Havana 425 mature leaves. A total of 264 unique sequences were obtained, ranging from 16 to 25 nucleotides in length. Eighty of these small RNAs identified known sequences in the genome of <i>N. tabacum</i>, 81% of which without mismatch. Most of the small RNAs corresponding to known sequences map in exons (47%) or intergenic regions (33%), some correspond to introns (10%) and only few correlate with 3'UTR (6%) and 5'UTR (4%). This is the first report of small RNAs sequenced in tobacco plants. Results are discussed and future work is presented.</p>	

## EPSO-Conference 2002 - Poster 1.45

Session	Basic Biological Processes
Title	Arabidopsis contains a large set of endogenous micro RNAs
Author(s)	Azeddine Si-Ammour, Hanspeter Schöb, Andreas Gisel and Frederick Meins Jr.  Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland  Author email: Azeddine.Si-Ammour@fmi.ch
<p>Abstract:</p> <p>Growing evidence suggests that naturally occurring, ca. 25-21-nt small RNAs -sometimes called microRNAs (miRNAs) - have important regulatory functions (1). Posttranscriptional gene silencing (PTGS) and antisense mediated gene silencing (ASGS) in plants, like RNA interference (RNAi) in animals, probably involves the processing of double stranded RNAs (dsRNA) by a Dicer-like activity to small ca. 21-22-nt small interfering RNAs (siRNAs), which then act as guides for the endonucleolytic degradation of cognate mRNAs. Recent studies suggest a similar function for an Arabidopsis microRNA (miRNA39), which is thought to be produced from an intergenic region and guides the sequence-specific cleavage of Scarecrow-like mRNA (2). Dicer-like activity can also generate ca. 21-nt small temporal RNAs (stRNA), first shown in <i>C. elegans</i> to trigger translational repression of cognate mRNAs and to control the timing of larval development. To better understand their function, we cloned a large number of microRNAs from Arabidopsis thaliana ecotype Columbia. Total RNA prepared from leaves of bolting plants was fractionated by polyacrylamide gel electrophoresis to give a fraction enriched in 15 to 25-nt RNAs. A protocol for cloning products of Dicer-like activity with 5'-monophosphate and 3'-hydroxyl termini was used (3). The clones were concatenated and sequenced. A total of 750 clones representing 350 unique sequences were analyzed. Of these, only 11 have been reported earlier (4, 5). These miRNAs were most frequently 21-22-nt in length. BLAST analyses showed that 65% are derived from intergenic regions. Some are identical in sequence or complementary to sequences present in transcribed regions, mainly in the 3' UTR. The possible functions of several informative miRNAs will be discussed.</p> <ol style="list-style-type: none"><li>1. Ambros (2001). <i>Cell</i>, 107:823-826.</li><li>2. Llave et al. (2002). <i>Science</i>, 297:2053-2056.</li><li>3. Hutvágner et al. (2001). <i>Science</i>, 293:834-838.</li><li>4. Llave et al. (2002). <i>Plant Cell</i>, 14:1605-1619.</li><li>5. Reinhart et al. (2002). <i>Genes Dev.</i>, 16:1616-1626.</li></ol>	

## EPSO-Conference 2002 - Poster 1.46

Session	Basic Biological Processes
Title	Identification of the RNA binding domain of Virp-1
Author(s)	M.A. Denti 1, M. Gozmanova 1,2, A E Martinez de Alba1 ,K. Kalantidis1, E. Marinou 1,2, Th.Saridaki 1,2, M. Tsagris 1,2 and M. Tabler 1  1 Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology, P.O. Box 1527, 71110 Heraklion Greece 2 and Dep. of Biology, University of Crete  Author email:tabler@imbb.forth.gr
<p>Abstract:</p> <p>Viroids are the smallest pathogens known, which consist of a circular, non-coding RNA which replicates in different host plants. Viroids are divided to 2 subgroups, one group which replicates predominantly in the nucleus, and one which replicates in the chloroplasts. Virp1 is a tomato protein, which binds specifically to potato spindle tuber viroid RNA, a viroid replicating in the nucleus of tomato and potato plants. In order to investigate the RNA binding domain and the specificity of binding, deletion and mutational analysis has been performed on the protein. The RNA binding domain could be localized. Further, we have isolated by RT-PCR the partial cDNAs of the homologous proteins from potato (a symptomatic host), <i>Nicotiana benthamiana</i> (a non symptomatic host) and <i>Nicotiana tabacum</i> (a non host for the specific strain of PSTVd used in this work). The c-terminal part of each of the proteins was expressed in <i>E.coli</i>, purified through affinity chromatography and is currently used for binding assays.</p>	

## EPSO-Conference 2002 - Poster 1.47

Session	Basic Biological Processes
Title	EC 5th Framework project: virus-induced gene silencing: unravelling the basis of a mechanism and its exploitation for the analysis of a multitude of individual gene functions in plants
Author(s)	E. Truve National Institute of Chemical Physics and Biophysics Akadeemia tee 23 12618 Tallinn Estonia  Author email: erkki@kbfi.ee
<p><b>Abstract:</b></p> <p>The objective of the project is to produce a detailed description of the molecular mechanisms underlying different stages of virus-induced gene silencing (VIGS) in plant cells. VIGS is a recently discovered sequence-specific RNA degradation mechanism in plants, that has a huge potential for the functional analysis of individual genes on the genome-wide scale, provided that its general principles are better understood. We plan to investigate the following aspects of VIGS: i) viral features detected by the plant's surveillance mechanism; ii) suppressors of VIGS; iii) host factors required for VIGS; v) role of DNA methylation in VIGS. The fulfilment of the project should pave the path for the construction of generally applicable efficient VIGS vectors for the functional analysis of multitude of plant genes.</p> <p>The following labs are participating in the network: National Institute of Chemical Physics and Biophysics, Tallinn, Estonia (Erkki Truve); The Sainsbury Laboratory, Norwich, UK (David Baulcombe); Agricultural Biotechnology Center, Gödöllő, Hungary (József Burgyán); Centro Nacional de Biotecnología, Madrid, Spain (Juan Antonio García); Wageningen University, Wageningen, The Netherlands (Marcel Prins); Trinity College, Dublin, Ireland (Tony Kavanagh); Institute of Molecular Biology and Biotechnology, Heraklion, Greece (Martin Tabler); Keygene N.V., Wageningen, The Netherlands (Michiel de Both); Friedrich Miescher Institute, Basel, Switzerland (Thomas Hohn); University of Basel, Basel, Switzerland (Manfred Heinlein). The project lasts from fall 2002 to fall 2005.</p>	

## EPSO-Conference 2002 - Poster 1.48

Session	Basic Biological Processes
Title	Gene expression profiling and genome-wide DNA methylation patterns in relation with hybrid vigor and stability of performance in maize
Author(s)	<p>Athanasios S. Tsaftaris<sup>1,2</sup>, Alexios N. Polidoros<sup>2</sup>, Nives Kovacevic<sup>1</sup>, Helen Tani<sup>1</sup>, Maria Kafka<sup>1</sup>, Nicolas Tarazas<sup>1*</sup>, Evaggelia Glalni<sup>1</sup> and Efthimia Hasiotou<sup>1</sup></p> <p>Dept of Genetics and Plant Breeding<sup>1</sup>, Aristotle Univesrity of Thessaloniki, Greece and          Institute of Agrobiotechnology 2, Center for Research and Technology Hellas, Greece          *Present address: Threpsi S.A., Greece</p> <p>Author email: tsaft@agro.auth.gr</p>
<p><b>Abstract:</b></p> <p>Heterosis is a genetic phenomenon, in which hybrids manifest superiority over the inbred parental genotypes, for several quantitative characters, including yield. While plant breeders and agronomists utilizing heterosis achieved an impressive yield increase in many plant species including maize, the biological basis of the phenomenon remains unknown. Recent biochemical and molecular investigations on heterosis indicate that quantitative variation of gene expression may be important in vigor manifestation. In the present study we examined gene expression differences between a heterotic and a non-heterotic hybrid and their parents, and compared these differences at different developmental stages. We used dot-blot hybridization to examine the expression variation of 35 genes and 2-D PAGE to examine the expression polymorphism of the majority of the proteins in these materials. Our data showed that the heterotic hybrid had a significant number of genes expressed over the quantity of the better parent and contained a significant number of proteins absent or very faint in the other materials. In addition, the non-heterotic hybrid had a significant number of genes expressed below the level of the worst parent.</p> <p>These results point to the significance of regulatory mechanisms involved in the quantitative modulation of gene expression. Since DNA methylation could be considered as a genome-wide general regulatory mechanism that affects the expression of many genes important for the manifestation of heterosis, we measured total DNA methylation in different maize inbreds and hybrids using HPLC chromatography, and site-specific DNA methylation changes related to density induced stress using the CRED-RA and RLGS techniques. We found developmental differences in the level of total DNA methylation among the different genotypes, which could be related to hybrid vigor. We also found that stressful growth conditions resulted in more methylated DNA (less expressed) and, in general, vigorous hybrids were more resistant to such density induced methylation changes. In addition, different transcription factors were profiled in maize parental inbreds, and heterotic and non-heterotic hybrids. Thus, the significance of the regulatory proteins modulating transcription was also studied.</p>	

## EPSO-Conference 2002 - Poster 1.50

Session	Basic Biological Processes
Title	Host Factors Involved in the Stable Integration of T-DNA into the Plant Genome
Author(s)	Lisa Valentine, Cynthia Ramos, Stanton B Gelvin* and Barbara Hohn Friedrich Miescher Institut, Maulbeerstrasse 66, Basel, Switzerland * Purdue University, West Lafayette, Indiana, USA Author email: lisav@fmi.ch
<p>Abstract:</p> <p>Genetic modification of plants is commonly achieved using the soil-borne pathogen <i>Agrobacterium tumefaciens</i>. The bacterium is capable of trans-kingdom gene transfer, the mechanism of which has been harnessed for biotechnology. The role of the bacterial virulence genes has been well characterised including (i) processing of the single-stranded Transferred DNA (T-DNA), (ii) components of the bacterial type IV secretion system and (iii) translocation of virulence factors into the plant cell. In contrast, little is known about the plant factors used by the bacterium to facilitate transport of the T-DNA across the cytoplasm, into the nucleus and ultimately integration in the plant genome. Elucidation of the path taken and the plant factors involved will be valuable in allowing recalcitrant species to be modified into more susceptible plants for biotechnological manipulations and conversely, allow modification of vulnerable species into resistant plants.</p> <p>To identify plant factors involved in <i>Agrobacterium</i> mediated transformation process, a screen of a tagged <i>Arabidopsis</i> library was initiated ( Nam et al., 1999). The screen involves assaying roots segments for tumour growth after infection with <i>Agrobacterium</i>. Classification of mutants specific for integration is possible using a reporter-based transient expression system to identify those mutants capable of successful nuclear import but with unsuccessful stable integration. These integration mutants are being further characterised using molecular techniques to identify the mutated gene and confirm its role in the process. In parallel in vitro assays are being used to analyse the integration specificity and quantify the integration deficiency (Ziemienowicz et al., 1999).</p>	

## EPSO-Conference 2002 - Poster 1.51

Session	Basic Biological Processes
Title	cDNA microarray analysis of carnation petal senescence
Author(s)	Hoeberichts FA, van Doorn WG, van Wordragen MF Agrotechnological Research Institute (ATO) PO Box 17 6700 AA Wageningen The Netherlands  w.g.vandoorn@ato.wag-ur.nl
<p>Abstract:</p> <p>cDNA microarrays consisting of over 2000 DNA fragments were used to study petal senescence in cut carnation flowers. The microarrays were hybridised with probes derived from ethylene-treated, silver thiosulphate (STS)-treated, sucrose-treated and untreated flowers. Ethylene advanced senescence whereas STS (an inhibitor of ethylene action) and sucrose delayed it. Changes in the time to visible senescence were reflected in gene expression: STS and sucrose both delayed expression of numerous clones, whereas ethylene advanced it. A putative RING zinc finger transcription factor and an EIN3-like protein were among the identified differentially expressed clones.</p>	

## EPSO-Conference 2002 - Poster 1.52

Session	Basic Biological Processes
Title	Development and growth of leaves: identification of genetic networks (DAGOLIGN)
Author(s)	Mieke Van Lijsebettens Department of Plant Systems Biology Ghent University K.Ledeganckstraat 35 9000 Gent Belgium <a href="mailto:mieke.vanlijsebettens@gengenp.rug.ac.be">mieke.vanlijsebettens@gengenp.rug.ac.be</a>
<p>Abstract:</p> <p>The DAGOLIGN Research Training Network was assigned within Framework V (starting date: October 2002; duration: 3 years; web site: <a href="http://www.psb.rug.ac.be/DAGOLIGN/">http://www.psb.rug.ac.be/DAGOLIGN/</a>). The network consists of six academical partners and one spin-off company (five European countries are involved).</p> <p>The project aims at identifying the molecular components of the genetic networks controlling the phyllotaxis, morphogenesis, growth and shape of leaves by using Arabidopsis with simple leaves and close relatives with dissected leaves.</p> <p>A first objective is to clone a number of genes controlling leaf phyllotaxis, architecture, development, growth and shape by means of a high throughput positional cloning or through differential cDNA display in Arabidopsis. The ectopic regulation of the knox pathway will be analysed in Arabidopsis relatives with highly lobed leaves.</p> <p>A second objective is to develop a multi-level analysis (growth model and micro-array) of wild type leaves, leaf mutants, double mutants and overexpression lines in Arabidopsis and lobed leaves of Arabidopsis relatives in order to identify regulatory genes for leaf morphogenesis and to determine the molecular components of the growth and developmental processes analysed.</p> <p>Genetic interactions will be measured by double mutant analyses; ectopic gene expression of molecular markers genes will be analysed in mutant backgrounds, in situ hybridisations or multiplex RT-PCR. Two-hybrid screening will identify interacting proteins of the studied genes.</p> <p>A third objective is to generate a relational knowledge database for multi-level integration of all the data on leaf mutants and genes obtained in this project for basic knowledge and application in rice. This leaf knowledge database will allow in depth characterisation of genotype/ phenotype relationships and the deduction of comprehensive models for leaf phyllotaxis, architecture, morphogenesis, growth and shape. It will be made publicly available over time.</p> <p>The Arabidopsis genes will be tested in rice to investigate whether the same set of genes determine leaf growth and architecture in both dicotyledonous and monocotyledonous plants and how leaf architecture and growth relate to yield.</p>	

## EPSO-Conference 2002 - Poster 1.53

Session	Basic Biological Processes
Title	Interference with African cassava mosaic virus replication by stable expression of different viral antisense RNAs in transgenic cassava
Author(s)	<p>Peng Zhang<sup>1,*</sup>, Nania Schärer-Hernández<sup>1</sup>, Johannes Fütterer<sup>1</sup>, Ingo Potrykus<sup>1</sup>, Johanna Puonti-Kaerlas<sup>2</sup> and Wilhelm Gruissem<sup>1</sup></p> <p>1 Institute of Plant Sciences, ETH-Zentrum / LFW E 17, CH-8092 Zürich, Switzerland 2 European Patent Office, D-80298 Munich, Germany</p> <p>* Author for correspondence, e-mail: zhang.peng@ipw.biol.ethz.ch</p>
Abstract	<p>African cassava mosaic virus (ACMV), the pathogen of African cassava mosaic disease, is a geminivirus transmitted by the whitefly (<i>Bemisia Tabaci</i>). Its genome consists of two circles of single-stranded DNA (ssDNA) denoted DNA A and DNA B. To develop strategies towards ACMV resistance, three antisense constructs have been designed for expression of viral antisense RNAs as 3'-untranslated regions (3'UTR) of a selectable marker gene. The targets for antisense interference are the viral mRNAs for Rep (AC1), TrAP (AC2) and REn (AC3), which are encoded on the complimentary sense of DNA A and play key roles in viral replication and transcriptional regulation.</p> <p>DNA inserts containing the viral antisense coding sequences of Rep, REn and TrAP were separately cloned into the 3' untranslated region of the hygromycin phosphotransferase gene, which is driven by the CaMV 35S promoter. Transgenic cassava plants were regenerated from hygromycin-resistant embryogenic suspension after Agrobacterium-mediated transformation. Southern and northern analyses verified the integration and stable expression of the three viral antisense genes in corresponding transgenic plant lines. Primary results of a viral replication assay showed that replication of ACMV was reduced in some transgenic lines. Selected plant lines will be tested for ACMV infection using plants from the green house.</p>

## EPSO-Conference 2002 - Poster 1.54

Session	Basic Biological Processes
Title	Evaluation of dsRNA technology for the production of virus resistant transgenic lines.
Author(s)	<p>Kalantidis K.1, Providaki M1., Missiou A. 2, Boutla A.1,2, Kotsis D. 1,2, Tabler M.1, and Tsagris M 1,2.</p> <p>1 Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, P.O. Box 1527, GR-71110 Heraklion/Crete, Greece.  2 Department of Biology, University of Crete, Vassilka Vouton, GR-71110 Heraklion/Crete, Greece.</p> <p>e-mail: kriton@imbb.forth.gr</p>
<p><b>Abstract:</b></p> <p>Posttranscriptional gene silencing (PTGS) is a recently discovered defense mechanism against invasive RNAs, which is widely conserved in different biological kingdoms, from protozoa to vertebrates, including plants. Double stranded (ds) RNA triggers the sequence-specific degradation of homologous target RNAs. In a first step the dsRNA is processed to 21-25nt long RNA fragments, called short interfering RNA (siRNA). This naturally occurring process can be used in biotechnology for specific gene suppression but also for the generation of plants resistant to viruses. We have used this natural process in an attempt to engineer three plant species resistant to various viruses. Conserved sequence regions of, Cucumber Mosaic Virus (CMV), Potato Virus Y (PVY) and of Plum Pox Virus (PPV), three viruses that cause great yield losses in agriculture, were used to construct plasmids that upon introduction into plants are able to generate “pan-handle” or “hairpin” shaped, dsRNA molecules with homology to the respective viral genes. All constructs were introduced via <i>A. tumefaciens</i> transformation. The CMV gene construct was introduced in susceptible oriental tobacco (var. Basmal) , the CP-PVY gene was introduced in American tobacco (var. Virginia) and potato (var. Spunta) whereas the PPV gene construct was transferred to <i>N. benthamiana</i> plants. Transgenic plants were challenged with the respective viruses and in each case we could identify resistant lines. The percentage of resistant lines ranged from 27 % to almost 70 % of the total transgenic lines generated. Resistance had usually the character of immunity, however, also recovery phenotypes were observed. In addition, a strong correlation between resistance and the presence of the siRNAs was observed showing that resistance was achieved through RNA silencing in spite the fact that all the above viruses carry suppressors of silencing.</p>	

## EPSO-Conference 2002 - Poster 1.55

Session	Basic Processes III (development)
Title	Odshatter Resistance: Exploitation of Arabidopsis genes to develop a productivity trait in Oilseed Rape
Author(s)	Guy Vancanney, Pascale Redig, Robin Child, Martin Yanofsky and Johan Botterman Bayer BioScience N.V. (formerly Aventis CropScience N.V.) Jozef Plateaustraat 22 B-9000 GENT Belgium
<p><b>Abstract:</b></p> <p>Plants have developed several fruit structures to propagate themselves through the distribution of seeds. Arabidopsis and Brassicas form pods or siliques which release the seed following the separation of the valves. This active process during fruit maturation is called dehiscence. Shedding of the seed before and during crop harvest represents an inherent agronomic problem in oilseed rape. Several genes regulating pod dehiscence have been identified in Arabidopsis through mutant analysis. The genes are specifically expressed in pods and either promote or inhibit dehiscence: The scope of this work is to define the role of these genes in pod development using them as tools to control valve separation in Arabidopsis and shatter control in oilseed rape (<i>Brassica napus</i>).</p> <p>We demonstrate that podshatter can be controlled through the inhibition or overexpression of specific regulatory genes in <i>B. napus</i>.</p>	