

## EPSO-Conference 2002

**Session            Plants as key to food quality and health  
improvement  
Poster 4.1 – 4.11**

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## EPSO-Conference 2002 - Poster 4.1

Session	Plants as Key to Food Quality and Health Improvement
Title	Regulation of Terpenoid biosynthesis in cotton and <i>Artemisia annua</i>
Author(s)	<p>Xiao-Ya Chen, Shan Lu, Yan-Hua Xu, Guo-Dong Wang, Qian-Jin Li, Jun-Wei Jia, Luo Bin</p> <p>National Key Laboratory of Plant Molecular Genetics          Institute of Plant Physiology and Ecology          Shanghai Institutes for Biological Sciences          Chinese Academy of Science          Shanghai 200032,          P. R. China</p> <p>Author email: xychen@iris.sipp.ac.cn</p>
<p><b>Abstract:</b></p> <p>Terpenoids comprise the largest family of natural products. Our laboratory focused on elucidation of biosynthetic pathways of terpenoid in cotton (<i>Gossypium</i> spp.) and <i>Artemisia annua</i> L. Many <i>Gossypium</i> species, including most of the cotton cultivars, accumulate cadinene-type sesquiterpenes, such as gossypol, in subepidermal glands of aerial tissues and in root epidermal cells. Formation of these defense compounds in cotton plants and suspension cultured cells may also be induced by fungal and bacterial infection and by other stress factors. We have cloned and characterized three enzymes that catalyze early and consecutive steps of gossypol biosynthesis, they are farnesyl diphosphate synthase (FPS), (+)-d-cadinene synthase (CAD, a sesquiterpene cyclase), and (+)-d-cadinene 8-hydroxylase (CYP706B1, a P450 monooxygenase). Hydroxylation of (+)-d-cadinene at C-8 is a critical step for the formation of gossypol. By comparative analysis of transcripts in seeds of glandless and glanded cotton cultivars, we isolated two P450 cDNAs that are highly expressed in glanded seeds, but not in glandless seeds. One of them, CYP706B1, was found to encode (+)-d-cadinene 8-hydroxylase. CYP706B1 is encoded by a single gene in <i>G. arboreum</i>, thus it holds a greater potential in manipulation of gossypol biosynthesis in cottonseeds via genetic engineering.</p> <p>We isolated a laccase cDNA, named GaLAC, from <i>Gossypium arboreum</i> cells treated with <i>Verticillium dahliae</i> elicitor. Transcription level of GaLAC in roots was found higher in glanded cultivars than in a glandless cultivar. An <i>in vitro</i> assay showed that the yeast-expressed GaLAC protein was active toward a number of phenolic compounds, and higher activities were obtained with ABTS and sinapinic acid as substrates. Preliminary results of chemical and histochemical analyses showed that laccase might be related to cross-linking sesquiterpene aldehydes to the cell wall.</p> <p>Plants of <i>Artemisia</i>, including <i>A. annua</i>, contain a wealth of monoterpenes and sesquiterpenes. Preliminary analysis suggested that formation of different terpenes in <i>A. annua</i> are differentially regulated. We have isolated from this medicinal species cDNAs encoding three monoterpene synthases which showed circadian rhythm in the shifting of its transcript abundance. The circadian pattern of a monoterpene cyclase gene expression may give a new insight into the regulation of plant primary and secondary metabolism.</p>	

## EPSO-Conference 2002 - Poster 4.2

Session	Plants as Key to Food Quality and Health Improvement
Title	Inducible Cell-Specific Activation Tagging of Genes Affecting Reproduction in <i>Arabidopsis thaliana</i> .
Author(s)	Mark Curtis, Andrea Steimer, Baskar Ramamurthy, Ueli Grossniklaus.  Institute of Plant Biology, Zollikerstrasse 107, 8008 Zurich, Switzerland  Author email: <a href="mailto:mcurtis@botinst.unizh.ch">mcurtis@botinst.unizh.ch</a>
<p><b>Abstract:</b></p> <p>Arabidopsis seeds result from double fertilisation. One sperm migrates to the central cell, another to the egg, leading to co-ordinated development of endosperm and embryo, respectively. In apomictic species, clonal offspring of maternal origin form in the absence of meiosis and fertilisation, resulting from deregulated ancestral sexual processes in time and space (Grossniklaus, 2001). During apomictic reproduction, deregulated sexual development results from (i) circumvention of meiotic reduction (apomeiosis); (ii) activation of the egg without fertilisation (parthenogenesis); and (iii) autonomous endosperm formation. These apomictic elements likely depend on deregulated gene expression in the nucellus (apomeiosis) and the egg cell (parthenogenesis).</p> <p>Reversible mis-expression of genes in time and space is not currently possible. We are developing a new system that allows inducible, highly specific (deregulated), expression of tagged genes. This approach will specifically target reproductive development, but has universal application, providing gain-of-function mutants that are rarely produced by classical mutagenic screens.</p> <p>Early nucellar- and egg cell-specific promoters, expressing the chimeric transcription factor XVE, will be used to transactivate genes adjacent to a <i>lexA</i> operator. We have produced activation constructs, using the Gateway<sup>TM</sup> recombination system, from which we can generate alternative vectors with different cell-specific cis-acting elements.</p> <p>Transgenic plants expressing the chimeric transcription factor will be transformed with T-DNAs containing a <i>lexA</i> operator, positioned such that a gene adjacent to the T-DNA may be expressed in these specific cell-types, following chemical induction.</p> <p>Plants will be screened for apomictic qualities. Apomeiotic mutants are likely to be either sterile or produce triploid (3n) progeny after fertilization. Sterile mutants are easily identified and triploids can be identified using a simple flow cytometry screen. Parthenogenetic mutants will be identified using a conditionally male sterile <i>Arabidopsis</i> line. Plants showing aspects of seed development under conditions favouring male sterility will identify genes that, when mis-expressed, produce parthenogenetic qualities.</p>	

## EPSO-Conference 2002 - Poster 4.3

Session	Plants as Key to Food Quality and Health Improvement
Title	Microarray analysis of gene expression in the ripening pear.
Author(s)	Sandra Fonseca ICAT, Ed. ICAT Campo Grande 1749-016 Lisboa Portugal  Author email: scfons@yahoo.com
<p><b>Abstract:</b></p> <p>Fruit ripening is a complex developmental process that involves changes in gene expression and enzyme activity. In this study, we used the microarray technology in order to monitor gene expression during pear fruit development and ripening. The 1364 clones were printed in a high-density microarray. To organize and visualize complex data sets we used hierarchical cluster analysis and self-organizing maps.</p> <p>In good accordance with the progressive fruit softening, we found several genes coding for cell wall modification enzymes to be overexpressed during ripening, an ACC oxidase from the ethylene biosynthesis pathway and with also a capsanthin/capsorubin synthase involved in pigment biosynthesis.</p> <p>Another class of genes induced during ripening is the one that has been traditionally related to defence mechanisms. Also interesting was the overexpression during ripening of an early light induced protein (ELIP) and transcripts coding for possible stress proteins.</p> <p>We found to be overexpressed during fruit development but repressed during fruit ripening several putative cell cycle/cytoskeleton and the same profile was found in genes usually correlated to the cold / drought stress.</p> <p>Information obtained from this study is a first contribution for the understanding of the transcriptional changes occurring during developing and ripening fruits.</p>	

## EPSO-Conference 2002 - Poster 4.4

Session	Plants as Key to Food Quality and Health Improvement
Title	Regulation of ferritin expression in Arabidopsis
Author(s)	Irene Murgia <sup>1</sup> , Delia Tarantino <sup>1</sup> , Massimo Delledonne <sup>2</sup> and Carlo Soave <sup>1</sup> .  <sup>1</sup> Dip. di Biologia, Università degli Studi di Milano, via Celoria 26, 20133 Milano, Italy <sup>2</sup> Dip. Scientifico e Tecnologico, Università degli Studi di Verona, Strada Le Grazie 15 - Ca' Vignal - 37134 Verona - Italy  irene.murgia@unimi.it
<p><b>Abstract</b></p> <p>Iron, an essential element for living organisms is a double-edge sword: on one side, bound to heme groups, Fe-S clusters, or associated with proteins it is essential for many cellular processes like those involved in electron transport. On the other side, in the free ionic form, iron is toxic as it catalyses the formation of reactive oxygen species (ROS) through the Fenton reaction. Levels of free iron are finely regulated and ferritins are one of the players in this game.</p> <p>Plants and animal ferritins are iron storage proteins formed by 24 subunits arranged to form a protein coat able to sequester up to 4500 iron atoms in a non-noxious form. Iron itself or oxidative stresses regulate ferritin levels both in animal and plant cells. Still unknown transcription factors bind to iron dependent regulatory sequences (IDRS) in the promoter of plant ferritin genes causing repression of ferritin transcription under low iron supply. Knowledge of mechanisms regulating plant ferritin levels is important for either engineering plants more tolerant to iron deficiency or for producing plants for human consumption with a higher content of available iron.</p> <p>Our goal is to identify molecules involved in the signaling pathway leading to Arabidopsis ferritin accumulation as well as the physio-pathological conditions regulating that accumulation. We show that nitric oxide (NO), a signaling molecule implicated in plant growth, development and defense also mediates iron induced Atfer1 ferritin accumulation. The pathway is ser/thr phosphatase-dependent, necessitates protein synthesis and is apparently cGMP independent. NO<sup>+</sup> is the active NO redox form implicated in that regulation. Moreover, NO mediates ferritin regulation through the IDRS sequence of the Atfer1 promoter.</p> <p>IDRS is not the only regulatory element in the Atfer1 promoter as senescence strongly activates Atfer1 promoter, through an IDRS-independent mechanisms.</p> <p>Murgia et al (2002) Plant J. 30(5), 521-528.  Murgia et al (2001) Plant Physiol.Biochem. 39, 1-10</p>	

## EPSO-Conference 2002 - Poster 4.5

Session	Plants as Key to Food Quality and health Improvement
Title	Investigation of introduced medicinal and aromatic plants for their antioxidant, antimicrobial and immunomodulating properties
Author(s)	<p>Vitalija Povilaityte, Rimantas Venskutonis</p> <p>Department of Food Technology, Kaunas University of Technology, Radvilenu pl. 19, LT – 3028, Kaunas, Lithuania</p> <p>Ona Ragazinskiene, Silvija Rimkiene Medicinal Plants Department, Kaunas Botanical Garden of Vytautas Magnus University, Ž.E. Zilibero 6, Kaunas, LT-3018, Lithuania</p> <p>E-mail: Vitalija.Povilaityte@ctf.ktu.lt</p>
<p>Abstract:</p> <p>A large and diverse range of introduced plants are cultivated in the collection of Medicinal and Aromatic plants at the Kaunas Botanical Garden of Vytautas Magnus University since 1924 as an important sources of plant products and natural food additives, as well as for scientific research, development of local plantings, cosmetic and pharmaceutical industry. The collection covers an area of 2771 m<sup>2</sup> and holds 408 plots of 215 species belonging to 163 genera and 60 families. The species in the collection are classified according to the main secondary metabolites. Most of these plants serve as potential genetic sources of future scientific investigations and are used to increase biodiversity. Their adaptation, vegetation changes, morphological characteristics and reproductive biology is analyzed during several years. The introduction of plants to different geographic conditions has influence on the synthesis of secondary metabolites, especially to the compounds of the phenylpropanoid pathway, which is responsible for the synthesis of a diverse array of flavonoids, tannins, hydroxycinnamate esters, stilbenes, lignans and the structural polymer lignin. These compounds are often induced by stress and serve specific roles in plant protection: pathogen defense, ultraviolet screening, or structural components of the cell wall. Many of them have antioxidant, anticarcinogenic, antimicrobial, anti-inflammatory and other pharmacological properties. Therefor the biologically active compounds of <i>Echinacea purpurea</i> (L.) Moench, <i>E.pallida</i> (Nutt.) Nutt., <i>E. angustifolia</i> DC., <i>Perilla frutescens</i> (L.) Britton, <i>Viola tricolor</i> L., <i>Rhaponticum carthamnoides</i> (DC.) Ilijin, <i>Potentilla fruticosa</i> L., <i>Lavandula angustifolia</i> Mill., <i>Atropa belladonna</i> L., <i>Salvia officinalis</i> L., <i>Nigella sativa</i> L., <i>Nigella damascena</i> L. and <i>Humulus lupulus</i> L. have been analyzed in different food, model and physiological systems. These plants have been considered as potential source of phenolic compounds, responsible for antioxidant, antibacterial, and immunomodulating properties on which we are focused. Many laboratories have been involved in these studies, including botanists, pharmacists, physiologists and food specialists. Most of plants are from Mediterranean region and it is interesting to analyze them not only as potential source of biological active compounds but also in the fundamental research to understand the changes of secondary metabolites synthesis under agro-climatic stress conditions.</p>	

## EPSO-Conference 2002 - Poster 4.6

Session	Plants as Key to Food Quality and Health Improvement
Title	Molecular breeding of strawberry cv Firework for enhanced disease resistance and taste improvement by introduction of thauII and rs-afp3 genes.
Author(s)	Konstantin Schestibratov, Sergey Dolgov  Artificial Climate Station "Biotron" Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS. Science Avenue 6 142290 Pushchino Moscow Region Russia
<p>Abstract:</p> <p>Strawberry is an especially suitable target for improvement through direct gene manipulations because of the genetic limitations associated with high heterozygosity and polyploidy which hamper the conventional breeding programs. The development of in vitro regeneration and transformation systems for the cultivated strawberry, <i>Fragaria ananassa</i>, has opened up the opportunity for strawberry improvement through genetic engineering. An efficient genetic transformation protocol has been developed for strawberry cv. Firework using <i>Agrobacterium tumefaciens</i>. The leaf disks were inoculated with disarmed supervirulent strains CBE21:pBI121, EHA105:p35SGUSint, CBE21:pBIThau35 and CBE21:pPCV002rs. The pBIThau35 vector was derived from pBI121 by replacement uidA gene fragment by the preprothaumatin fragment. Mature protein thaumatin II have an antifungal activity, possibly mediated by membrane permeabilization. The sweet taste of thaumatin may be the secondary property of this protein. Rs-afp3 is plant defensin gene from <i>Rafanus sativus</i>. It is recently characterized as small cyst-rich antimicrobial peptide. The frequency of transgenic shoots obtained using pBI121 ranged from 1.2% to 3.6% of the original treated explants. Histochemical assays for GUS activity indicated that the 35S promoter was highly active in meristematic and vascular tissues. In strawberry fruits gus activity has been shown only in the outer surface of the berry receptacle. Transformation efficiency of leaf explants by binary vector p35SGUSint was up to 1.1%. However, fluorometric assay showed higher expression levels of transgenic lines with intron containing uidA gene than without it. Efficiency of transformation mediated by CBE21:pBIThau35 ranged from 1 to 11.2%. PCR and Western blot analysis confirmed integration of nptII and thauII into the plant genome and expression of thaumatin II protein at the high level. Transgenic shoots obtained after inoculation of leaf explants by CBE21:pPCV002rs regenerated from 0.9-4.9% of leaf disks.</p>	

## EPSO-Conference 2002 - Poster 4.7

Session	Plants as Key to Food Quality and Health Improvement
Title	Maximising the Health Benefits of Garlic
Author(s)	Brian Thomas Horticulture Research International Wellesbourne Warwickshire CV35 9EF U.K.  brian.thomas@hri.ac.uk
Abstract	<p>Fruits and vegetables are consumed directly by humans as fresh produce and after processing. They are identified as essential sources of health related compounds including micronutrients, vitamins and beneficial phytochemicals. Changes in lifestyle and decline in consumption is leading to deficiencies of key compounds derived from these sources. A future challenge for plant scientists is to enable the market to be supplied with produce that is maximally effective in delivering the potential health benefits to the population. This requires understanding the genetics physiology and biochemistry of the compound in question to maximise the potential of crops and optimised agronomy and post harvest handling to maximise realisation of the potential. Such work needs to be carried out in collaboration with nutritionists and health scientists.</p> <p>We are using this approach in an EC programme on enhancing the health benefits of Garlic involving 15 laboratories across Europe. Cardiovascular diseases and cancer are by far the leading causes of death and morbidity in the EU. Garlic has been used for a long time as food with many therapeutic effects. However, detailed studies on the mechanisms underlying the interference of garlic with cardiovascular diseases and cancer are lacking. Moreover, because little is known about the compounds of garlic responsible</p>

## EPSO-Conference 2002 - Poster 4.8

Session	Plants as Key to Food Quality and Health Improvement
Title	Wheat Quality Genomics: Evaluation of Spectroscopic techniques as functional genomics tools
Author(s)	G A Toole 1, N Wellner 1, R H Wilson 1, D J Leader 2, E N C Mills 1  1. Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK 2. Wheat Improvement Centre, Syngenta, Norwich Research Park, Colney, Norwich, NR4 7UH, UK  Author email: geraldine.toole@bbsrc.ac.uk
<p><b>Abstract:</b></p> <p>Vibrational spectroscopy, specifically Near Infra Red (NIR), is widely used by wheat breeders to analyse intact grains for protein, moisture and hardness (Martin et al. 1993, Delwiche &amp; Massie 1996, Delwiche &amp; Hruschka 2000). However, Fourier Transform Infrared (FT-IR) and Raman spectroscopy can provide more detailed information about specific components and their physical state within the seed. The use of chemometrics to analyse the spectra can maximise their discriminatory ability. This work aims to develop high to medium throughput screens for quality based on spectroscopic analysis of wheat, and to develop a better fundamental understanding of the role attributable to the components within the seed in determining baking quality.</p> <p>NIR spectroscopy was conducted using a Perten 7000 NIR/VIS single grain analyser (Perten Instruments North America, Springfield, IL) to collect data at all wavelengths. Large numbers of spectra may be obtained for individual grains, these are then analysed chemometrically to determine both differences between varieties and also show outliers within a population of similar grains. In this way we can screen a large number of grains, generated within a genetic breeding programme, and select individual outliers. These outliers can then be analysed more intensely using FT-IR and Raman spectroscopy.</p> <p>FT-IR is conducted using a 'Golden Gate' ATR (Attenuated Total Reflectance). This allows spectra to be obtained not only for any part of the grain surface, but also for the inner endosperm, by cutting away a part of the grain. By producing thin sections of statistically outlying grains we are able to remove the cell contents without disturbing the endosperm cell walls, thus allowing further investigation of grain composition and structure, using FT-IR and Raman spectroscopy.</p> <p>Using these methods we aim to develop high to medium throughput screens for functional genomics in wheat and establish a database of grain quality related chemical and biophysical parameters.</p> <p><b>References:</b>            Martin CR, Rousser R, Brabrc DL 1993. Development of a single kernel wheat characterization system. Transactions ASAE 36:1399-1404            Delwiche S R, Massie DR 1996. Classification of wheat by visible &amp; near-infrared reflectance from single kernels. Cereal Chemistry 73:399-405            Delwiche, S R, Hruschka W R. 2000. Protein content of bulk wheat from Near-Infrared Reflectance of individual kernels. Cereal Chemistry 77:86-88</p>	

## EPSO-Conference 2002 - Poster 4.9

Session	Plants as Key to Food Quality and Health Improvement
Title	Rice functional genomics studies in China
Author(s)	<p>Hong-Wei Xue</p> <p>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</p> <p>Author email: hwxue@iris.sipp.ac.cn</p>
<p>Abstract:</p> <p>China has involved in the international rice genome project, for further studies on functional genomics, several aspects have been focused including expression profiles studies through DNA chips; generation of the mutant populations via different strategies, and studies on transcriptomes.</p> <p>Our research with a concentration on 1) generation of rice mutant populations via transposon-tag, T-DNA tag and EMS mutagenesis, around 10000 (via transposon- and T-DNA tag) and 50000 (via EMS mutagenesis) rice lines have been achieved; and 2) transcriptomes studies, with a focus on the isolation of cDNAs encoding transcription factors on genome level and related expression profiling analysis with gene chips. Update progresses will be presented.</p>	

## EPSO-Conference 2002 - Poster 4.10

Session	Plants as Key to Food Quality and Health Improvement
Title	PlanTIS – a novel tool for <i>in vivo</i> imaging transport and distribution of radiolabeled carbon or nitrogen in plants
Author(s)	Gerd Roeb <sup>1</sup> , Siegfried Jahnke <sup>1</sup> , Karl Ziemons <sup>2</sup> , Horst Halling <sup>2</sup> and Ulrich Schurr <sup>1</sup>  <sup>1</sup> Institute of Phytosphere, <sup>2</sup> Central Electronic Laboratory Forschungszentrum Jülich 52425 Jülich Germany  Author email: s.jahnke@fz-juelich.de
<p>Abstract:</p> <p>PlanTIS (= Plant Tomographic Imaging System) is a novel tool in plant biology which will be designed to significantly enlarge techniques to measure transport and distribution of short-lived isotopes in plants. Positron emitting isotopes (e.g. <sup>11</sup>C or <sup>13</sup>N) enable the measurement of transport of labelled compounds in plants under <i>in vivo</i> conditions. Reactions of plants to changing environmental conditions or exchange of compounds at the interface plant/atmosphere or plant/soil can also be studied with this method. Detection is non-invasive and experiments can be repeatedly performed due to the short half-life. Up to now, spatio-temporal information of radiotracer distribution within bulky organs (e.g. corn ears, fruits, or turnips) was not available. PlanTIS will open new insights since it will allow imaging of radiotracer with spatial resolution of &lt; 2 mm. It will not only be used for single-plant experiments but also to screen transport processes within sets of smaller plants (e.g. <i>Arabidopsis</i>). PlanTIS is currently under development and we expect to have it ready for plant research in the middle of 2003. Different departments of the Research Centre Jülich join their expertise within the PlanTIS project. The Central Electronic Laboratory accounts for detector design and data acquisition systems. The Institute of Nuclear Chemistry produces short-lived isotopes and can synthesize labelled compounds. The Institute of Phytosphere constructs the application facilities (cuvettes, gas exchange devices) and performs experiments with short-lived isotopes on plants.</p>	

## EPSO-Conference 2002 - Poster 4.11

Session	Food Quality
Title	Proteomics of <i>Medicago truncatula</i> seed filling.
Author(s)	Karine Gallardo, Christine Le Signor, Françoise Moussy, Gérard Duc, Richard Thompson and Judith Burstin  INRA-URGAP Legume Unit BP 86510 21065 Dijon France
<p><b>Abstract:</b></p> <p>The objective of the Plant Breeding and Genetics Research Unit at the INRA of Dijon is to improve seed quality in legumes, especially in peas, by plant breeding and integrated agronomic practice. To provide new information regarding genes involved in legume seed quality, we undertook a proteomic study of the model species <i>Medicago truncatula</i> during seed development. The analyses of two-dimensional (2-D) protein patterns of seeds collected during development clearly show a progressive accumulation of the most abundant proteins between 12 and 20 days after pollination (DAP). Matrix-assisted laser-desorption ionization time of flight mass spectrometry (MALDI-TOF) analyses of 31 abundant proteins revealed the major storage protein families, the vicilins, legumins and convicilins. To focus on key events occurring in legume seeds during synthesis and deposition of storage proteins we quantitatively compared 2-D protein patterns of <i>Medicago truncatula</i> seeds collected 12 DAP (before the accumulation of storage proteins) and after 14, 16, 18 and 20 days. Statistical analyses of the 273 detected protein spots revealed a significant variation in the quantity of 133 polypeptides of low abundance during these five stages. Some of them were associated with stages preceding and/or following storage protein accumulation, whereas others show various patterns during storage protein accumulation. Thirty four spots showing significant variations were analyzed by MALDI-TOF or Electrospray ionization tandem mass spectrometry and classified according to their function (e.g. cell division, photosynthesis, metabolism, translation, folding, stresses). These data will be used to provide reference maps of <i>Medicago truncatula</i> seed proteins at various stages of development to enable us to focus on the effects of genetic and environmental factors.</p>	