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20TH INTERNATIONAL SYMPOSIUM ON PLANT LIPIDS

20th International Symposium on Plant Lipids ISPL2012

8th-13th July 2012
Seville, SPAIN

CSIC

20th International Symposium on Plant Lipids
ISPL2012

Seville, 8-13 July 2012

PRODUCTION:
VIAJES CAJASOL
Santo Domingo de la Calzada 5, 1º - 41018 Seville, Spain

LAYOUT & DESIGN:
METAPRINT, S.L.
metaprint@metaprint.es

Seville, 2012

INDEX

ORGANISERS
DELEGATE INFORMATION
AWARDS
SPONSORS

20th International Symposium on Plant Lipids
ISPL2012

Seville, 8-13 July 2012

ORGANISING COMMITTEE

Plant Lipid Metabolism Research Line. Instituto de la Grasa. CSIC. Seville. SPAIN.

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SYMPOSIUM OFFICIAL WEB ADDRESS AND EMAIL CONTACTS

www.ispl2012.org
ispl2012@ig.csic.es
secretary_ispl2012@ig.csic.es

VENUE

Hotel Meliá Lebreros ****
Luis Morales 2, 41018-Seville, SPAIN
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melia.lebreros@melia.com

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virgendelosreyes@mediumhoteles.com

SOCIAL EVENTS

Opening Mixer*
Sunday 8th July 21:00-22:30
Welcome Cocktail at Hotel Meliá Lebreros

General Excursion
Wednesday 11th July 11:30-21:00
Trip to Cadiz. Departure from Meliá Lebreros Hotel at 11:30 am. Bus trip to Cadiz, panoramic city tour, lunch at the Hotel Playa Victoria and free time to enjoy the beach until 19:30 returning to Seville.

Gala Dinner
Thursday 12th July 21:00-24:00
Gala Dinner at Restaurant Robles Peyré.
Francos 42, Seville.

AWARDS

Terry Galliard Award

In recognition of the great contribution to the international lipid research from Professor Terry Galliard, the organizer of the first ISPL in Norwich in 1974, Terry Galliard Memorial Lecture was started on the 11th ISPL in 1994. The Terry Galliard Award will be given to a scientist who contributes significantly to the field of plant lipid research, is a member of the plant lipid family and has participated in several of the ISPL. So far nine Terry Galliard lectures have been held, and the Terry Galliard Medal was first awarded to each of the T. Galliard lecturer in 2000. Past winners of the Terry Galliard Award have been:

1994 . . . . . Norio Murata (Okazaki, Japan)
1996 . . . . . John Shanklin (Upton, USA)
1998 . . . . . John Harwood (Cardiff, UK)
2000 . . . . . John Ohlrogge (East Lansing, USA)
2002 . . . . . Ernst Heinz (Hamburg, Germany)
2004 . . . . . Hartmut Lichtenthaler (Karlsruhe, Germany)
2006 . . . . . John Browse (Pullman, USA)
2008 . . . . . Sten Stymne (Alnarp, Sweden)
2010 . . . . . Christoph Benning (East Lansing, USA)

The recipient of this year's Terry Galliard Award is Dr. Ivo Feussner from Georg-August-University, Albrecht-von-Haller-Institute for Plant Sciences, Department of Plant Biochemistry, Göttingen, Germany. Ivo is an outstanding scientist who continues to make highly significant contributions to the field of plant lipid research and has been selected by a selection committee, formed by sixty members of the international plant lipid scientific community.

Paul K. Stumpf Award

In recognition of Paul Stumpf's great contributions to plant lipid biochemistry, funds were obtained to support a P.K. Stumpf Award at the International Symposia on Plant Lipids. The first of these awards was made at the Bordeaux meeting in July, 2008. The Award will be given to a scientist at the early stages of their career (where `early stages' are defined as someone studying for their Ph.D. or within 6 years of obtaining their Ph.D.). Past winners of the Paul K. Stumpf Award have been:

2008 . . . . . Sébastien Baud (Versailles, France)
2010 . . . . . Yuki Nakamura (Singapore, Singapore)

The recipient of this year's P.K. Stumpf Award is Dr. Noemi Ruiz Lopez from Rothamsted Research, Harpenden, United Kingdom. Noemi is a promising scientist in the field of plant lipid research and has been designated by the ISPL2012 Scientific Committee.

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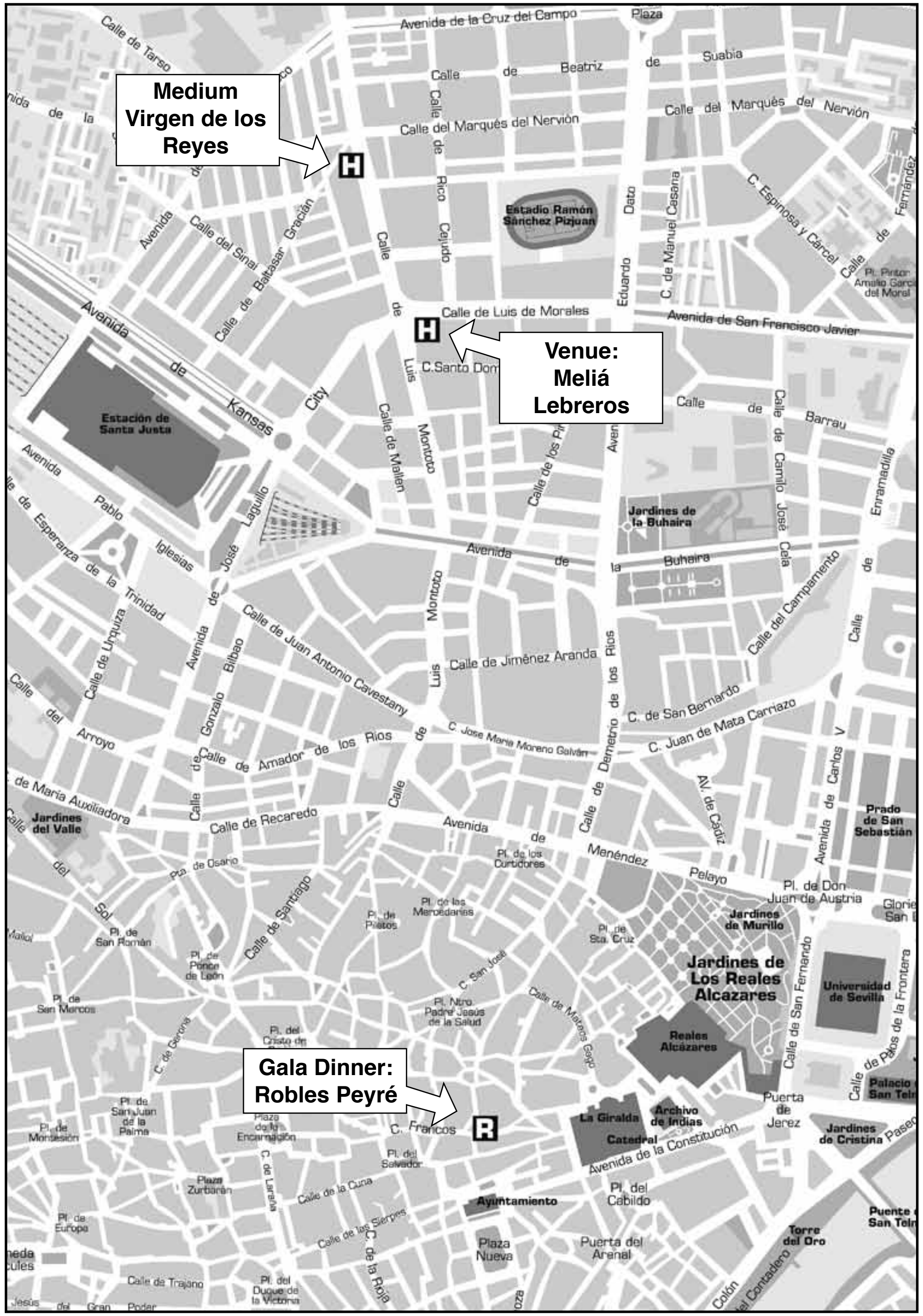
TIME TABLE
SYMPOSIUM MAP

	Sunday July 8	Monday July 9	Tuesday July 10	Wednesday July 11	Thursday July 12	Friday July 13
08:45 hrs.		Opening Session				
09:00 hrs.		**S1** New methods and technologies **Yonghua Li-Beisson**	**S4** Storage lipids: accumulation and modification **Mee-Len Chye**	**S7** Isoprenoids **Damaso C. Hornero Mendez**	**S8** Sphingolipids and sterols **Edgar Cahoon**	**S11** Plant lipid biotechnology **Johnathan Napier**
10:00 hrs.						
11:00 hrs.						
		S2 Fatty acid biosynthesis and modification **Penny Von Wettstein-Knowles**	**S5** Surface lipids/Extracellular lipid metabolism **Rene Lessire**		**S9** Lipid trafficking and signalling **Christoph Benning**	**S12** Future developments **John Ohlrogge**
12:00 hrs.						
13:00 hrs.						Closing Session
						
14:00 hrs.						
15:00 hrs.		Poster Session	Terry Galliard Lecture		Poster Session	
16:00 hrs.						
		S3 Chloroplast lipid metabolism **Peter Doermann**	**S6** Oxylipins **Ivo Feussner**		**S10** Algal and fungal lipids **John Harwood**	
17:00 hrs.						
18:00 hrs.						
19:00 hrs.						
20:00 hrs.						
21:00 hrs.						
22:00 hrs.						
23:00 hrs.						
					GALA DINNER	
24:00 hrs.						


Medium Virgen de los Reyes
Venue: Meliá Lebreros
Gala Dinner: Robles Peyré
Estadio Ramón Sánchez Pizjuán
Estación de Santa Justa
Jardines de la Buhaira
Jardines del Valle
Jardines de Murillo
Jardines de Los Reales Alcazares
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Plaza Nueva
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Pl. del Cabildo
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Torre del Oro
Avenida de la Cruz del Campo
Calle del Marqués del Nervión
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C. Francos
Pl. de Don Juan de Austria
Pl. del Salvador
Plaza Zurbarán
Pl. de Europa

SCIENTIFIC PROGRAM

SCIENTIFIC PROGRAM

20th International Symposium on Plant Lipids
ISPL2012

Sunday 8th July

19:00-21:00 **Registration**
21:00-22:30 Opening Mixer

Monday 9th July

08:45-09:00 **Opening Session**
09:00-11:00 **Session 1 "New methods and technologies"**
Yonghua Li-Beisson

S1 O1 Diagnosing bottlenecks to the accumulation of unusual fatty acids in transgenic seeds.
P.D. Bates and **John Browse**. Institute of Biological Chemistry, Washington State University, Clark Hall, Pullman, WA 99164-6340, USA.

S1 O2 Plant lipidomics: talkin 'bout a revolution!.
Richard P. Haslam, L.V. Michaelson and J.A. Napier. Biological Chemistry, Rothamsted Research, Harpenden, U.K.

S1 O3 Glycerolipid metabolic switching in plant and algal developmental processes.
Yuki Nakamura. Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan.

S1 O4 Mass-spectrometry based high-resolution metabolite imaging to unravel the surface lipids of Arabidopsis.
Basil J. Nikolau, Y.-J. Lee and Z. Song. Iowa State University, Ames, IA50011, USA.

S1 O5 Advanced engineering of lipid pathways in Nicotiana benthamiana leaves using a draft genome assembly and the V2 viral silencing-suppressor protein.
Fatima Naim, K. Nakasugi, E. Hilario, J. Taylor, S. Singh, R. Crowhurst, R. Hellens, P. Waterhouse and **Craig Wood**. CSIRO Plant Industry, Canberra Australia. University of Sydney, Sydney Australia. Institute of Plant and Food Research, New Zealand.

S1 O6 Non-invasive solution: advantages and challenges of nuclear magnetic resonance (NMR) application for oil seed crops.
Ljudmylla Borisjuk [(1)], T. Neuberger [(2)] and H. Rolletschek [(1)]. [(1)] Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, [(2)] Department of Bioengineering, Pennsylvania State University, University Park, PA 16802, USA.

11:00-11:30 Coffee Break

11:30-13:30 **Session 2 "Fatty acid biosynthesis and modification"**
Penny Von Wettstein-Knowles

S2 O1 Regulation of fatty acid synthesis in Canola and understanding desaturase regioselectivity.
John Shanklin, C. Andre and E. Whittle (BNL); J. Guy, M. Moche, J. Lengqvist, and Y. Lindqvist, (KI) ; and R. Haslam, (RR). Brookhaven National Laboratory, Upton, NY 11973, USA. Karolinska Institute Stockholm, Sweden. Rothamstead Research, UK.

S2 O2 Alkamide biosynthesis in Echinacea.
Robert E. Minto [(1),] M.R. Shepard [(1)], L. Rizshsky [(2)], H. Jin [(2)], A.S. Ransdell [(1)], H.W. Scott [(1)], and B.J. Nikolau [(2)]. [(1)] Department of Chemistry and Chemical Biology, Indiana University Purdue University – Indianapolis, Indianapolis, USA; [(2)] Department of Biochemistry, Biophysics & Molecular Biology, Iowa State University, Ames, USA.

S2 O3 Members of the WRINKLED clade orchestrate tissue-specific regulation of fatty acid synthesis in Arabidopsis thaliana.
Sebastien Baud. Jean-Pierre Bourgin Institute, INRA Versailles, Versailles, France.

S2 O4 Characterization of a bifunctional protein from Tetrahymena involved in ether lipid biosynthesis.
F. Dittrich-Domergue, L. Fouillen, S. Pascal, J. Joubès, R. Lessire and Frederic Domergue. Laboratoire de Biogenèse Membranaire. Univ. Bordeaux Ségalen, Bordeaux, France.

S2 O5 A new pathway for the synthesis of the very long chain mono-unsaturated fatty acid components of Arabidopsis membrane lipids.
M. Dauk, H. Ramadan, H. Yang, I. Ramirez, L. Forseille and **Mark Smith**. National Research Council Canada, Saskatoon, Canada.

S2 O6 Acyl carrier protein thioesterase activity (FATA) is essential for embryo development in Arabidopsis thaliana.
M. Siebers [(1)], V. Svetlichnyy [(2)] and P. Dörmann [(1)]. [(1)] Institute for Molecular Physiology and Biotechnology of Plants, University of Bonn, Bonn, Germany. [(2)] Institute for the Reproduction of Farm Animals Schönow Inc., Germany.

13:30-15:00 Lunch

15:00-16:00 **Poster Session**

16:00-16:30 Coffee Break

16:30-18:30 **Session 3 "Chloroplast lipid metabolism"**
Peter Dörman

S3 O1 Dynamic changes in glyceroipid metabolism under phosphate-starved conditions.
M. Shimojima [(1)], Y. Madoka [(1)], Y. Shimomura [(3)], M. Murakawa [(3)], K. Yamamichi [(2)], R. Koizumi [(3)], K. Endo [(4)], K. Ozaki [(4)] and **Hiroyuki Ohta** [(1)]. [(1)] Center for Biol. Resour. Inform., Tokyo Tech. [(2)] Biomaterial Anal.Center, Tokyo Tech. [(3)] Grad School Biol. Sci., Tokyo Tech. [(4)] Global R&D, Biol. Science, Kao Corp., Japan.

S3 O2 Identification of the missing epimerase involved in galactolipid synthesis in cyanobacteria – Are galactolipids necessary for the thylakoid membranes?
Koichiro Awai [1], H. Ohta [2] and N. Sato [3]. [1] Division of Global Research Leaders, Shizuoka University, Japan. [2] Center for Biological Resources and Informatics, Tokyo Institute of Technology, Yokohama, Japan. [3] Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan.

S3 O3 Requirement of phosphatidylglycerol for development of embryo and chloroplasts in Arabidopsis.
Hajime Wada, R. Tanoue, K. Katayama, S. Tanabashi, M. Kobayashi, N. Nagata, H. Akbari and M. Frentzen. Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan. Faculty of Science, Japan Women's University, Tokyo, Japan. Institute for Biology I, RWTH Aachen University, Aachen, Germany.

S3 O4 Updating the galactolipid synthesis model.
Asdrúbal Burgos, J. Szymanski, and L. Willmitzer. Max Planck Institute for Molecular Plant Physiology, Potsdam-Golm, Germany.

S3 O5 Search for genes involved in membrane lipid homeostasis in Arabidopsis thaliana based on a screening of a collection of EMS mutants resisting to galvestine-1, an inhibitor of galactolipid synthesis.
Laurence Boudière, C. Cataye, J. Jouhet, D. Falconet, C. Carles, R. Blanvillain, L. Nussaume, T. Desnos, J. Cintrat, B. Rousseau, R. Lopez, M.A. Block and E. Maréchal. LPCV CEA Grenoble, Grenoble, France.

S3 O6 Biophysic properties of biomimetic membranes obtained by self-assembly of natural chloroplast lipids.
A. De Ghellinck [1], C. Cataye [2], B. Demé [1], G. Fragneto [1], M.A. Block [2], E. Maréchal [2] and **Juliette Jouhet** [2]. [1] Institut Laue-Langevin, Grenoble, France. [2] Laboratoire de Physiologie Cellulaire Végétale, CNRS, CEA, INRA, Université Joseph Fourier, Grenoble, France.

Tuesday 10th July

9:00-11:00 **Session 4 "Storage lipids: accumulation and modification"**
Mee-Len Chye

S4 O1 Molecular biology of triacylglycerol biosynthesis in developing flax seed.
X. Pan, A.D. Wickramarathna, R.M.P.Siloto and **Randall J. Weselake**. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.

S4 O2 Accumulating triacylglycerol in leaves via the monoacylglycerol acyltransferase pathway.
Surinder Singh, J. Petrie, T. Vanhercke and P. Shrestha. Food Futures National Research Flagship and CSIRO Plant Industry, Canberra, Australia.

S4 O3 Phosphatidylcholine diacylglycerol exchange and acyl-editing are major mechanisms affecting fatty acid composition in seed triacylglycerols.
Chaofu Lu, J. Browse, P. Bates, A. Snapp, Z. Hu, Z. Ren and A. Carlsson. Montana State University, Bozeman, USA. Washington State University, Pullman, USA. Swedish University of Agricultural Sciences, Sweden.

S4 O4 Olive pollen and seeds as models for studies on lipid mobilization machinery during germination process.
Agnieszka Zienkiewicz [1,2], K. Zienkiewicz [1,3], J.D. Rejón [1], J.D. Alché [1], A.J. Castro [1] and M.J. Rodríguez-García [1]. [1] Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, CSIC, Granada, Spain. [2] Chair of Plant Physiology and Biotechnology, Department of Physiology and Molecular Biology of Plants, Nicolaus Copernicus University, Torun, Poland. [3] Department of Cell Biology, Nicolaus Copernicus University, Torun, Poland.

S4 O5 How to design new sunflower oils: triacylglycerols assembly target.
Monica Venegas-Calerón, J.A. Aznar-Moreno, M. Payá-Milans, J. Pérez-Hormaeche, J.D. Fernández-García, J.J. Salas, R. Garcés and E. Martínez-Force. GGBLS, Instituto de la Grasa, CSIC, Seville, Spain.

S4 O6 New oil body associated proteins.
I. López-Ribera, J.L. La Paz, N. García-Muniz, M. Miquel, T. Jouenne and **Carlos M. Vicient.** CRAG-Center of Agrigenomics Research (CSIC-IRTA-UAB-UB), Bellaterra (Cerdanyola del Vallés), Barcelona, Spain.

11:00-11:30 Coffee Break

11:30-13:30 **Session 5 "Surface lipids/Extracellular lipid metabolism"**
René Lessire

S5 O1 Dissecting the alkyl hydroxycinnamate biosynthetic pathway.
Dylan K. Kosma [1], A. Rice [1], I. Molina [2], O. Rowland [3], F. Domergue [4], J.B. Ohlrogge [1] and M. Pollard [1]. [1] Department of Plant Biology, Michigan State University, East Lansing, Michigan, USA. [2] Department of Biology, Algoma University, Sault Ste. Marie, Ontario, Canada. [3] Laboratoire de Biogenèse Membranaire, Université Victor Ségalen Bordeaux 2, CNRS, Bordeaux, France. [4] Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada.

S5 O2 Three suberin biosynthetic genes in potato: how they contribute to the polymer?.
Olga Serra [1], P. Boher [1], S. Chatterjee [2], R.E. Stark [2], M. Molinas [1] and **M. Figueras** [1]. [1] Cork Laboratory, University of Girona, Girona, Spain. [2] Department of Chemistry, City College of New York, Graduate Center and Institute for Macromolecular Assemblies, City University of New York, New York, USA.

S5 O3 Reconstitution of plant alkane biosynthesis in yeast demonstrates that Arabidopsis CER1 and CER3 are core components of a VLC-alkane synthesis complex.
Amélie Bernard [1,2], F. Domergue [1,2], S. Pascal [1,2], R. Jetter [3], C. Renne [4], J.-D. Faure [4], R.P. Haslam [5], J.A. Napier [5], R. Lessire [1,2] and J. Joubès [1,2]. [1] Université de Bordeaux, Laboratoire de Biogenèse Membranaire, Unité Mixte de Recherche 5200, Bordeaux, France. [2] Centre National de la Recherche Scientifique, Laboratoire de Biogenèse Membranaire, Unité Mixte de Recherche 5200, Bordeaux, France. [3] Departments of Botany and Chemistry, University of British Columbia, Vancouver, Canada. [4] Institut Jean-Pierre Bourgin, Unité Mixte de Recherche 1318, Institut National de la Recherche Agronomique AgroParisTech, Centre de Versailles-Grignon, Versailles, France. [5] Rothamsted Research, Harpenden, United Kingdom.

S5 O4 A bifunctional AP2/ERF-type transcription factor represses cuticular wax biosynthesis and activates dark-inducible genes under dark conditions in Arabidopsis.
Y.S. Go [1], S.B. Lee [2], H. J. Kim [2], J.Y. Kim [2] and **Mi Chung Suh** [2]. Departments of [1] Plant Biotechnology and [2] Bioenergy Science and Technology, Chonnam National University, Gwangju, Korea.

S5 O5 Arabidopsis ECERIFERUM2 is a component of the fatty acid elongation machinery required for fatty acid extension to exceptional lengths.
Tegan M. Haslam, A. Manas Fernandez and L. Kunst. University of British Columbia, Vancouver, BC, Canada.

S5 O6 Biosynthesis and functions of free and combined fatty alcohols associated with suberin.
Sollapura J. Vishwanath [1], F. Domergue [2], D.K. Kosma [3], I.P. Pulsifer [1] and O. Rowland [1]. [1] Department of Biology and Institute of Biochemistry, Carleton University, Ottawa, Ontario, Canada. [2] Laboratoire de Biogenèse Membranaire, Université Victor Ségalen Bordeaux 2, CNRS-UMR 5200, Bordeaux, France. [3] Department of Plant Biology, Michigan State University, East Lansing, MI, USA.

13:30-15:00 Lunch

15:00-16:00 **Terry Galliard Lecture**

Conserved functions of oxylipins in flowering and non-flowering plants.
Ivo Feussner. Georg-August-University, Albrecht-von-Haller-Institute for Plant Sciences, Department of Plant Biochemistry, Göttingen, Germany.

16:00-16:30 Coffee Break

16:30-18:30 **Session 6 "Oxylipins"**
Ivo Feussner

S6 O1 Oxylipin metabolites of the polyunsaturated acylethanolamide, N-linolenoylethanolamine, specifically mediate chloroplast disassembly in cotyledons of Arabidopsis seedlings.
J. Keereetaweep [1], E.B. Blancaflor [1,2] and **Kent D. Chapman** [1]. [1]University of North Texas, Center for Plant Lipid Research, Denton, USA. [2] Samuel Roberts Noble Foundation, Plant Biology Division, Ardmore, USA.

S6 O2 bHLH transcriptional factors MYL1, MYL2 and MYL3 modulate jasmonate metabolism and anthocyanin production.
Yuko Sasaki-Sekimoto [1], Y. Jikumaru [1], H. Saito [2], S. Masuda [3], Y. Kamiya [1], H. Ohta [3] and K. Shirasu [1]. [1] RIKEN Plant Science Center, 1-7-22 Suehiro-cho Tsurumi-ku Yokohama, Japan. [2] Graduate school of Bioscience and Biotechnology, Tokyo Institute of Technology,4259 Nagatsuta-cho Yokohama, Japan. [3] Center for Biological Resources and Informatics, Tokyo Institute of Technology, 4259 Nagatsuta-cho Yokohama, Japan.

S6 O3 Factors affecting hydroperoxide lyase performance in the synthesis of oxylipin volatiles responsible for virgin olive oil quality.
A. Sanchez-Ortiz, A.G. Perez and **Carlos Sanz**. Department of Physiology and Technology of Plant Products. Instituto de la Grasa (CSIC), Seville, Spain.

S6 O4 Air-transferred oxylipin volatiles from herbivore-infested tomato plants change uninfested conspecific plants to be defensive.
Koichi Sugimoto, K. Matsui, R. Ozawa and J. Takabayashi. Center for Ecological Research, Kyoto University, Otsu, Shiga, Japan. Graduate School of Medicine, Yamaguchi University, Yamaguchi, Japan.

S6 O5 MGDG with C12 and C10 oxo acids are formed after tissue disruption in Arabidopsis.
A. Nakashima, Y. Iijima, H. Tasaka, K. Aoki, D. Shibata and **Kenji Matsui**. Graduate School of Medicine, Yamaguchi University, Yamaguchi, Japan. Kazusa DNA Research Institutes, Kisarazu, Chiba, Japan. Kanagawa Institute of Technology, Kanagawa, Japan.

S6 O6 Oxylipin pathways in marine diatoms: occurrence, regulation and ecological role.
Giuliana d'Ippolito, A. Cutignano, C. Gallo and A. Fontana. Istituto di Chimica Biomolecolare (ICB) CNR, Pozzuoli (Napoli), Italy.

Wednesday 11th July

9:00-10:30 **Session 7 "Isoprenoids"**
Damaso Hornero Mendez

S7 O1 Isoprenoid precursor availabilities modulate protein prenyltransferase substrate specificities and activities in plants in vivo.
A. Hemmerlin [1], A. Huchelmann [1], M. Rohmer [2] and **Thomas J. Bach** [1]. [1] Institut de Biologie Moléculaire des Plantes, CNRS UPR 2357, Département "Réseaux Métaboliques", Université de Strasbourg, Strasbourg, France. [2] Institut de Chimie, Laboratoire de Chimie et de Biochimie des Microorganismes, UMR CNRS/Université de Strasbourg 7177, Strasbourg, France.

S7 O2 Accumulation of fatty acid phytyl esters in Arabidopsis thaliana during senescence.
Katharina vom Dorp [1], F. Lippold [2], V. Wewer [1], J. Lindberg Yilmaz [3], I. Lager [4], F. Kessler [5], S. Stymne [4] and P. Dörmann [1].
[1] Institute of Molecular Physiology and Biotechnology of Plants (IMBIO), University of Bonn, Bonn, Germany. [2] Aevotis GmbH, Potsdam, Germany. [3] Scandinavian Biotechnology Research AB, Alnarp, Sweden. [4] Swedish University of Agricultural Sciences (SLU), Department of Plant Breeding and Biotechnology, Alnarp, Sweden. [5] Laboratory of Plant Physiology, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland.

S7 O3 Triterpene production in Panax ginseng C.A. Meyer and its modulation by HMG-CoA reductase and oxidosqualene cyclases.
Y.-J. Kim, J. Oh, D.-C. Yang and **Ok Ran Lee**. Department of Oriental Medicinal Materials and Processing, College of Life Science, Kyung Hee University, Suwon, Korea.

S7 O4 Trisporoids composition in Blakeslea trispora under the lycopenogenesis stimulation.
Olga A. Vereshchagina, A.S. Memorskaya and V.M. Tereshina. Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia.

10:30-11:00 **Paul K. Stumpf Lecture**

The metabolic engineering of omega-3 long chain polyunsaturated fatty acids in transgenic plants – less is more.
Noemí Ruiz-Lopez. Rothamsted Research, Harpenden, Herts, UK.

11:00-11:30 Coffee Break

11:30-13:30 **GENERAL EXCURSION**

Thursday 12th July

9:00-11:00 **Session 8 "Sphingolipids and sterols"**
Edgar Cahoon

S8 O1 56-Amino acid small subunits of serine palmitoyltransferase stimulate sphingolipid synthesis, impact mycotoxin sensitivity and are essential for pollen viability in Arabidopsis.
A.N. Kimberlin [(1)], S. Majumder [(2)], M. Chen [(1)], G. Han [(2)], J.M. Stone [(1)], T.M. Dunn [(2)] and **Edgar B. Cahoon** [(1)]. [(1)] Center for Plant Science Innovation and Department of Biochemistry, University of Nebraska-Lincoln, Beadle Center, Lincoln, Nebraska, USA. [(2)] Department of Biochemistry and Molecular Biology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA.

S8 O2 The effect of long chain base sphingolipids on rates of programmed cell death in stressed plant cells.
Cara T. Daly, N.M. Islam, C.K.-Y. Ng and P.F. McCabe. School of Biology and Environmental Science, University College Dublin, Dublin, Ireland.

S8 O3 A novel protein anchor for lipid-specific plasma membrane raft targeting in plant cells.
S. Raffaele, C. Popp, A. Perraki, J.-M. Crowet, J.-L. Cacas, I.K. Jarsch, T.F. Stratil, L. Lins, T. Ott and **Sébastien Mongrand**. Laboratoire de Biogenèse Membranaire, CNRS UMR 5200 / Université Bordeaux Segalen, Bordeaux Cédex, France.

S8 O4 The role of mannosylated sphingolipids in Arabidopsis: unexpected roles in both growth and defence.
Johnathan A. Napier, J. Mortimer, S. Albrecht, P. Dupree, R.P. Haslam and L.V. Michaelson. Department of Biological Chemistry, Rothamsted Research, Harpenden, UK. Department of Biochemistry, University of Cambridge, Cambridge, UK.

S8 O5 Degradation of long-chain base 1-phosphate (LCBP) in Arabidopsis: functional characterization of LCBP phosphatase and LCBP lyase.
Hiroyuki Imai, M. Kato, N. Shimada, N. Nakagawa and M. Ishiguro. Department of Biology, Konan University, Okamoto, Higashinada-ku, Kobe, Japan.

S8 O6 Quantification of free sterols, sterol esters, sterol glucosides and acylated sterol glucosides in plants by Q-TOF mass mpectrometry.
Vera Wewer, I. Dombrink, K. vom Dorp and P. Dörmann. Institute for Molecular Physiology and Biotechnology of Plants, University of Bonn, Germany.

11:00-11:30 Coffee Break

11:30-13:30 **Session 9 "Lipid trafficking and signalling"**
Christoph Benning

S9 O1 Long chain acyl-CoA synthetase activities involved in lipid flux from ER to plastid.
Martin Fulda and D. Jessen. Georg-August-University Goettingen, Germany.

S9 O2 Role of Arabidopsis acyl-CoA-binding proteins in lipid trafficking.
Mee-Len Chye. School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong.

S9 O3 Phloem-mediated long-distance lipid signaling in plants.
U. Benning, B. Tamot and **Susanne Hoffmann-Benning.** Michigan State University, Department of Biochemistry and Molecular Biology, East Lansing, MI, USA.

S9 O4 CGI-58 regulates triacylglycerol homeostasis and lipid signaling pathways in plants through interaction with the peroxisomal transport protein PXA1.
S. Park [(1)], S.K. Gidda [(2)], C.N. James [(3)], P.J. Horn [(3)], N. Khuu [(2)], D.C. Seay [(1,4)], J. Keereetaweep [(3)], K.D. Chapman [(3)], R.T. Mullen [(2)] and **John M. Dyer** [(1)]. [(1)]United States Department of Agriculture-Agricultural Research Service, US Arid-Land Agricultural Research Center, Maricopa, USA. [(2)]Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada. [(3)]Department of Biological Sciences, Center for Plant Lipid Research, University of North Texas, Denton, USA. [(4)]Department of Natural Sciences, Del Mar College, Corpus Christi, TX, USA.

S9 O5 The phosphatidate phosphohydrolase PAH is involved in ABA signaling and freezing stress response in Arabidopsis.
Mie Shimojima [(1)], A. Numata [(2)], M. Ishi [(2)], T. Tsuzuki [(3)], Y. Yamaoka [(4)], Y. Madoka [(1)], T. Kinoshita [(3)], I. Nishida [(4)] and H. Ohta [(1)]. [(1)] Center for Biological Resources and Informatics, Tokyo Institute of Technology, Japan. [(2)] Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan. [(3)]Division of Biological Science, Nagoya University, Japan. [(4)] Graduate School of Graduate School of Science and Engineering, Saitama University, Japan.

S9 O6 pect1-4 mutation affects the chotochrome oxidase pathway capacity of mitochondrial respiration in Arabidopsis thaliana.
M. Otsuru, Y. Yu, Y. Fujiki and **Ikuo Nishida**. Saitama University, Shimo-Okubo 255, Sakura-Ku, Saitama, Japan.

13:30-15:00 Lunch

15:00-16:00 **Poster Session**

16:00-16:30 Coffee Break

16:30-18:30 **Session 10 "Algal and fungal lipids"**
John Harwood

S10 O1 High-throughput screening of lipid mutants in the model green microalgae Chlamydomonas reinhardtii.
H.M. Nguyen, C. Cagnon, H. Goold, S. Cuiné, A. Beyly, P. Carrier, P. Auroy, F. Beisson, G. Peltier and **Yonghua Li-Beisson**. CEA/CNRS/Aix-Marseille Université, CEA Cadarache, France.

S10 O2 Lipid characterization of Nitzschia lembiformis and Rhodomonas salina grown in different media: a case study.
Asher Wishkerman, R. Valmaña, A. Estevez, C. Ibáñez and R. Trobajo. IRTA-Sant Carles de la Rapita, Sant Carles de la Rapita, Tarragona, Spain.

S10 O3 Deciphering lipid biosynthesis in non-model microalgae to manipulate value-added compounds productivities.
Inna Khozin-Goldberg, S. Leu, A. Zarka, N. Shtaida , S. Sitnik, F. Guihéneuf and S. Boussiba. Microalgal Biotechnology Laboratory, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Israel.

S10 O4 Improvement of polyunsaturated fatty acid productivity in oleaginous fungus Mortierella alpina 1S-4 by overexpression of its acyl-CoA synthetase genes.
Eiji Sakuradani [(1)], T. Asaoka [(1)], H. Kikukawa [(1)], T. Okuda [(1)], A. Ando [(1)], M. Ochiai [(2)] and J. Ogawa [(1)]. [(1)] Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Oiwakecho, Kitashirakawa, Sakyoku, Kyoto, Japan. [(2)] Suntory Business Expert Ltd, 1-1-1 Wakayamadai, Shimamotocho, Mishimagun, Osaka, Japan.

S10 O5 Phosphatidic acids in Aspergillus niger under heat shock.
Vera M. Tereshina [(1)], A. S. Memorskaya [(1)] and E.R Kotlova [(2)]. [(1)] Winogradsky Institute of Microbiology Russian Academy of Sciences, Moscow, Russian Federation. [(2)] Komarov Botanical Institute Russian Academy of Sciences, St. Petersburg, Russian Federation.

S10 O6 Triacylglycerol metabolism in the microalga Chlamydomonas reinhardtii.
Christoph Benning [(1)], X. Li [(2,3)], B. Liu [(1)], R. Miller [(3,4)], B. Sears [(3)], C.-H. Tsai [(2,3)] and J. Warakanont [(3)]. [(1)] Department of Biochemistry and Molecular Biology, [(2)] Department of Energy Plant Research Laboratory, [(3)] Department of Plant Biology, [(4)] Cell and Molecular Biology Program, Michigan State University, East Lansing, Michigan, USA.

Friday 13th July

9:00-11:00 **Session 11 "Plant lipid biotechnology"**
Johnathan Napier

S11 O1 Capturing genetic diversity for advanced biofuels in Camelina.
Jillian Silva, U. Iskandarov, R. Cahoon and E. Cahoon. Center for Plant Science Innovation and Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, USA.

S11 O2 Enhanced production in yeast and substrate specificities of four variants of type 1 diacylglycerol acyltransferase from Brassica napus.
Michael S. Greer, M. Truksa, S.-C. Lung, and R.J. Weselake. Alberta Innovates Phytola Centre, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.

S11 O3 Engineering "Super DGATs": targeted mutations in type-2 diacylglycerol acyltransferase lead to enhanced performance in multiple transgenic systems.
Jay Shockey [(1)], R. Siloto [(2)], C. Mason [(1)], A.H.J. Ullah [(1)], K. Sethumadhavan [(1)], S. Boone [(1)] and R. Weselake [(2)]. [(1)] USDA-ARS, Southern Regional Research Center, New Orleans, LA, USA. [(2)] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.

S11 O4 Metabolic engineering of Yarrowia lipolytica for the production of omega 3 fatty acids: the role of different acyltransferases in influencing fatty acid desaturation, elongation, and lipid production.
Naren Yadav, H. Zhang, Q. Zhu, Z. Xue, D. Xie, B.D. Tyreus, R. Short, P.L. Sharpe, R. Hong, X. Fan, B. Boonyaratanakornkit and C. Dellomonaco. Biochemical Sciences and Engineering, Central R&D, Dupont Co., Wilmington, DE, USA.

S11 O5 Metabolic engineering plant seeds with fish oil-like levels of DHA.
James Petrie and S. Singh. CSIRO Food Futures National Research Flagship, Canberra, Australia.

S11 O6 The molecular basis of high and super-high oleic safflower seed oils.
Craig Wood, Q. Liu, J. Cao, X.-R. Zhou, A. Green and S. Singh. CSIRO Plant Industry, Canberra, Australia.

11:00-11:30 Coffee Break

11:30-13:30 **Session 12 "Future developments"**
John B. Ohlrogge

S12 O1 Plant lipids in 2030.
John B. Ohlrogge [(1)] and S. Stymne [(2)]. [(1)] Department of Plant Biology, Michigan State University, East Lansing, MI, USA. [(2)] Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Alnarp, Sweden.

S12 O2 Improved soybean seed quality traits for food, feed, fuel and industrial applications.
Howard Damude, K. Meyer, Z. Li, S.C. Falco and A. Kinney. DuPont Pioneer, DuPont Experimental Station, Wilmington, DE, USA.

S12 O3 Comparison of transcriptome changes associated to oil accumulation in oil palm mesocarp and in oil seeds.
F. Bourgis [(1)], A. Kilaru [(2)], X. Cao [(3)], E. Legrand [(1)], B. Beauvoit [(4)], M. Maucourt [(4)], C. Deborde [(4)], A. Moing [(4)], G.-F. Ngando-Ebongue [(5)], N. Drira [(6)], J. Ohlrogge [(3)] and **Vincent Arondel** [(1)]. [(1)] Laboratoire de Biogenèse Membranaire, CNRS UMR5200, Université Bordeaux Segalen, Bordeaux, France. [(2)] Department of Biological Sciences, East Tennessee State University, Johnson City, USA. [(3)] Great Lakes Bioenergy Research Centre, Michigan State University, East Lansing, USA. [(4)] UMR 1332 Biologie du Fruit et Pathologie, Plateforme Métabolome du Centre de Génomique Fonctionnelle Bordeaux, INRA Université de Bordeaux, Villenave d'Ornon, France. [(5)] Centre de Recherches sur le Palmier à Huile (CEREPAH) de la Dibamba, IRAD, Douala, Cameroon. [(6)] Laboratoire de Biotechnologie Végétale, Faculté des Sciences de Sfax, Sfax, Tunisia.

S12 O4 RDR1 and SGS3, components of RNA-mediated gene silencing, are required for regulation of cuticular wax biosynthesis in developing inflorescence stems of Arabidopsis.
Patricia Lam, L. Zhao, H.E. McFarlane, M. Aiga, V. Lam, T.S. Hooker and L. Kunst. Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada.

S12 O5 Transgenic nutritional enhancement: the production of omega-3 long chain polyunsaturated fatty acids in plants.
O. Sayanova, N. Ruiz-Lopez, R. Haslam, S. Usher, M. Venegas-Calerón and **Johnathan A. Napier**. Department of Biological Chemistry, Rothamsted Research, Harpenden, UK.

13:15-13:30 **Closing Session**

13:30-15:00 Lunch

ORAL COMMUNICATIONS

Diagnosing bottlenecks to the accumulation of unusual fatty acids in transgenic seeds

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Engineering of oilseed plants to accumulate unusual fatty acids (FA) in seed triacylglycerol (TAG) requires not only the unusual FA biosynthetic enzymes but also efficient utilization of the unusual FA by the pathways of TAG synthesis in the host-plant. Competing pathways of diacyglycerol (DAG) and subsequent TAG synthesis ultimately effect TAG FA composition. The membrane lipid phosphatidylcholine (PC) is the substrate for many FA modifying enzymes (desaturases, hydroxylases, etc) and DAG can be derived from PC for TAG synthesis. The relative proportion of PC-derived DAG versus *de novo* synthesized DAG utilized for TAG synthesis, and the ability of each pathway to utilize unusual FA substrates are unknown for most oilseed plants, including Arabidopsis. Through metabolic labeling experiments we demonstrate that the relative flux of *de novo* DAG into the PC-derived DAG pathway *versus* direct conversion to TAG is ~14/1in wild-type Arabidopsis. Expression of the *Ricinus communis* FA hydroxylase reduced the flux of *de novo* DAG into PC by ~70%. TAG synthesis directly from *de novo* DAG did not increase, resulting in less total synthesis of labeled lipids. Hydroxy-FA containing *de novo* DAG was rapidly synthesized, but it did not accumulate and appeared to be in a futile cycle of synthesis and degradation. However, FA hydroxylation on PC and conversion to DAG allowed some hydroxy-FA to accumulate in *sn*-2 position of TAG. We conclude that the flux of DAG through PC represents a major bottleneck for accumulation of unusual FA in TAG of transgenic Arabidopsis seeds.

Plant Lipidomics: talkin ‘bout a revolution!

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The field of plant lipidomics is the logical outcome of the history and tradition lipid biochemistry. At its heart is the desire to understand the role of lipids in plant cellular functions. The desire to grasp the diversity of cellular lipids has led to the application of new technology, most notably mass spectrometry, but also complementary approaches for the extraction, detection and characterization of lipids. Here we describe how improvements in chromatographic separation and mass spectrometry have allowed us to determine not only the lipid composition (i.e. parts list) of cells and tissues, but also address questions regarding lipid substrates and products that have previously overwhelmed traditional analytical technologies. The development of high throughput extraction, sample delivery and data processing will be discussed. Although it is clear that the development of these integrated methods has greatly advanced our capacity for understanding the biological roles of plant lipids; the molecular composition of the plant lipidome is complex and much remains to be understood.

Glycerolipid metabolic switching in plant and algal developmental processes

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The dynamic alteration of glycerolipid profiles is associated with various biological processes. We focus on the reproductive processes of Arabidopsis flowers as a model to investigate its dynamic changes in glycerolipidome. By utilizing a transgenic plant showing a synchronized flower development, we profiled levels of glycerolipid classes including phosphoinositides and expression pattern of relevant lipid biosynthetic genes. The results revealed active changes in lipid profiles and expression patterns of the relevant lipid biosynthetic genes during flower development. To study the spatiotemporal impact of glycerolipid metabolism in different tissues, we currently try to develop a new technique “metabolic switching system” by transiently altering the expression of a key enzyme in glycerolipid metabolism. The system works robustly to change the proportion of glycerolipids, and to reveal a phospholipid-protein interaction in coordinating inflorescence development. This suggests that “metabolic switching system” is a powerful tool to investigate the spatiotemporal effect of glycerolipid metabolism. The system is also being applied in Chlamydomonas to switch the glycerolipid metabolism in favor of triglycerides accumulation with the scope of potential bioenergy creation.

Mass-spectrometry based high-resolution metabolite imaging to unravel the surface lipids of Arabidopsis

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The cuticle is a complex and unique mixture of lipids that covers the very outer surface of all aerial organs of terrestrial plants. This lipid-coat functions as a water-barrier and a protective layer against the environmental stresses. Despite the identification and characterization of many mutants that affect the deposition of the cuticle (called eceriferum (*cer*) mutants), there are many unanswered questions concerning the regulation of the metabolism that generates this protective barrier. Because the cuticle is the product of a single cell layer of the plant and it is unidirectionally secreted to the surface, addressing these questions requires the combined application of new technologies to investigate the distribution of cuticle metabolites at the cellular and subcellular levels, combined with the molecular and biochemical characterization of the isolated *CER* genes. We have developed high spatial resolution techniques that use mass spectrometry to image epicuticular lipids of the surfaces of *Arabidopsis thaliana*. This technology has the ability to image the distribution of metabolites to a resolution of ~12 μm, which is less than the size of a typical plant cell. We combine this new technology with biochemical and genetic characterizations of Arabidopsis *cer* mutants to provide a more accurate annotation of *cer* gene functions.

Advanced engineering of lipid pathways in *Nicotiana benthamiana* leaves using a draft genome assembly and the V2 viral silencing-suppressor protein

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The *Nicotiana benthamiana* leaf transient assay is emerging as a useful platform for plant lipid research, such as the characterisation of wax ester synthesis pathways and oils containing long-chain fatty acids. The assay is ideal for the overexpression of transgenes, however, the simultaneous silencing of endogenes is not possible and therefore the flux of substrates entering transgenic pathways is compromised. Here we introduce the viral silencing-suppressor protein, V2, and a draft *N.benthamiana* genome database, and demonstrate their utility for plant lipid research. We simultaneously over-expressed Arabidopsis fatty acid metabolism genes, *AtFAE1* and *AtDGAT1*, which together convert the limiting substrate 18:1 into plant oils containing 20:1. This transgenic pathway is limited due to the rapid and efficient conversion of 18:1 to 18:2 in leaves; the enzyme NbFAD2 is highly active in young leaves. Searching the draft genome database revealed two *NbFAD2* genes, *NbFAD2.1* and *NbFAD2.2.* This resource was used to accurately design hairpin RNAi against *NbFAD2* genes, *hpNbFAD2*. Combinations of V2, hpNbFAD2, AtFAE1 and AtDGAT1 resulted in 12-fold and 17-fold increases in 18:1 and 20:1, respectively, indicating that the use of hairpin RNAi shunts endogenous lipid metabolism into newly-introduced transgenic pathways. We provide a web-searchable database that covers over 80% of the 3.2 Gb haploid genome and represents an immense increase in genomic resources available to guide plant lipid research. Overall these new tools, V2 and the *N.benthamiana* genome, now allow accurate gene replacement and the rapid assembly of engineered lipid pathways.

Non-invasive solution: advantages and challenges of nuclear magnetic resonance (NMR) application for oil seed crops

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Improvement of oil crops relies on deep understanding of various aspects of the plant development, and requires advanced analytical tools which can be applied for both *in vitro* and *in vivo* studies. Nuclear magnetic resonance (NMR) technique allows to visualize non-invasively internal structures and metabolites and therefore has the potential to monitor physiological processes crucial for seed development. Application of the NMR based technologies for oil seed crops is not yet a routine approach mainly because restricted availability, high costs of the MRI systems and relatively low throughput.

We provide some breakthrough approaches toward increasing resolution and throughput of quantitative imaging for a number of model plants such as oil seed rape, tobacco and oat. We also explored advantages of NMR-based modeling and combining NMR studies with flux balance analysis and other techniques for solving outstanding issues in metabolite delivery, seed development and oil storage. Special attention deserves application of NMR for study biodiversity of oil crop seeds and characterization of ex situ gene bank collections.

Overall, it is our believe that establishment of NMR-platform for oil crops will facilitate progress in both understanding mechanisms that control oil storage and valorization of ex situ gene bank resources for breeding and biotechnology.

Regulation of fatty acid synthesis in canola and understanding desaturase regioselectivity.

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I will address two key issues: 1) understanding feedback regulation of fatty acid biosynthesis as a basis for increasing oil yield and 2) controlling the chemical structure of the stored fatty acid. We employed a *Brassica napus* embryo cell culture to investigate feedback inhibition of fatty acid biosynthesis with the goal of identifying the signal for feedback inhibition and the target of the inhibition. 18:1-ACP was identified as the signal for feedback inhibition and ACCase as the target for inhibition. The inhibition is characterized by rapid onset, full reversibility and its occurrence at physiological concentrations of 18:1-ACP. In a second project we seek to understand the mechanism underlying regioselectivity to facilitate engineering of desaturase enzymes to produce novel desired products. We determined the crystal structure of the castor D9 18:0-ACP desaturase in complex with ACP, and used High Ambiguity Driven biomolecular DOCKing (HADDOCK) to model the ACP binding mode onto our crystal structure of the ivy D4 16:0-ACP desaturase. The castor and ivy enzymes bind ACP in two distinct orientations. Both are potentially available in the castor desaturase, but the D9 binding mode is favored because charge repulsion between desaturase D280 and the oxygens of ACP phosphoserine 38 prevents the D4 mode. Position 280 of the ivy desaturase is a lysine which forms a hydrogen bond with ACP phosphserine 38, stabilizing the D4 binding mode. We are now exploiting our understanding of ACCase feedback inhibition and desaturase regioselectivity to increase oil yield and to create novel regioselectivities.

Alkamide biosynthesis in *Echinacea*

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Echinacea, a coneflower genus native to North America, includes the most widely used medicinal plants of the United States and Europe. Alkamides, one of their bioactive natural products, lie at the nexus between acyl lipid and amino acid metabolism. Drawing on metabolomic, transcriptomic, and isotopic tracer studies, we have identified the origins of isobutyl and 2-methylbutylamine units and reevaluated the biosynthesis of the short and medium chain, highly unsaturated fatty acyl groups. The first plant amino acid decarboxylase capable of acting on branched-chain amino acids has been identified. Based on stable-isotope GC/LC-MS data, inhibitor studies, and positional analysis via HSQC NMR experiments, a model in which acyl chains are formed through extra-chloroplastic fatty acid biosynthesis in *E. purpurea* was developed. The origin of the polyacetylenic acyl moiety of the alkamides may lie with microsomal fatty acid FAD2-like desaturase/acetylenase enzymes. We have identified from *Echinacea* and functionally characterized in *Yarrowia lipolytica*, two FAD2-like acetylenases. Collectively, our experiments are defining the pathways involved in alkamide biosynthesis.

Members of the WRINKLED clade orchestrate tissue-specific regulation of fatty acid synthesis in *Arabidopsis thaliana*.

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Acyl lipids are essential constituents of plant cells where they have four main functions: (i) glycerolipids are basic components of the membranes, (ii) triacylglycerols (TAGs) are the major form of carbon and energy store in seeds and fruits, (iii) cuticular lipids serve as vital hydrophobic barrier, and (iv) minor amounts of plant lipids participate in signaling pathways. Even though the structure and properties of these acyl lipids vary greatly, they derive from the same fatty acid (FA) and glycerolipid biosynthetic pathway. Once the metabolic pathway producing FAs elucidated, the question of the regulation of this pathway arose. In Arabidopsis, the coordinated regulation of genes encoding enzymes involved in FA biosynthesis, including their strong activation in tissues where the demand for acyl chains is high (e.g. in seeds), suggested that transcriptional controls occur. Such regulation may determine the rate of FA synthesis. This has been further established by the isolation of WRINKLED1 (WRI1), a member of the AP2-EREBP family of transcription factors. WRI1 is induced in early-maturing embryos, promoting sustained rates of FA synthesis in tissues dedicated to oil storage. Confirmed target genes of WRI1 encode enzymes of late glycolysis and the FA biosynthetic network. KO *wri1* lines, despite their reduced seed oil content, retain some capacity for FA synthesis, suggesting that the FA biosynthetic network may be controlled by a set of cooperating and/or redundant elements. In order to isolate and characterize some of these regulators, a yeast one-hybrid screen was performed using the promoter of BCCP2, a target of WRI1. This gene codes for the BCCP subunit of the heteromeric acetyl-CoA carboxylase (HtACCase). Among the candidates isolated, WRI3 and 4, two transcription factors of the AP2-EREBP family were identified that appeared to be closely related to WRI1. If the spectrum of their target promoters seemed to be redundant with that of WRI1, their expression patterns suggested different biological functions. This hypothesis was confirmed by the fine characterization of corresponding simple, double, and triple mutants. Whereas WRI1 is the only member of the WRI clade triggering high rates of FA production in seeds, the three WRIs cooperate to provide acyl chains for the synthesis of cuticular lipids in flowers and stems. As a consequence of a lack of cutin and epicuticular waxes, flowers of the *wri1wri3wri4* mutants exhibit organ fusions resulting in the production of aborted siliques.

Characterization of a bifunctional protein from Tetrahymena involved in ether lipid biosynthesis.

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Primary fatty alcohols are found throughout the biological world, either in free form or in a combined state. They are produced by Fatty Acyl Reductases (FARs) and corresponding genes have been isolated from various organisms such as plants, animals, insects and protozoa. In plants, fatty alcohols are common components of plant surface lipids as free alcohols, wax esters or alkyl hydroxycinnamates. In animals, fatty alcohols primarily serve for the synthesis of ether lipids and plasmalogens.
The lipid profile of the free-living ciliate *Tetrahymena thermophyla* is characterized by high amounts of ether lipids as well as by the presence of minor quantities of wax esters. Using the Tetrahymena genome database (http://www.ciliate.org), we found a unique sequence related to Fatty Acyl Reductase. Interestingly, this FAR protein contains at its C-terminus a second domain belonging to the acyl-transferase (AT) superfamily. We therefore named this protein TtFARAT.
For the functional characterization of TtFARAT, we first expressed in yeast the complete open reading frame as well as each domain (TtFAR and TtAT) on its own. Expression of TtFARAT or TtFAR resulted in the production of high levels of free hexadecanol and octadecanol, with wax esters levels remaining close to background. Expression of the TtAT domain alone did not result in any clear lipid phenotype. On the other hand, functional complementation of the *cmy228* yeast mutant (gat1.gat2. + [pGAL1::GAT1 URA3]) by TtAT (as well as by TtFARAT) indicated that TtAT acylates glycerol-3-phosphate (G3P) and/or dihydroacetone-3-phosphate (DHAP). Using *in vitro* assays, we could show that the TtAT domain has strong preferences for DHAP and hexadecanoyl-CoA as substrates, implying that TtAT is a dihydroacetone-3-phosphate-O-acyltransferase (DHAP-AT). Finally, co-expression studies with Tetrahymena Alkyl-DHAP-Synthase allowed us to reconstitute the biosynthesis of ether lipids for the first time in yeast.

A new pathway for the synthesis of the very long chain mono-unsaturated fatty acid components of arabidopsis membrane lipids

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Very Long Chain Mono-Unsaturated Fatty Acid (VLC-MUFAs) with chain lengths of 24 and 26 carbons are major components of Arabidopsis sphingolipids and of the phospholipids phosphatidylserine (PS) and phosphatidylethanolamine (PE). Data from forward and reverse genetic studies, and from lipidomic analysis, will be presented demonstrating that an Arabidopsis fatty acid desaturase that acts on VLC-saturated fatty acids is required to maintain levels of VLC-MUFAs in these membrane lipids. This desaturase is localized in the ER membrane and catalyses the n-9 desaturation of 24:0 and 26:0. These observations are contrary to the existing hypothesis that VLC-MUFAs result from elongation of monounsaturated long chain fatty acids and demonstrate a new fatty acid desaturation pathway. Other members of the gene family were also demonstrated to encode VLCFA desaturases with differing regioselectivity, their biological role in Arabidopsis is currently unknown.

Acyl Carrier Protein Thioesterase Activity (FATA) is essential for embryo development in *Arabidopsis thaliana*

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Chloroplasts are the sites of the *de novo* synthesis of the predominant proportion of fatty acids in plant cells. Fatty acids derived from chloroplasts are essential for the establishment of the glycerolipid bilayer in plastidial and extraplastidial membranes, and for the production of storage oil in the seeds. Acyl-acyl carrier protein (ACP) thioesterases hydrolyze acyl-ACPs in the chloroplast releasing free fatty acids. Fatty acids are exported from the plastid and activated by long chain fatty acid-CoA synthetases (LACS) to serve as substrates for glycerolipid synthesis at the ER. In higher plants, two different forms of acyl-ACP thioesterases exist, FATA and FATB, which differ in amino acid sequence and substrate specificity. FATA shows highest activity with oleoyl-ACP (18:1-ACP). In Arabidopsis, there are two loci predicted to encode type A acyl-ACP thioesterases, FATA1 and FATA2. There is one FATB enzyme in Arabidopsis which shows broad substrate specificity for long chain acyl groups (16:0, 18:0, 18:1). The *fatb* mutant of Arabidopsis contains reduced amounts of 16:0 in seed lipids. For the two *FATA* genes, only mutants carrying insertions 5' of the start codon were available which still show considerable expression of *FATA1* and *FATA2* transcripts. To study the role of FATA in the regulation of plant lipid biosynthesis, we obtained two Arabidopsis single mutants (*fata1, fata2*) which carry insertions between the start and stop codon. Expression analysis by RT-PCR indicated that *fata1* and *fata2* represent null alleles. Analysis of the F2 progeny derived from the cross of *fata1*, *fata2* revealed that the loss of the two *FATA* genes is embryo-lethal. The *fata1fata2* embryo development is arrested at late heart stage. Fatty acid composition of the *fata1fata2* embryos is most similar to that of wild type torpedo stage embryos. Thus, embryo development in the *fata1fata2* double mutant comes to a halt when lipid export from the chloroplast is up-regulated at the onset of storage lipid biosynthesis.

Dynamic Changes in Glycerolipid Metabolism under Phosphate-Starved Conditions

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Glycerolipid metabolism is dramatically changed under nutrient starvation. Particularly in plants, it is well known that phosphate (Pi) starvation induces global changes in membrane lipids from phospholipids to glycolipids, which are crucial to adapt Pi-limited conditions. TypeB MGDG synthases (MGD2 and MGD3) have major roles to synthesize galactolipids under Pi-starved conditions. We recently found that MGD3 overexpressor became more tolerant to phosphate limited conditions. It suggests that biosynthesis of galactolipids is still limiting step in wild type plants under Pi-starved conditions. We also found that leaf TAG levels are increased upon Pi starvation. In a starch-deficient mutant (*pgm1*) grown under phosphate-depleted conditions, the TAG levels were 10-fold higher than that of wild type under nutrient-sufficient conditions. Similar increase in TAG content was observed in a double mutant of soluble type phosphatidate phosphatases (*pah1pah2*). Since PAH1/PAH2 is involved in the membrane lipid alteration under Pi-limited conditions, impairment of the membrane lipid change may cause the accelerated accumulation of storage lipids. Link between membrane and storage lipid syntheses in plant leaves will be discussed in this presentation.

Identification of the missing epimerase involved in galactolipid synthesis in cyanobacteria – Are galactolipids necessary for the thylakoid membranes?

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The thylakoid membranes of oxygenic photosynthetic organisms are dominated by the galactolipids, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). These galactolipids occupy more than 80% of the thylakoid membranes, but it is unknown why the lipids of the membranes utilize galactose instead of glucose as a polar head.
In plants, MGDG is synthesized by transfer of galactose from UDP-Gal to diacylglycerol (DAG) by MGDG synthases. On the other hand, in cyanobacteria, it is hypothesized by biochemical analyses more than three decades ago that first a glucose molecule of UDP-Glc is transferred to DAG for synthesis of monoglucosyldiacylglycerol (MGlcDG) by MGlcDG synthase (*mgdA*) and then isomerizes to MGDG by unknown epimerase reaction. To identify the reason of galactolipid utilization in the thylakoid membranes, we took advantage of this cyanobacterial MGDG synthetic pathway. We have tried to identify the genes involved in this pathway to prove the hypothesis and found two genes for sugar transferases *mgdA* and DGDG synthase (*dgdA*). Here, we report the identification of the final piece, the gene for the epimerase (*mgdE*) by a comparative genomic analysis. The gene product did not have typical epimerase motif but had the Rossmann fold for NAD binding, frequently found in oxidoreductases. Co-expression of *mgdA* and *mgdE* in *E. coli* accumulates MGDG, confirming the epimerase reaction by *mgdE*. Knock out mutants of *mgdE* gene were viable on solid agar plates but not in liquid medium. These mutants are expected to accumulate MGlcDG instead of MGDG. From the results obtained, we will discuss requirement of galactolipids in the thylakoid membranes.

Requirement of phosphatidylglycerol for development of embryo and chloroplasts in Arabidopsis

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In higher plants, PG is synthesized in three subcellular compartments; in plastids such as chloroplasts, in the endoplasmic reticula (ER), and in mitochondria. PG synthesized in chloroplasts plays an important role in photosynthesis. By contrast, PG synthesized in mitochondria is utilized for biosynthesis of cardiolipin. Although PG is also synthesized in the ER, the role of ER-synthesized PG remains to be clarified.

We previously isolated a *pgp1* mutant of Arabidopsis, which has a T-DNA insertion in the *PGP1* gene for PG phosphate synthase involved in the biosynthesis of PG in plastids and mitochondria. The *pgp1* required sucrose for growth, and the development of chloroplasts in the mutant leaf and embryonic cells was severely arrested, demonstrating that biosynthesis of PG in plastids is essential for the development of chloroplasts. In addition to the *pgp1*, we isolated *pgp2* mutant, which has a partial deletion in the *pgp2* gene for PGP synthase presumably located in ER. The *pgp2* mutant did not show any phenotype. However, the development of embryo in the double mutant made by crossing the *pgp1* with *pgp2* was delayed and could not reach to mature stage under standard growth conditions. These findings indicate that PG plays critical roles in the development of embryo and chloroplasts in Arabidopsis

Updating the galactolipid synthesis model

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Recent analytical advances in the lipids field, involving the quantitative analysis of a broad range of lipid molecular species using mass spectrometry based platforms, allow us to re-asses and fine tune the knowledge of classic plant lipid studies. In this study, we focus on the galactolipid synthesis model and confront it with results of UPLC-MS measurementes of lipid abundance. Classic studies unraveled the two-pathway oringin of galactolipids. Although galactose is added to diacylglycerol (DAG) in the plastid, the utilized DAG can be produced either in the chloroplast or in the endoplasmic reticulum (ER). One of the observations that we can make on Arabidopsis lipid composition is the existence of two pools of 34-C digalactosyldiacylglycerol (DGDG), one pool has a saturated 16-C chain, whereas the other has an unsaturated 16-C chain. These two pools are consistent with the two pathway origin of galactolipids but at the same time challenge the currently accepted model of galactolipid synthesis. It is assumed that under normal conditions the enzyme MGD1 catalyzes the synthesis of monogalactosyldiacylglycerol (MGDG) in the inner envelope of the chloroplast using as a substrate plastidial and ER DAG pools. The conversion of MGDG to DGDG is catalyzed in turn by the enzyme DGD1 in the outer enevelope. To date, the location of a key enzyme, the 16/C desaturase FAD5, has not been considered. The enzyme was found in the chloroplast envelope, but still ignored if in the inner or outer membrane. Notheless, in both cases FAD5 would desaturate 16-C, and there would be no DGDG pool with saturated 16-C. We propose a model where MGDG synthesis occurs in both envelope membranes and FAD5 locates to the inner envelope, providing a spatial separation that allows 16-C in the outer envelope to remain saturated. The study exemplifies the use of a lipidomic approach to re-evaluate known biosynthetic pathways and assign a putative subcellular localization of a protein based on a lipid composition.

Search for genes involved in membrane lipid homeostasis in *Arabidopsis thaliana* based on a screening of a collection of EMS mutants resisting to galvestine-1, an inhibitor of galactolipid synthesis.

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Monogalactosyldiacylglycerol (MGDG) and digalactosyl¬diacylglycerol (DGDG) are the most abundant membrane lipids in the chloroplast of plant cells. MGDG and DGDG are synthesized in the inner and outer membranes of the envelope that surrounds this organelle, by the action of MGDG synthases (MGD1, MGD2 and MGD3) and DGDG synthases (DGD1 and DGD2) respectively. The chloroplast contains the photosynthetic membranes (the thylakoids) in which the photosystems (multi-protein complexes that capture light energy required for photosynthesis) are embedded. Galactolipids are known to be essential for the structure (and function) of these multi-protein complexes. They are also critical for the thylakoid biogenesis and are a source of lipid for other cell membranes, outside the chloroplast, when plants are exposed to environmental stresses like phosphate deprivation.
Based on a high throughput chemical screen, we have previously characterised a novel compound, called galvestine-1, which inhibits MGDs by competition with its diacylglycerol substrate. To dissect the mode of action of galvestine-1 in *Arabidopsis thaliana*, a screening of EMS (ethyl methanesulfonate) mutants (~ 54,500 mutations) has been initiated in the first year of my PhD project. The aim of this screening was to select galvestine-1-resisting mutants.
In the second year of my PhD, one part of the project is the purification of the mutations of these selected mutants by backcrossing, in order to isolate the focused mutations. Then, the mutants will be characterized by observation of their phenotypes, including variations of the expression of genes involved in lipid metabolism, presence of corresponding proteins, and eventually lipid profiles. Another part of the project is the mapping of these mutants by backcrossing with another ecotype to identify the chromosomes bearing the mutations and progress to find the mutated genes.
These genes will be studied in order to understand their roles after the galvestine-1 treatment and this analysis will provide data on the mechanism of the action of galvestine-1. In the future, this study aims to advance our knowledge on the homeostasis of membrane lipid in plant cells.

Biophysic properties of biomimetic membranes obtained by self-assembly of natural chloroplast lipids

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Chloroplasts are delineated by an envelope made of two membranes, the outer and the inner envelope membranes (oem and iem, respectively), and contain stacks of flattened photosynthetic membranes (thylakoids). Chloroplast membranes differ from all other cellular membranes by their low proportion of phospholipids, represented mainly by phosphatidylglycerol (PG), and their very high content of glycolipids, mainly mono- and digalactosyldiacylglycerol (MGDG, DGDG).

The biogenesis of chloroplast membranes raises two important questions closely linked to the biophysical properties of their lipids.
[(1)] The biosynthesis of PG, MGDG and DGDG occurs in the chloroplast envelope. In the case of galactolipid synthesis, the pathway involves the production of phosphatidic acid (PA); the hydrolysis of PA into diacylglycerol (DAG); the galactosylation of DAG into MGDG and then into DGDG. In addition, PA is an essential activator of MGDG synthesis. Since the involved enzymes are distributed in the iem and the oem, with different orientation of the catalytic sites which may face opposite sides of the bilayer, the topology of the pathway raises critical and still unresolved questions. To operate, DAG and PA should for instance relocate from the inner monolayer to the outer monolayer of the iem. Reverse relocations should also occur to allow the insertion of PA and DAG formed in extraplastidial membranes. To comprehend the complete biosynthetic scheme of chloroplast lipids, we thus need to understand the precise dynamics of DAG and PA in membranes of natural chloroplast lipid composition.
[(2)] Lipids produced in the envelope are subsequently used for the high demand of membranes required for thylakoid biogenesis. These photosynthetic membranes self assemble very rapidly in the form of flattened sacks, which stacking was shown to involve, at least partly, photosystem subunits. Since the inhibition of MGDG synthesis, by genetic and chemical genetic approaches, leads to the appearance of iem-thylakoid connections, we address the question of the possible role of natural galactolipids in the early steps of thylakoid biogenesis.

Both questions are here addressed using non-invasive and non-destructive biophysical techniques, i.e. neutron reflectometry and neutron diffractometry. We present our results indicating that galactolipids have remarkable and unsuspected biophysical properties that favour lipid trafficking and thylakoid stacking independently of protein presence.

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Molecular biology of triacylglycerol biosynthesis in developing flax seed.

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During seed development in flax (*Linum usitatissimum*), triacylglycerols (TAGs) may be synthesized through a combination of diacylglycerol acyltransferase (DGAT) and phospholipid:diacyglycerol acyltransferase (PDAT) activities. DGAT catalyzes the acyl-CoA-dependent synthesis of TAG whereas PDAT catalyzes the transfer of a fatty acyl chain from nitrogenous phospholipid to *sn*-1, 2-diacylglycerol to generate TAG. The flax genome was shown to contain a single *DGAT1*, three *DGAT2* and six *PDAT* genes. Phylogenetic analysis indicated that *PDATs* are divided into three families, each containing two genes. Expression profile analysis indicated that *DGAT1*, two *DGAT2s* and three *PDATs* are preferentially expressed in developing flax seed embryos. Functional expression of the corresponding cDNAs in *Saccharomyces cerevisiae* (strain H1246) showed that DGAT1 is the most active enzyme. When the yeast were cultured in the presence of alpha-linolenic acid (alpha-18:3), however, two of the recombinant PDATs also resulted in substantial accumulation of TAG. Further investigation indicated that these two PDATs can also be stimulated by culturing yeast in presence of different polyunsaturated fatty acids (PUFAs), including alpha-linoleic, arachidonic, stearidonic or eicosapentanoic acid. One of these PDATs appeared to preferentially catalyze the synthesis of trilinolenin upon culturing yeast in the presence of alpha-18:3. TAG accumulation, however, was not stimulated by oleic or linoleic acid, using this isoform of PDAT, which suggests this enzyme has a restricted preference for substrates containing PUFAs. This TAG-biosynthetic enzyme might have practical applications for increasing the accumulation of PUFAs in oilseeds for food, feed and industrial applications.

S4 01 - Tuesday 10th July
Storage lipids: accumulation and modification

Accumulating triacylglycerol in leaves via the monoacylglycerol acyltransferase pathway

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Monoacylglycerol acyltransferases (MGATs) are predominantly associated with lipid absorption and resynthesis in the animal intestine where they catalyse the first step in the monoacylglycerol (MAG) pathway by acylating MAG to form diacylglycerol (DAG). Typical plant TAG biosynthesis routes such as the Kennedy pathway do not include an MGAT step. Rather DAG and TAG are synthesised *de novo* from glycerol-3-phosphate (G-3-P) by a series of three subsequent acylation reactions although a complex interplay with membrane lipids exists. We will show that heterologous expression of a mouse MGAT acyltransferase in *Nicotiana benthamiana* significantly increases TAG accumulation in leaf tissues despite the low levels of endogenous MAG substrate available. In addition, DAG produced by this acyltransferase can serve as a substrate for both native and coexpressed diacylglycerol acyltransferases (DGAT). Based on *in vitro* yeast assays and expression results in *N. benthamiana*, we propose that co-expression of a MAG synthesising enzyme such as *A. thaliana* GPAT4 and a MGAT or bifunctional M/DGAT can result in DAG and TAG synthesis from G-3-P via a route that is independent and complementary to the endogenous Kennedy pathway and other TAG synthesis routes. The possibility of this novel method of increasing oil content in leaf and other vegetative tissues by recruiting MAG as a substrate for TAG biosynthesis will be discussed.

Phosphatidylcholine diacylglycerol exchange and acyl-editing are major mechanisms affecting fatty acid composition in seed triacylglycerols

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Seed storage triacylglycerols (TAG) have numerous applications including food and industrial uses, which are affected by the fatty acid composition. It is essential to understand the mechanisms of fatty acid modification and their assembly into TAG during seed development. We have previously discovered a new enzyme, phosphatidylcholine diacylglycerol cholinephosphotransferase (PDCT), that catalyzes the phosphocholine headgroup exchange between PC and DAG, thus playing an important role during TAG synthesis. We also showed that acyl-editing is the major pathway for newly synthesized fatty acids entering PC for further modification. We have now identified the candidate genes encoding the acyl-CoA lyso-PC acyltransferases (LPCATs) in Arabidopsis that are responsible for acyl-editing. Mutant characterization and radiolabeling experiments indicate that PDCT and LPCATs are responsible for nearly 70% of newly synthesized 18:1 (oleic acid) entering PC for desaturation. Therefore PDCT and LPCATs are major mechanisms for acyl fluxes during TAG synthesis, and represent promising targets for genetic manipulation to improve seed oil fatty acid composition. For example, we show that PDCT is required for efficient metabolism of hydroxy fatty acids in transgenic Arabidopsis seeds.

Olive pollen ans seeds as models for studies on lipid mobilization machinery during germination process

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In both, pollen grains and seeds the process of germination is accompanied by an intense metabolic activity of cells and complex structural changes at the sub-cellular level. Germination is associated also with degradation and mobilization of the reserves accumulated during pollen and seed maturation. In olive, the major amount of these reserves consists of storage lipids deposited in discrete spherical organelles called oil bodies (OBs). These structures represent the primary energy reserve to support periods of active metabolism. Our preliminary studies showed that during olive seed and pollen germination a gradual decrease of OBs occurs in the cells of olive cotyledon and in the pollen tube, respectively. Here, we report the spatio-temporal organization of lipase, phospholipases A and lipoxygenase during *in vitro* olive pollen and seed germination. The association of phospholipases with OBs was shown in mature pollen grain, whereas lipase and lipoxygenase appeared on the OBs' surface during pollen hydration. During pollen tube growth on a medium lacking any carbon source (sucrose) the sugar depletion did not affected pollen tube growth, but caused: 1) faster migration of OBs from pollen grain into the pollen tube, 2) significant increase in the level of lipase and lipoxygenase during pollen germination comparing to pollen tubes growing on medium with sucrose, 3) substantially higher lipase and LOX activities. Treatment of germinating pollen with lipase and LOX inhibitors strongly affected the pollen tube growth in both, sugar containing and sugar-depleted germination medium. According to our results the storage lipids present in the mature olive pollen grain supply sufficient energy for proper pollen germination and that lipid mobilization machinery is directly connected to this process, but its functioning depends on extracellular conditions during *in vitro* pollen germination.

In the mature olive seed the cytoplasm of cotyledon cells is completely filled by protein bodies (PBs) and OBs. In the living cells of olive cotyledons just after seed imbibition, we localized the PLA1 and PLA2 activities only on the OBs surface, but not in the PBs area. At the same time, lipase activity was localized only in the in PBs. These results, together with the fact that lipase and LOX appeared on the OBs surface later in olive germination course, support the hypothesis that phospholipases as first have the access to OBs membrane, causing its reorganization or hydrolysis, which in turn allow the direct TAG mobilization by lipases and LOX.

Interestingly, during olive seed germination the lipase and LOX activities were found in the PBs matrix, accompanying the gradual relocation of lipidic material from OBs to PBs area revealed by Sudan Black staining. These results strongly indicate the PBs of olive seed as multifunctional structures, spatially and functionally connected to OBs breakdown and storage lipids mobilization during seed germination.

Our results showed that germinating pollen grain and seed of olive are excellent models for studies of lipids breakdown process. These two models represent two distinct mechanisms of spatio-functional organization of storage lipid mobilization in the cells of different biological role and fate. However, our studies showed that both, germinating pollen grain and seed serve also as good examples of the common pattern of lipids mobilization during the periods of active metabolism in oleaginous plants.

How to design new sunflower oils: triacylglycerols assembly target.

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Consumers are demanding healthier fats for the food industry. Since most vegetable fats are rich in unsaturated fatty acids, these vegetable oils are often chemically modified by means of hydrogenation and/or transesterification to increase their content of solids. These processes produce fats containing *trans* fatty acids and/or saturated fatty acids in the triacylglycerol (TAG) *sn*-2 position, another alternative is to use palm oil, rich in palmitic acid, both being related with negative effects on human health.

In this regard, stearic acid is not related to cardiovascular diseases, unlike other saturated fatty acids. For this reason, it would be interesting to develop a fat with a higher stearic acid content that to be used for the manufacture of margarines and related products, without any chemical transformation, vegetable fats rich in distearic TAGs species will be a healthy alternative to palm and chemically modified oils.

In sunflower seeds, TAG biosynthesis is biased towards the production of monounsaturated TAG species. Biochemical characterization of acyltransferases has being carried out to determine the enzymatic activities responsible for this bias. Furthermore, we have selected new sunflower lines with a higher content of stearic-oleic-stearic triglyceride species by mutagenesis and recombination from sunflower lines with high stearic acid content. In this communication, analytical, genetic and biochemical studies done so far will be presented.

New oil body associated proteins

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We used two transcriptomic approaches (mRNA sequencing and microarray hybridization) in order to characterize gene expression in maize scutellum during seed maturation and germination. Many genes involved in metabolism are expressed during seed maturation, predominantly in the parenchymal cells. One of the genes predominantly expressed in scutellum during seed maturation is *obap1*, which has a pattern of expression very similar to oleosin. OBAP1 fused to YFP co-localize with oleosins. Arabidopis insertional mutants of *obap* genes do not show any significant phenotype. OBAP1 protein contains hydrophobic regions and deletion analyses demonstrated that one of them is the responsible of the interaction with the oil bodies. A proteomic analysis allowed us to identify new proteins associated with oil bodies during seed maturation and germination. In addition to oleosins, caleosins and steroleosins, we also observed other less abundant proteins. Eighteen proteins were differentially accumulated in oil bodies of germinating scutella compared to dry seeds. Prohibitin 2, stress-inducible Tim17 and manganese superoxide dismutase were overaccumulated during germination. The abundance of cupin2, two different protein disulfide isomerases, a triosephosphate isomerase, a class IV heat shock protein, the embryonic protein DC-8, the 60S ribosomal protein P0, a nucleoside-diphosphate kinase, and a rubber elongation factor protein decreased during germination. Some of them are located in organelles other than oil bodies, suggesting that oil bodies may interact with these organelles. We also suggest that oil bodies may act as transient storage depots for proteins that are temporally in excess.

Dissecting the alkyl hydroxycinnamate biosynthetic pathway

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Alkyl hydroxycinnamate esters represent the junction of two distinct metabolisms, the very-long-chain-fatty acid (VLCFA) and phenylpropanoid pathways. Alkyl hydroxycinnamates are typically described as associated with suberized tissues. Whereas the suberin polymer is insoluble, alkyl hydroxycinnamates are distinct from suberin as they can be readily extracted in chloroform and other organic solvents. Well-known examples of alkyl hydroxycinnamates include the alkyl ferulates found in the periderm of potato (*Solanum tuberosum*) and other tubers. “Root waxes” of Arabidopsis contain a large proportion of alkyl hydroxycinnamates which are dominated by alkyl caffeates. Here we address several issues on the localization, synthesis, and biodiversity of alkyl hydroxycinnamates including: 1) the identification and characterization of an acyltransferase responsible for the synthesis of alkyl caffeates, 2) the identification of genes responsible for synthesizing and supplying the fatty alcohol substrates required for alkyl hydroxycinnamate synthesis, and 3) an initial survey of the phylogenetic distribution of alkyl hydroxycinnamates in root waxes.

Three suberin biosynthetic genes in potato: how they contribute to the polymer?

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Suberin is a complex cell wall polymer used by land plants to regulate apoplastic water transport and to restrict infection. It consists of a fatty acyl- and glyceryl-based polyester esterified to ferulic acid. Suberin deposition takes place in barrier layers such as the periderm in mature stems, roots and tubers and in the exodermis and endodermis of the primary root. Understanding suberin biosynthesis at the genetic and molecular level is central for plant science.
To advance the molecular study of suberin, we first sought suberin candidate genes by means of subtractive libraries from cork oak bark and potato skin [1-2]. Among the genes upregulated in these suberized tissues, we selected key putative suberin biosynthetic genes for reverse genetics in potato. To date, three genes encoding a fatty acid elongase (StKCS6), a fatty acid hydroxylase (CYP86A33), and a fatty .-hydroxyacid/fatty alcohol hydroxycinnamoyl transferase (FHT) have been characterized in the tuber periderm by RNAi silencing [3-5]. Overall, analyses of the silenced lines has provided significant insights into the structure and function of suberin. We showed that suberin and its associated wax share the same precursors and that both suberin composition and amount affect the sealing properties of periderm barrier. We also demonstrated that .-functionalized fatty acids but not ferulate esters are critical to assemble the polyester in its typical lamellar structure. This last result is contrary to the models proposed to date for suberin structure, in which ferulate esters are crucial for the formation of lamellae.
To gain deeper insight into suberin molecular structure, we have initiated the appication of new analytical methodologies such as 13C CP-MAS NMR to intact periderm from the three silenced lines. Preliminary results showed a distinctive spectrum for each line, validating the technique for the screening of modified suberins and complementing the results obtained by chemical analyses.
On the other hand, since FHT showed transcriptional expression in suberized tissues, promoter-based studies have been conducted. The results showed the capability of FHT promoter to direct the expression of target genes to suberized tissues for biotechnological or research applications.

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Reconstitution of plant alkane biosynthesis in yeast demonstrates that Arabidopsis CER1 and CER3 are core components of a VLC-alkane synthesis complex

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In land plants, very-long-chain (VLC-) alkanes are major components of cuticular waxes that cover aerial organs mainly acting as a waterproof barrier to prevent non-stomatal water loss. Although thoroughly investigated, plant alkane synthesis remains largely undiscovered. The Arabidopsis CER1 protein is recognized as an essential element of wax alkane synthesis; nevertheless, its function remains elusive. In this study, a screen for CER1 physical interaction partners was performed. The screen revealed that CER1 interacts with the wax-associated protein CER3 and ER-localized cytochrome b5 (CYTB5s). The functional relevance of these interactions was assayed through an iterative approach utilizing yeast as heterologous system. In a yeast strain manipulated to produce VLC-acyl-CoAs, a strict CER1 and CER3 co-expression resulted in VLC-alkane synthesis. The additional presence of CYTB5s was found to enhance CER1/CER3 alkane production. Site directed mutagenesis showed that CER1 histidine-clusters are essential for alkane synthesis while CER3's are not, suggesting that CYTB5s are specific CER1 co-factors. Collectively, our study reports on the identification of plant alkane synthesis enzymatic components and supports a new model for alkane production in which CER1 interacts with both CER3 and CYTB5 to catalyze the redox-dependent synthesis of VLC-alkanes from VLC-acyl-CoAs.

A bifunctional AP2/ERF-type transcription factor represses cuticular wax biosynthesis and activates dark-inducible genes under dark conditions in Arabidopsis

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As sessile organisms, plants must adapt to changing environmental conditions for optimal growth and survival. The aerial parts of plants are covered by lipophilic cuticular layer, which is involved in prevention of non-stomatal water loss, acclimation to high light, and scattering of UV radiation. Under dark conditions, total cuticular wax loads were decreased by ~ 20% in Arabidopsis stems and leaves, and the expression levels of representative genes involved in cuticular wax biosynthesis were significantly reduced. To understand regulatory mechanisms of cuticular wax biosynthesis under dark condition, we isolated genes encoding dark-inducible AP2/ERF-type transcription factor, which show higher expression in Arabidopsis stem epidermal peels than in stems. Overexpression of an AP2/ERF-type transcription factor caused alteration of the ultrastructure of cuticular layers and reduction in cuticular wax content, whereas total wax loads were increased in the knock-out plants. The AP2/ERF-type transcription factor harbors transcriptional activation domain at the N-terminus and binds to the ethylene-responsive GCC-box. Microarray results from Arabidopsis overexpressing the AP2/ERF-type transcription factor showed that genes required for cuticular wax biosynthesis were down-regulated and dark-inducible genes were up-regulated compared with wild-type. Up- or down-regulation of genes controlled by the AP2/ERF-type transcription factor was confirmed by quantitative RT-PCR and transcriptional activation assay in tobacco protoplasts. The AP2/ERF-type transcription factor is essential for down-regulation of wax biosynthetic genes and up-regulation of dark-inducible genes under dark conditions. Taken together, this study demonstrates a transcriptional repressor that regulates cucticular wax biosynthesis and provides a new mechanism of plant adaptation to unfavorable light environments.

Arabidopsis ECERIFERUM2 is a component of the fatty acid elongation machinery required for fatty acid extension to exceptional lengths

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Plants produce very long chain fatty acids (VLCFAs) for the synthesis of sphingolipids, suberin, seed oil, tryphine, and cuticular waxes. An ER-bound fatty acid elongase (FAE) complex is responsible for the elongation of VLCFAs in two carbon increments by condensing (n)-acyl-CoAs with malonyl-CoA, and derivatizing the (n+2)-ß-diketone condensation products to (n+2)-acyl-CoAs. FAE complex substrate specificity is determined by a ketoacyl-CoA synthase enzyme that catalyzes the condensation reaction. In *Arabidopsis thaliana*, characterized KCS enzymes involved in cuticular wax synthesis can only elongate VLCFAs up to 28 carbons (C28) in length, despite the predominance of C29-C31 monomers in Arabidopsis wax. This suggests that additional proteins are required for fatty acid elongation beyond C28. The wax-deficient mutant *cer2* lacks waxes longer than C28, suggesting that CER2, a putative BAHD acyltransferase, is required for C28 elongation. Although the previous annotation of CER2 was rationally based on sequence homology, we have found firm evidence that CER2 cannot act as a BAHD acyltransferase. Using a biochemical assay in *Saccharomyces cerevisiae* we demonstrate conclusively that CER2 is directly involved in fatty acid elongation beyond C28. Our results shed light on a novel intricacy of VLCFA elongation, which is necessary for the synthesis of cuticular wax.

Biosynthesis and functions of free and combined fatty alcohols associated with suberin

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Primary fatty alcohols occur widely in free form or in a combined state (e.g. wax esters and alkyl hydroxycinnamates) in organisms of all life kingdoms. They are found in plant extracellular lipid/phenolic-based barriers such as the cuticle coating the aerial surfaces of plants, suberin found in the cell walls of various external and internal tissue layers including roots, and likely in sporopollenin found in the outer walls (exine) of pollen grains. These barriers are important in protecting against water loss and pathogen attack. Three of the eight-member gene family encoding alcohol-forming Fatty Acyl Reductases (FARs) from *Arabidopsis thaliana*, *FAR1*, *FAR4* and *FAR5*, are expressed at sites of suberin deposition (e.g. root endodermal layer and in response to wounding). Analysis of suberin composition of single mutants with T-DNA insertions in *FAR1*, *FAR4*, or *FAR5* showed that suberin composition was modified in each *far* mutant; specifically, C18:0-OH was reduced in *far5*, C20:0-OH was reduced in *far4*, and C22:0-OH was reduced in *far1*, indicating that *FAR1*, *FAR4*, and *FAR5* each generates a distinct chain-length of fatty alcohol found in root, seed coat, and wound-induced suberin. We have further analysed double and triple *far* mutant lines developed using conventional genetic crossing and artificial microRNA mediated gene silencing. The double mutants *far1 far4, far1 far5, far4 far5* showed reduced levels of both respective chain lengths of fatty alcohols in root and seed coat suberin, and the triple mutant *far1 far4 far5* showed reduced levels of all three C18:0 to C22:0 primary fatty alcohols. The C18-C22 alkyl hydroxycinnamates of suberin-associated root waxes were also reduced in these lines. The triple mutant seeds were found to have increased seed coat permeability to tetrazolium salts and were sensitive to abscisic acid (ABA) hormone during germination.

Conserved functions of oxylipins in flowering and non-flowering plants

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Lipid peroxidation is common to all biological systems and metabolites derived there from are collectively named oxylipins. They may either originate from chemical oxidation or are synthesized by the action of enzymes. The isolation of oxylipin forming and metabolizing enzymes as well as the identification of the oxylipin perception machinery in Arabidopsis revealed new insights on oxylipin function in flowering plants. We aim to identify enzymes that are involved in oxylipin metabolism in yet not analyzed genera in order to explore their function, since a still increasing amount of information on eukaryotic and prokaryotic genomes is being generated. In the moss *Physcomitrella patens* we identified and characterized a plastidial oxylipin pathway that shows similarities to flowering plants and mammals. Interestingly is as in flowering plants involved in regulation of sporophyte formation, the sexual organ of the moss. However, knowledge on extra plastidial oxylipin metabolism and perception is lacking so far for the moss. A comparable situation may be found in cyanobacteria, where similar reactions may be catalyzed either by lipoxygenases alone or by bifunctional fusion proteins. According to these findings a new picture for oxylipin formation arises that suggests that first formation of hydroxy fatty acids may first have evolved during evolution in prokaryotes and later on in eukaryotes additional enzymes may have been acquired that lead to formation of more complex oxylipins.

Oxylipin metabolites of the polyunsaturated acylethanolamide, N-linolenoylethanolamine, specifically mediate chloroplast disassembly in cotyledons of Arabidopsis seedlings.

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N-Acylethanolamines (NAEs) are fatty acid derivatives conjugated to ethanolamine via an amide linkage, and this class of lipids regulates diverse physiological processes in both animal and plant systems. Polyunsaturated NAEs are the most prevalent species in seeds of Arabidopsis, and their levels drop dramatically with seed germination and seedling establishment. Evidence *in vitro* and *in vivo* indicates that polyunsaturated NAEs can be depleted during seedling establishment by two competing pathways-- hydrolysis and/or lipoxygenase (LOX)-mediated oxidation. Our hypothesis is that the normal seedling establishment requires the progressive metabolic depletion of NAEs. Exogenous addition of several different NAE types reduces Arabidopsis seedling growth generally, but treatment of seedlings with exogenous N-linolenoylethanolamine (NAE18:3) specifically resulted in a concentration-dependent disruption of chloroplasts over a three day period. Chloroplast disassembly was documented by loss of autofluorescence in confocal scanning fluorescence microscopy and a reduction of extractable chlorophyll from cotyledons. Removal of NAE18:3 resulted in recovery of seedling growth, cotyledon pigmentation and normal chloroplast appearance. Comprehensive oxylipin profiling studies with mutants and pharmacological treatments that impair NAE hydrolysis or oxidation suggests that it is a LOX-mediated oxidation product of NAE18:3 that is responsible for the deleterious effects on chloroplast organization and seedling morphology. Our results indicate that NAE-oxylipin metabolites themselves may function as potent bioactive lipid mediators. We suggest that a combination of regulatory effects is mediated by NAEs depending upon type, and that the collective effects of higher NAE tissue metabolite levels in seeds and seedlings are to arrest normal seedling growth and development, although the precise target(s) of NAE action remain to be elucidated.

bHLH transcriptional factors MYL1, MYL2 and MYL3 modulate jasmonate metabolism and anthocyanin production.

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Jasmonic acid (JA) and its derivatives such as JA-Ile play crucial roles in developmental processes and defense responses against wounding and pathogens. COI1 mediates jasmonate signaling by promoting the JA-Ile-dependent degradation of JAZ proteins. JAZ proteins are repressors of MYC2, a JA responsive transcription factor. After the degradation of JAZs by SCFCOI1, MYC2 is thought to be released to regulate the expression of jasmonate-responsive genes. However, information on other transcriptional factors that regulate jasmonate signaling is rather limited.

Based on our co-expression analysis, we focused on a subset of bHLH transcription factors designated as MYL1, MYL2 and MYL3. We obtained and analyzed T-DNA insertional mutants of each *MYL* gene. Contrary to the *myc2* phenotype, *myl1myl2myl3* mutants were hypersensitive to methyl jasmonate (MJ). To characterize genes regulated by *MYL1*, *MYL2* and *MYL3*, we performed GeneChip analyses of *myl1myl2myl3* and Col. We observed a set of genes that showed enhanced jasmonate responsiveness in *myl1myl2myl3*. For example, *PAP1* and *MYB113*, positive regulators of anthocyanin biosynthesis, showed increased expression in *myl1myl2myl3* after MJ treatment. We confirmed that anthocyanin levels in *myl1myl2myl3* were higher than Col after MJ treatment, and therefore we conclude that *MYL1*, *MYL2* and *MYL3* negatively regulate anthocyanin biosynthesis. We also found that jasmonate metabolic genes show increased expression in MJ treated *myl1myl2myl3*. In wounded *myl1myl2myl3* leaves, the contents of JA and JA metabolite levels were higher than Col. These results suggest that MYL1, MYL2 and MYL3 function as negative regulators of jasmonate metabolism.

Factors affecting hydroperoxide lyase performance in the synthesis of oxylipin volatiles responsible for virgin olive oil quality

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Hydroperoxide lyase (HPL) catalyzes the cleavage of polyunsaturated fatty acid hydroperoxides to aldehydes that are involved in the plant defense response against pest and pathogen attack. Moreover, it plays a major role in forming the aroma profile of many plant fruits and flowers. In this sense, oxylipin volatiles derived from the lipoxygenase (LOX) pathway are the most important quantitative and qualitative compounds in virgin olive oil (VOO) aroma. Among them, straight-chain six-carbon (C6) aldehydes are of the most sensorial significance, but it is somehow modulated by the presence of five-carbon (C5) compounds, generated through a sideway LOX homolytic activity. High-quality VOO is today demanded by consumers not only because of its potential health benefits but also due to its particular organoleptic properties. Thus, production of flavorful olive oils is considered a priority in olive breeding programs. These oxylipin volatiles are synthesized *de novo* when enzymes and substrates meet as tissues are disrupted during olive oil processing. This process is carried out only by physical methods, involving three basic steps, olive fruit crushing, paste kneading, and oil separation. Previous experimental findings suggest that the biosynthesis of VOO aroma compounds depends mainly on the availability of non-esterified polyunsaturated fatty acid to be metabolized through the LOX pathway during the process to obtain this oil. Results also suggest that VOO aroma compound contents depend on the enzymatic activity load of LOX during the oil extraction process. A set of experiments was conducted in order to study the limitation related to the step catalyzed by HPL within the LOX pathway, involving modification of this enzymatic activity load and substrate contents during the oil extraction process. HPL enzymatic activity seems to be constitutive and cultivar dependent, displaying a lower degree of limitation than LOX activity for the biosynthesis of VOO aroma.

Air-transferrd oxylipin volatiles from herbivore-infested tomato plants change uninfested conspecific plants to be defensive.

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Herbivore-infested plants emit various kinds of volatiles to defend themselves against herbivores. Green leaf volatiles, such as (Z)-3-hexenal, (Z)-3-hexenol, and their esters, are major volatile compounds emitted from herbivore-infested plants. These compounds are synthesized from oxygenated fatty acids in damaged plants. It is known that the compounds have a role to induce defensive responses in the neighboring undamaged plants.
Using an air-flow, we observed induced defense of uninfested tomato plants against common cutworms (CCM) when the plants were exposed to the volatiles emitted from conspecific plants infested by CCM. We found that the plants accumulated a glycoside, (Z)-3-hexenyl vicianoside, which had negative impacts on the performance of CCM. Thus, taking in external (Z)-3-hexenol and subsequent glycosylation would be one of the defensive responses in undamaged tomato plants exposed to volatiles from infested plants. When plants of different species were exposed to authentic green leaf volatiles, the plants accumulated the different types of (Z)-3-hexenyl glycosides. These data suggest that taking in external green leaf volatiles and subsequent glycosylation would be one of the widely conserved responses in plants.
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MGDG with C12 and C10 oxo acids are formed after tissue disruption in Arabidopsis.

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When plants are damaged by microbes or herbivores, they rapidly form and emit green leaf volatiles (GLVs) consisting of (Z)-3-hexenal, (Z)-3-hexen-1-ol, and (E)-2-hexenal. It has been believed that hydrolysis of lipids by a lipase to form free fatty acids is the first step of GLV biosynthesis; however, the amount of the counterpart of the biosynthetic pathway, namely, 12-oxo-(Z)-9-dodecenoic acid (C12 oxo acid), is much less than that expected from those of GLVs. In order to clarify the reason for the lower yield of C12 oxo acid, we first conducted an *in vitro* experiment using monogalactosyl diacylglycerol (MGDG) purified from *Trifolium repens* leaves. The result indicated that MGDG could be the substrate for lipoxygenase (LOX) and hydroperoxide lyase (HPL) at least *in vitro*; thus, it would be possible that GLV pathway proceeded without the action of a lipase. With LC-MS/MS analyses on crude lipid extract prepared from homogenized Arabidopsis leaves, we detected MGDG with hydroxylated C12 and/or C10 oxo acids. Digesting the crude lipids with pancreatin yielded free forms of hydroxylated C12 and C10 oxo acids, which supported the formation of MGDG with hydroxylated C12/C10 oxo acids. The amounts of free C12/C10 oxo acids released from MGDG after lipase-digestion were only ca. 20% of those of GLVs, thus, it was assumed that a portion of the oxo acids formed by HPL were converted into the other, still unknown, metabolites.

Oxylipin pathways in marine diatoms: occurrence, regulation and ecological role

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Diatoms are unicellular photosynthetic microorganisms considered the most important group of eukaryotic phytoplankton. They are responsible for 40% marine primary productivity and they are the base of marine food chain. Diatoms produce a plethora of bioactive oxylipins, such as hydroxyacids, ketoacids, epoxyalcohol and unsaturated aldehydes, by lipoxygenases with different regio- and stereo-specificity. This process is triggered by lypolitic acyl hydrolase activity that feeds the downstream lipoxygenase pathway, and that is able to release free C16 and C20 fatty acids from glycolipids. The molecules were characterized by NMR, LC/MS/MS and GCMS. Recently we reported a new MS/MS method to establish the position of fatty acid oxidation, based on the diagnostic mass fragmentation pattern of epoxyalcohol. This was an important tool to explore the occurrence and distribution of oxylipin pathways in different species of marine diatoms as well as in different strain of the same species.
The ecological meaning of oxylipins in marine diatoms was related to interaction with predators, and the physiological role as signalling molecule was investigated along the growth curve.

Isoprenoid precursor availabilities modulate protein prenyltransferase substrate specificities and activities in plants *in vivo.*

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In plants, the post-translational covalent modification of proteins with farnesyl (F) or geranylgeranyl (GG) moieties implies the involvement of both the cytosolic mevalonate (MVA) and the plastidial methylerythritol phosphate (MEP) pathways for the synthesis of the isoprenyl groups. This was demonstrated by incorporation of radiolabeled MVA [1], but also of deoxyxylulose (DX) [2] into proteins. Recently we proved that in our major test system, specifically transformed tobacco BY-2 cells, under standard growth conditions MEP-derived geranylgeranyl diphosphate (GGPP) was used as a substrate by protein GGPP transferase type 1 (PGGT-1) and could not be replaced by a MVA-derived isoprenyl group [3]. We extended now our studies to Arabidopsis plants, also using specific KO mutants, and thereby verified on the basis of the same visualization systems that MEP-dependent isoprenylation is a consequence of limited PGGT-1 substrate specificity *in vivo*. We also analyzed in detail the involvement of both pathways in providing isoprenyl residues to modify proteins supposed or even being reported to become farnesylated. Under certain circumstances the MEP pathway can be used for delivering the substrate to modify such protein substrates, too. Modulation of isoprenoid metabolic pools, either by treating plant cells with chemicals or through overexpression of specific enzymes revealed disparities in modifying farnesyl- or geranylgeranyl-specific protein substrates. To examine the physiological regulation that might lead to involve two biosynthetic pathways for protein isoprenylation, we have set up a screening system, aiming at identification of metabolites or compounds that act as molecular switch for using one or the other pathway under particular conditions.

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Accumulation of fatty acid phytyl esters in *arabidopsis thaliana* during senescence

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During senescence or stress (e.g. dark treatment, nitrogen starvation), thylakoid membranes are disintegrated, releasing a large amount of potentially harmful breakdown products into the stroma and cytosol. Degradation of chlorophyll and galactolipids results in the accumulation of free phytol and free fatty acids, which have detergent-like characteristics. During stress, these lipid catabolites are converted into fatty acid phytyl esters which are deposited in the plastoglobules of the chloroplast. We could show that one function of fatty acid phytyl esters is the reversible deposition of lipid building blocks (phytol, fatty acids) during periods of chlorotic stress. Fatty acid phytyl esters can be broken down for re-synthesis of chloroplast lipids after growth conditions have improved. We identified two genes (Phytyl Ester Sythase 1, *PES1*; Phytyl Ester Synthase 2, *PES2*) primarily responsible for the synthesis of fatty acid phytyl esters in Arabidopsis using a bioinformatic approach. In silico data and RT-PCR experiments revealed a strong expression of *PES1* and *PES2* during senescence and nitrogen deprivation. Heterologous expression of PES1 and PES2 in yeast showed the production of fatty acid phytyl esters, triacylglycerol and sterol esters. *In vitro* assays with recombinant proteins demonstrated that acyl-CoAs, acyl-ACPs and galactolipids can serve as acyl donors for the acylation reaction. Furthermore, heterologously expressed PES1 and PES2 proteins harbor diacylglycerol acyltransferase activity which is even higher than the phytyl ester synthesis activity. A double knockout mutant of Arabidopsis (*pes1 pes2*) showed a 90% reduction of fatty acid phytyl esters and a 30% decrease in triacylglycerol levels in the leaves.

Triterpene production in *Panax ginseng* C.A. Meyer and its modulation by HMG-CoA reductase and oxidosqualene cyclases

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Isoprenoids, also known as terpenoids, represent the most diverse group of natural products found in all plant organisms. Ginsenosides are glycosylated triterpenes, which are considered to be the major pharmaceutically active ingredient in ginseng known as an adaptogenic herb. The backbone of ginsenosides, which is categorized as protopanaxadiol (PPD), protopanaxatriol (PPT), and oleanane saponin, is synthesized via the isoprenoid pathway by cyclization of 2,3-oxidosqualene mediated with dammarenediol synthase or ß-amyrin synthase. Functional characterization of several oxidosqualene cyclases such as dammarenediol and squalene epoxidase, and ß-amyrin synthase are demonstrated in literature. However, the possible roles of the rate-limiting 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) which catalyzes the first committed step of the mevalonate pathway for the isoprenoid biosynthesis in ginseng plant was remained uncovered. Heterologous overexpression of HMGR in Arabidopsis showed subcellular localization in ER and spherical vesicular structure similar to the previously reported endogenous HMGR. It suggests that HMGR is involved in directing flux of pathway intermediates into specific isoprenoid compounds. Mevinolin, a competitive inhibitor of HMGR, treatment on ginseng adventitious roots caused significant reduction of total ginsenoside, which explains the important role of mevalonate pathway for the ginseng saponin production. Promoter::GUS expression of ginseng *HMGR* gene showed dark-induced *HMGR* expression in hypocotyl region and prolonged dark condition of 3-year-old ginseng resulted with reduced total ginsenoside contents, which suggest that there is a tight regulation of light and dark dependent secondary metabolite biosynthesis in ginseng plant.

Trisporoids composition in *Blakeslea trispora* under the lycopenogenesis stimulation

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The hetherotallic fungus *B. trispora* is used as an industrial producer of two carotenoids – ß-carotene and lycopene. Sexual hormone, trisporic acids (TAs), is the strongest stimulator of carotenogenesis in *B. trispora*. That is why the combined culture of (+) and (-) strains is used in biotechnology. To enhance the process ß-ionon and 2-amino-6-methyl-pyridine (MAP) are applied. These two stimulators have different ways of action, which let us suggest the additional intensification of lycopenogenesis being possible using the combination of them. So this work was aimed to study carotenoid and TAs composition under ß-ionon and MAP treatment.

We showed the considerable stimulation of lycopenogenesis and essential changes in trisporoids composition under the action of ß-ionon and MAP. The main trisporoids in control culture were TAs and trisporines/ trisporols. The common tendency in MAP and ß-ionon action in changing the trisporoid composition was a sharp decrease of TAs and the emergence of an unknown substance with absorption maximum at 250 nm. The difference in their action was refered to trisporines/ trisporols level. It was a little lowered by MAP and 2,5-3-fold reduced by ß-ionon. The use of their combination led to an even stronger inhibition of trisporines/ trisporols (3-fold) and TAs (not detected) synthesis. Under the stimulation of ß-ionon and MAP production of lycopene was increased and the inhibition of TAs formation was shown, which means that the action of stimulators is not mediated by the enhancement of TAs synthesis.

The metabolic engineering of omega-3 long chain polyunsaturated fatty acids in transgenic plants – less is more.

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There is now considerable evidence as to the importance of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) in human health and nutrition. Unfortunately, current sources are either in severe decline (fish oils) or expensive (via microbial fermentation), leading to the search for an alternative source. We have transgenically assembled the primary biosynthetic pathway for LC-PUFAs in both model plants and crop species. Our data indicate that whilst the transgenic synthesis of C20 LC-PUFAs such as eicosapentaenoic acid is clearly feasible, a number of factors may limit the efficient heterologous reconstitution of this pathway. We have attempted to address this problem in a systematic manner by firstly identifying different metabolic “bottlenecks” via lipidomics and then seeking genetic interventions to overcome them. It seems likely that a generic bottleneck resides within the primary LC-PUFA biosynthetic pathway as a result of the “substrate dichotomy” between the lipid-dependent desaturases and the acyl-CoA-dependent elongases which catalyze the reactions. It is possible to overcome this bottleneck, through the use of acyl-CoA dependent desaturases, but not without impact on the seed lipidome. Perhaps surprisingly, whilst the model species Arabidopsis accumulates EPA to modest levels, the crop species *Camelina sativa* appears a superior host for transgenic accumulation of novel lipids. Elucidating the molecular and biochemical basis for this is the emerging challenge for the future and will require a systems-based approach which will allow us to manipulate lipid metabolism in a predictive manner.

56-Amino acid small subunits of serine palmitoyltransferase stimulate sphingolipid synthesis, impact mycotoxin sensitivity and are essential for pollen viability in arabidopsis

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Sphingolipid synthesis in plants is tightly regulated to provide sufficient amounts of these lipids for growth and endomembrane function and to mediate levels of sphingolipid metabolites that control basic cellular processes such as program cell death (PCD). Increasing evidence points to serine palmitoyltransfersase (SPT), the first enzyme in sphingolipid long-chain base synthesis, as the key regulated step in eukaryotic sphingolipid synthesis. We have previously shown that the core Arabidopsis SPT consists of a heterodimer of AtLCB1 and AtLCB2 subunits that are able to weakly rescue long-chain base auxotrophy when co-expressed in a yeast lcb1.lcb2. mutant. Through homology searches using amino acid sequences for mammalian small subunits of SPT (ssSPT), two Arabidopsis genes designated *AtssSPTa* and *AtssSPTb* were identified that encode 56 amino acid polypeptides. Co-expression of *AtssSPTa* or *AtssSPTb* was shown to enhance SPT activity of the AtLCB1-AtLCB2 heterodimer by =100-fold in the yeast lcb1. lcb2. mutant. Consistent with the subcellular localization of AtLCB1-AtLCB2, AtssSPTa and AtssSPTb were found to be ER-associated. Though both genes are constitutively expressed, *AtssSPTa* transcripts were >400-fold more abundant in pollen than those for *AtssSPTb*. Consistent with this, homozygous *AtssSPTa* T-DNA lines were not recoverable, and 50% pollen lethality was detected in heterozygous *AtssSPTa* T-DNA mutants, which was rescued by expression of the wild-type *AtssSPTa* gene. In addition, over-expression lines of *AtssSPTa* displayed enhanced sensitivity to the PCD-inducing mycotoxin fumonisin B1 (FB1), whereas *AtssSPTa* RNAi lines showed enhanced resistance to FB1. These results show that AtssSPT polypeptides play a critical role in sphingolipid synthesis and cellular functions in Arabidopsis through their ability to stimulate SPT activity. The larger role of AtssSPTs as components of the intricate SPT regulatory network that is responsive to sphingolipid homeostasis will be discussed.

The effect of long chain base sphingolipids on rates of programmed cell death in stressed plant cells.

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Plant cells growing in culture undergo programmed cell death (PCD) if the number of cells drops below a critical density. It has previously been shown that conditioned media can save these cells from death. Conditioned media is media in which cells have been growing and therefore contains secreted cell signals. When diluted cell cultures are supplemented with conditioned media, PCD is not activated, therefore, some of the signals in conditioned medium affect PCD. Previous research tells us that long chain base sphingolipids influence cell death. We have used HPLC-coupled with electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS) to profile the long chain base sphingolipids present in the conditioned media of carrot and Arabidopsis and have found phytosphingosine, sphingosine and dihydrosphingosine. We have induced PCD in Arabidopsis cell suspension cultures using heat stress, or by culturing below the critical density, and have tested LCB (±P) sphingolipids to determine if they influence PCD singly, or in concert. We have found that sphingosine, phytosphingosine-1-phosphate and sphingosine-1-phosphate save heat stressed cells but do not save diluted cells. Additionally, we have also found sphingosine and phytosphingosine (but not dihydrosphingosine) in cyanobacterial (NOSTOC) conditioned media and have found that NOSTOC conditioned media also protects heat stressed plant cells from PCD.

A novel protein anchor for lipid-specific plasma membrane raft targeting in plant cells

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Structural domains and clusters of surface-exposed residues mediate the association of peripheral proteins with the plasma membrane (PM). Post-translational linkage of fatty acid moieties (e.g. myristoylation, palmitoylation) to N- or C-terminal residues are known to serve as auxiliary modifications to stabilize PM association of such proteins. However, in plants only transmembrane domains have been described to mediate tight insertion of proteins into the bilayer.
Here, we describe a novel membrane anchoring mechanism used by plant-specific remorin proteins that are highly enriched in membrane domains called 'membrane rafts'. Their short C-terminal anchor (RemCA) is indispensable and sufficient for tight direct protein-lipid interactionsm particularly with raft-enriched negatively charged lipis (such as polyphosphoinostides and GIPC). Fusion of RemCA to soluble proteins results in their entire immobilization at the PM in planta and targeting to detergent-insoluble membrane (DIM) fractions. Deletion of RemCA abolishes Remorin function in the control of PVX virus propagation. Strength of PM binding mediated by RemCA corresponds to those of classical transmembrane domains. RemCA folds into an amphipathic helical structure and is required for selective binding to negatively charged lipids. Using this structural information, peptides sharing RemCA amino acid composition and the predicted fold were found in various peripheral PM proteins across organismic kingdoms suggesting convergent evolution of this peptide fold.

The role of mannosylated sphingolipids in Arabidopsis: unexpected roles in both growth and defence

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GONST1 (At2G13650) has previously been identified as one of a small family of Golgi-resident nucleotide sugar transporters present in Arabidopsis. Functional complementation of the yeast Golgi GDP-Man transporter mutant *vrg4-2* confirmed the biochemical activity of GONST1 but little else is known about this transporter.
In this presentation, we will provide evidence that GONST1 specifically transports GDP-mannose into the Golgi as part of the process of GIPC mannosylation. The consequences of impaired synthesis of mannosylated sphingolipids on Arabidopsis growth and development will be discussed, as will interactions with the SA defence response. To our knowledge, this is the first example of the altered sugar decoration of sphingolipids resulting in phenotypic changes to plant development.

Degradation of long-chain base 1-phosphate (LCBP) in Arabidopsis: functional characterization of LCBP phosphatase and LCBP lyase

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Sphingolipid metabolites, long-chain base 1-phosphates (LCBPs), are involved in ABA signaling pathways. The LCBPs synthesized by long-chain base kinase are dephosphorylated by LCBP phosphatase (SPP) or degraded by LCBP lyase (DPL). Transient expression of green fluorescent protein fusion in suspension-cultured Arabidopsis cells showed that SPP1 and DPL1 are localized in the endoplasmic reticulum. The level of dihydrosphingosine 1-phosphate was increased in loss-of-function mutants (spp1) compared with wild-type (WT) plants, suggesting a role of SPP1 in regulating LCBP levels. The rate of decrease in fresh weight of detached aerial parts was significantly slower in *spp1* and *dpl1* mutants than in WT plants. A stomatal closure bioassay showed that the stomata of *spp1* mutants were more sensitive than the WT to ABA, suggesting that SPP1 is involved in guard cell signaling. The *dpl1* plants exhibit hypersensitivity to FB1 in rosette leaves and seedlings. In contrast, the response of *spp1* plants to FB1 is similar to that in WT plants. In this symposium, lipid composition and expression of genes involved in sphingolipid metabolism will be reported and discussed in *spp1* and *dpl1* mutants.

Quantification of free sterols, sterol esters, sterol glucosides and acylated sterol glucosides in plants by Q-TOF mass spectrometry

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Sterol lipids represent one of the three major membrane lipid classes in plants, in addition to glycerolipids (phospho- and galactolipids) and sphingolipids. Plants contain free sterols, which are abundant in extraplastidial membranes, and conjugated sterols which can be glycosylated (sterol glucosides) or acylated (sterol esters) or both (acylated sterol glucosides). Sterol glucosides and acylated sterol glucosides are also constituents of the extraplastidial membranes, but the non-polar sterol esters localize to oil bodies in the cytoplasm. In contrast to animals, where cholesterol is the major sterol, the phytosterols sitosterol, stigmasterol and campesterol are predominant in plants. Furthermore, sterol esters and acylated sterol glucosides contain fatty acids of different chain lengths and degrees of unsaturation, resulting in a great diversity of molecular species. To study the biological function of sterol lipids in plants, we developed a method for the comprehensive sterol lipid quantification using Q-TOF (quadrupole time of flight) mass spectrometry. Lipids are extracted from plants in the presence of internal standards, purified by solid phase extraction and measured by nanospray Q-TOF MS by direct infusion without prior chromatography. This method allows the direct measurement of intact conjugated sterols in small tissue samples at high sensitivity. We applied this method to the quantification of sterol lipids in different tissues of *Arabidopsis thaliana* and *Lotus japonicus*, grown in the presence or absence of phosphate. Sterol lipids were also quantified in leaves of barley plants exposed to short term drought stress. Furthermore, the role of sterol lipids during plant-microbe interactions was analyzed in roots and nodules of *Lotus japonicus,* after infection with the symbiotic nitrogen fixing soil bacterium *Mesorhizobium loti,* and during symbiosis with the arbuscular mycorrhizal fungus *Glomus intraradices.* The Q-TOF MS method was well suited to monitor adaptive changes in the amounts and molecular species distribution of the four sterol lipid classes in different plant species and under different growth conditions.

Long chain acyl-CoA synthetase activities involved in lipid flux from ER to plastid

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Plastidial fatty acid synthase provides substrate for prokaryotic lipid synthesis in the plastid as well as for eukaryotic lipid synthesis at the ER. In many plant species including Arabidopsis lipid synthesis at the ER is not only generating cytoplasmic lipids but is also producing precursors for plastidial lipids. The nature of the precursor lipid molecule could not be resolved unambiguously but accumulating evidence by Christoph Bennings lab suggests that phosphatidic acid (PA) may indeed be delivered from the ER to the plastid. The transport of the lipid precursor involves four TGD proteins which are thought to channel PA to a PA phosphatase located at the inner envelope to provide finally diacylglycerol to glycolipid biosynthesis.

In the current model the production of PA in ER-plastid contact zones is predicted to involve a phospholipase D which is converting PC to PA. By analyzing LACS deficient mutant plants we obtained evidence that substantial amounts of fatty acid moieties destined for the transported to the plastid are released from membrane lipids as free fatty acids and get activated to acyl-CoA before becoming incorporated into a lipid transport molecule. Implications of these findings will be discussed.

Role of Arabidopsis acyl-CoA-binding proteins in lipid trafficking

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Acyl-CoA-binding proteins (ACBPs) have been characterized from mammals, yeast, Drosophila and the model plants, Arabidopsis and rice. ACBPs contain a conserved acyl-CoA-binding domain which binds acyl-CoA esters and ACBPs can facilitate intracellular lipid transport. In Arabidopsis, a family of six genes encodes ACBPs which range from 10.4 kDa to 73.1 kDa. These ACBPs have been subcellularly localized to different compartments in the plant cell using autofluorescent-tagged proteins in confocal microscopy, immunoelectron microscopy and western blot analysis on subcellular fractions. Bacterial-expressed recombinant ACBPs have been generated and used for *in vitro* binding assays. Results from these assays indicate that these proteins exhibit differential binding affinities to acyl-CoA esters and phospholipids, implying that ACBPs have non-redundant biological functions *in vivo*. Investigations using knock-out *acbp* mutants and transgenic Arabidopsis lines that overexpress ACBPs demonstrate that Arabidopsis ACBPs can influence plant development and stress responses. Recent findings further suggest that the function of the various Arabidopsis ACBPs in binding lipids appears to be related to their biological functions *in vivo*.

Phloem-mediated long-distance lipid signaling in plants

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Unlike animals, plants cannot move to escape adverse conditions. As a consequence, they evolved mechanisms to detect changes in their environment, communicate these to different organs, and adjust development accordingly. One of these adaptations, the phloem, serves as a major trafficking pathway for assimilates, viruses, RNA, plant hormones, metabolites, and proteins with functions ranging from synthesis to metabolism to signaling. The study of signaling compounds within the phloem is essential for our understanding of plant communication of environmental cues. Determining the nature of signals and the mechanisms by which they are communicated through the phloem will lead to a more complete understanding of plant development and plant responses to stress.
Lipids are known to act as signals at the cellular level. However, their role in long distance signaling in the phloem has received little attention. It is a new area of research which is expected to contribute new concepts to plant development. In analogy to lipid signaling in the blood and to serve as long-distance signals, lipids would have to be released into the phloem, moved bound to a protein “chaperone”, be detected by a receptor and modulate transcription factor activity. Our study of Arabidopsis phloem exudates revealed lipids and lipid-binding proteins with potential roles in performing those functions. One phloem lipid-binding protein (PLAFP) is proposed to mediate interaction with lipids or membrane-bound proteins. We have shown that it binds one of the lipids, phosphatidic acid (PA), which is also present in the phloem. Modifying the amount/ presence of PLAFP in the plant leads to a modification in the phloem-lipid content, changes in development, and reduced drought resistance. We will present data showing the effect of PLAFP on development, phloem lipid profile; lipid-binding properties, expression in response to various stresses, and localization within the plant and on a cellular level.
Our data suggest that PLAFP, and with it bound PA, play a role in long-distance developmental signaling.

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CGI-58 regulates triacylglycerol homeostasis and lipid signaling pathways in plants through interaction with the peroxisomal transport protein PXA1

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Mutation of the Comparative Gene Identification-58 (*CGI-58*) gene in humans causes Chanarin-Dorfman syndrome, a rare genetic disorder characterized by an increase in triacylglycerol (TAG) and lipid droplet (LD) contents in non-lipid-storing cell types. Interestingly, disruption of the *CGI-58* homologue in Arabidopsis causes a similar accumulation of TAG and LDs in non-lipid storing tissues (e.g., leaves and stems), suggesting that the molecular mechanism(s) underpinning CGI-58 activity might be conserved in plants and animals. Key proteins known to be important for modulating the activity of CGI-58 in mammals through protein-protein interaction, however, are absent in plants. To elucidate the function of CGI-58 in plants, we used Arabidopsis CGI-58 as bait in a yeast two-hybrid screen and identified PXA1 as an interacting protein. PXA1 is a peroxisomal membrane protein that transports both fatty acids and lipophilic hormones (e.g., 12-oxophytodienoic acid [OPDA] and indole-3-butyric acid [IBA]) into the peroxisome for subsequent breakdown or metabolic conversion to jasmonic acid (JA) and indole-3-acetic acid (IAA), respectively. Other experiments have shown that disruption of *PXA1*, like *CGI-58*, results in the abnormal accumulation of TAG and lipid droplets in vegetative cells types, suggesting that CGI-58 and PXA1 are functionally related. In support of this premise, *cgi-58* mutant plants showed elevated accumulation of OPDA and reduced amounts of JA in response to plant wounding, similar to (but less severe than) *pxa1* mutants. In addition, *cgi-58* mutant plants were resistant to the effects of IBA (the peroxisomal precursor) in root elongation assays, but sensitive to IAA (the peroxisomal product). Unlike *pxa1* mutants, however, *cgi-58* mutant seeds can germinate and undergo seedling establish in the absence of exogenously provided sucrose. Collectively, these results indicate that CGI-58 and PXA1 function cooperatively in the regulation of fatty acid (and thus TAG) homeostasis in vegetative tissues, and also regulate a variety of lipid signaling pathways. Implications for the metabolic engineering of plants for enhanced oil production, as well as new insights to lipid metabolic disorders in humans, will be discussed.

The phosphatidate phosphohydrolase PAH is involved in ABA signaling and freezing stress response in Arabidopsis.

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Phosphatidate phosphohydrolase 1/2 (PAH1/2) is a soluble phosphatidate phosphatase which produces diacylglycerol (DAG) by dephosphorylating phosphatidic acid (PA). PAH plays an important role in lipid metabolism. In *pah1pah2*, phospholipids as phosphatidylcholine, phosphatidylethanolamine, PA increase more than wild type. PA is a metabolic intermediate playing a central role as a signal transducer of abscisic acid (ABA) stress response in higher plants.
Thus, we analyzed various stress responses of *pah1pah2* double knock-out mutant. With regard to ABA stress responses, germination of *pah1pah2* was more severely inhibited than that of wild type particularly with ABA treatment, indicating that germination of *pah1pah2* is hypersensitive to ABA. However, the inhibition of stomatal opening in response to ABA treatment was similar between wild type and *pah1pah2*, suggesting that, in ABA signaling, PAH is differently involved in seed germination and leaf drought stress. We also analyzed freezing stress response of *pah1pah2* double mutant. As a result, *pah1pah2* displayed less sensitivity to freezing than wild type with or without of cold acclimation, showing that *pah1pah2* acquired freezing tolerance. However, ion leakage of *pah1pah2* leaves with 1-day cold acclimation was higher than that of wild type. Therefore, it is suggested that *pah1pah2* has freezing tolerance in different mechanism from suppression of ion leakage.

pect1-4 mutation affects the chotochrome oxidase pathway capacity of mitochondrial respiration in *Arabidopsis thaliana.*

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Mitochondrial membranes are relatively enriched in phosphatidylethanolamine (PE), a nonbilayer-forming phospholipid, which is mainly synthesized via the CDP-ethanolamine pathway in Arabidopsis. To evaluate the importance of PE in mitochondrial functions, we used a mild *pect1-4* mutant, which exhibits 26% of the wild-type CTP:phosphorylethanolamine cytidylyltransferase (PECT) activity and displays pleiotropic growth and developmental defects.
We compared leaf respiration capacity using wild-type and *pect1-4* seedlings grown for 2 w to 5 w. Wild-type and *pect1-4* seedlings exhibited comparable total respiration (TR) capacities between 2 w and 3 w, although TR decreased by 34-41% during this period. Wild-type TR capacity then increased twofold by 5 w, whereas *pect1-4* TR capacity increased only 1.2-fold by 5 w, resulting in a 43% decrease compared with that of the wild type. Decreases in *pect1-4* TR capacity were ascribed to a partial inhibition of the cytochrome oxidase pathway (CP) capacity. Respiratory capacity measurements using purified mitochondria from 5-w-old plants were consistent with the above view. However, COX II protein levels were comparable between wild-type and *pect1-4* mitochondria, whereas the maximum COX activity was 20% lower in *pect1-4* mitochondria than in the wild-type mitochondria. *pect1-4* mitochondria contained 24% less PE than wild-type mitochondria, and PE/PC ratio decreased from 1.40 ± 0.25 in wild-type mitochondria to 0.85 ± 0.05 in *pect1-4* mitochondria. These results suggest that mitochondrial PE levels and/or PE/PC ratios might be important for COX activity.

High-throughput screening of lipid mutants in the model green microalgae *Chlamydomonas reinhardtii*

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The green algae *Chlamydomonas reinhardtii* is widely used as a unicellular model organism to study photosynthesis, chloroplast biogenesis, flagella function, and more recently lipid metabolism. When subjected to stress such as nitrogen depletion, *C. reinhardtii* accumulates up to 50% oil. However, nitrogen starvation limits biomass production and thus the overall lipid productivity. With the aim of isolating mutant lines with altered fatty acid composition or increased oil content under stress or non-stress conditions, a forward genetic approach has been developed within the framework of our HélioBiotec platform (http://www-heliobiotec.cea.fr/) at the Atomic Energy and Alternative Energies Commission (CEA).
A *C. reinhardtii* mutant library has been generated via transformation of the wild-type strain 137C with a paromomycin resistance cassette. Individual lines are cultivated either in culture tubes or in 96-well plate format. The neutral lipid content is first semi-quantitatively analyzed by flow cytometer after staining with Nile red. The triacylglycerol content of selected lines is then quantified by automated High Performance Thin layer Chromatography and densitometry. The mutant library is also screened for altered fatty acid composition. After direct trans-esterification of cells harvested either from liquid cultures or from agar-grown colonies, fatty acid profiles are analyzed by robotized GC-FID/MS. The lipidome of selected lines is characterized by LC-MS/MS.
Here, we report the methodology and the preliminary characterization of two mutants. One shows increased triacylglycerol accumulation under non-stress conditions and the other one has changes in fatty acid composition. Detailed characterization of these mutants is under way. On the side, a reverse genetic approach toward deciphering gene functions related to lipid metabolism in Chlamydomonas will also be presented.

Lipid characterization of *Nitzschia lembiformis* and *Rhodomonas salina* grown in different media: a case study.

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The interest in microalgae as lipid sources has increased immensely in recent years. Microalgal species have potential uses in aquaculture, biodiesel production, animal feed and pharmaceuticals. For biodiesel production, high biomass, lipid productivity and the ability to tolerate and grow in extreme environments are desirable characteristics.

This study examined the growth and lipid characterization of *Nitzschia lembiformis* (Class Bacillariophyceae) and *Rhodomonas salina* (Class Cryptophyceae) cultivated in three media (ESAW, Walne's and F/2) with three different nitrate concentrations (46.3, 62.2, 76.4 mg/L, respectively) for a growth period of 7 days.

N. lembiformis exhibited a great variety of fatty acids (23 types), with the total lipids in the range of 14.8-19.5 %, and a high TAG content (53.8 - 69.6 %). The total PUFA, saturated and monounsaturated fatty acids were 18 - 21.3, 32 - 34.1 and 40.6 - 42.5 %, respectively. However, *R. salina* had only 15 types of fatty acids, with the total lipid content in the range of 9.1 - 12.7 %, and a lower TAG content (41.5 - 48.3 %). The total PUFA, saturated and monounsaturated fatty acids were 47 - 53.6, 29.5 - 36 and 16.4 - 17 %, respectively. The analysis of fatty acid composition showed major differences between the saturation levels (e.g., palmitoleic, alpha linolenic, stearidonic and docosahexaenoic acids) but, in a comparative sense, the microalgae investigated were in the range of lipid profiles of other microalgae from similar genera.

The combination results of growth and lipid analysis showed that the microalgae can be used for both biodiesel production and aquaculture feedstock, although, of the two examined species, the diatom *N. lembiformis* was superior for most of the lipid parameters examined (Total lipid, TGA, saturated and monounsaturated) but it was ~ 50% lower in PUFA.

Deciphering lipid biosynthesis in non-model microalgae to manipulate value-added compounds productivities

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Long-Chain Polyunsaturated Fatty Acids (LC-PUFA) and carotenoids are among the most promising targets for high-value production from microalgae. At the Microalgal Biotechnology Laboratory we are studying lipid metabolism and developing genetic transformation approaches and molecular tools for engineering of economically important species, such as the green microalgae *Parietochloris incisa* and *Haematococcus pluvialis*. These oleaginous species accumulate valuable products, namely arachidonic-acid-rich triacylglycerols and the ketocarotenoid astaxanthin, respectively, in extraplastidial oil globules. Due to coordinated efforts of the consortium members of the FP 7 funded project GIAVAP (www.GIAVAP.eu), genome sequencing of both species is close to completion and transcriptome sequencing is underway. Aiming to improve algal strains by means of genetic engineering, we are attempting to identify key enzymatic steps and transcription factors affecting lipid accumulation in order to enhance lipid and target compound productivities.
This presentation will focus on our recent results on cloning, characterization and transcriptional profiling of genes involved in plastid acetyl-CoA supply, TAG biosynthesis and oil globule biogenesis. We will also report on identification, functional characterization and gene expression of two novel acyl-CoA: diacylglycerol acyltransferases type 1 from *P. incisa* (PiDGAT1) and the diatom *Phaeodactylum tricornutum* (PtDGAT1). Notably, the DGAT1 members of two evolutionary remote microalgae groups display similar fatty acid preferences when expressed in a *Saccharomyces cerevisiae* neutral lipid-deficient quadruple mutant strain (*DGA1, LRO1, ARE1, ARE2*). *In vivo* assays revealed that both recombinant DGAT1s are capable of incorporating n-3 and n-6 C18 and C20 PUFA into TAG; in addition, both recombinant proteins prefer endogenous saturated C16:0 and C18:0 over monounsaturated fatty acids.

Improvement of polyunsaturated fatty acid productivity in oleaginous fungus *Mortierella alpina* 1S-4 by overexpression of its acyl-CoA synthetase genes

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Filamentous fungus *Mortierella alpina* 1S-4 produces triacylglycerols rich in arachidonic acid (AA). The microbial lipid production reaches 20 g/L with 30-70% AA in the total fatty acids. A multiple transformation system for *M. alpina* 1S-4 and its derivative mutants allowed manipulation of genes involved in polyunsaturated fatty acid (PUFA) biosynthesis for improvement of the production of various PUFAs. On overexpression or RNA interference of endogenous fatty acid desaturase or fatty acid elongase genes, the fatty acid compositions in the transformants from *M. alpina* 1S-4 were modified. It is of great interest to elucidate the mechanisms in accumulation of a large amount of lipids in mycelia, and the physiological function of lipid accumulation and AA biosynthesis in *M. alpina* 1S-4. *M. alpina* 1S-4 has some homologue enzymes involved in lipid biosynthesis in some cases. Genes encoding several types of acyl-CoA synthetases (ACS) were found in *M. alpina* 1S-4, which catalyze the conversion of a free fatty acid to an acyl-CoA. An acyl-CoA unit plays an important role in biosynthesis and conversion of lipids. In this research, we assessed the effects of overexpression of ACS genes in *M. alpina* 1S-4 with aiming at the enhancement of lipid productivity by molecular breeding.

Phosphatidic acids in *Aspergillus niger* under heat shock

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Unlike heat influences in tolerance zone the heat shock (HS) causes the growth arrest and results in significant biological phenomena - acquired thermotolerance. In membrane lipids of three investigated fungus we observed the common regularity – the shift of phosphatidic acids (PA) under HS. The goal of the work was the investigation of lipids synthesis under HS conditions using labeling of cells with [2-14.] sodium acetate in pulse-chase manner to find out the reason of PA shift.

Under HS the exchange of membrane lipids was intensified.The incorporation of label in all phospholipids was increased for10-30%, but in DAG and TAG the label was increased remarkably (two fold). After removing of the labeled substrate and replacement of culture medium HS during 3 h led to the decreasing of labeled phosphatidylethanolamines (PE) and particularly phosphatidylcholines (PC) value on the background of increase of labeled PA These date give evidence, that the origin of PA is the PC and PE degradation by phospholipase D.

PA, as PC and PE, was the main component of the membrane lipids under HS. We propose that PA performs the essential role in adaptation to HS. Perhaps, PA participate in formation of negative curvature of membranes and subsequent vesicle formation, endo- and exocytosis.

Triacylglycerol metabolism in the microalga *Chlamydomonas reinhardtii*.

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Under stress conditions, many microalgae accumulate highly reduced compounds such as triacylglycerols (TAGs) which serve as feed stock for biofuel production. Identification of the genes encoding the enzymes and regulatory factors that impact the biosynthesis and turnover of TAGs will enable us to better control these processes. The green algal model *Chlamydomonas reinhardtii* stores TAGs in lipid droplets following nitrogen (N) deprivation, while in response to N-resupply, it hydrolyzes TAGs to generate energy and membrane building blocks. We used insertional mutagenesis in combination with different detection methods to screen for mutants deficient in the biosynthesis and turnover of TAGs. Representative examples of mutants are *pgd1* (plastid galactoglycerolipid degradation) and *cht7* (compromised hydrolysis of triacylglycerols). The *pgd1* mutant shows a 50% reduction in TAG content following N deprivation. *In vivo* pulse chase labeling indicated that the galactoglycerolipid pool is involved in providing acyl groups for triacylglycerol synthesis. *In vitro* enzyme assays showed that the PGD1 protein is a galactolipase that digests monogalactosyldiacylglycerol. The *pgd1* mutant also provides evidence for a physiological role of TAGs, namely relieving a detrimental overreduction of the photosynthetic electron transport chain. The *cht7* mutant shows 10-fold higher TAG levels than the wild type after the induction of lipolysis. In addition, cell growth at this particular stage and zygote formation are strongly inhibited in *cht7*. The mutant phenotypes of *cht7* suggest a regulatory role of the affected protein in linking developmental transitions with changes in metabolism.

Capturing genetic diversity for advanced biofuels in Camelina

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The rising cost of petroleum-derived fuels and their limiting supplies justify the need for alternative sources of these non-renewable commodities. To this end we are exploring the use of oilseeds to produce liquid biofuels. Our research to date has focused on *Camelina sativa* as an oilseed platform for bioengineering of renewable sources of jet fuel. Camelina is a non-food oilseed crop that can accumulate up to 40% oil in seeds, and while its oil does have some limitations as a desirable biofuel, genetic transformation of camelina is possible. Several plant species, including some in the genus Cuphea, accumulate high levels of short- and medium-chain fatty acids in seed oil. These fatty acids are similar in structure to the hydrocarbon compounds found in jet fuel and thus could potentially be used as a renewable source of jet fuel. Consequently, we aim to engineer short- and medium-chain fatty acid biosynthesis in camelina using genetic resources from plant species that naturally accumulate these types of fatty acids. Thus far, several homozygous transgenic camelina lines expressing different C8, C10, C12, and C14 fatty acid synthesis and triacylglycerol biosynthetic specific genes from Cuphea and other plant species have been generated. The transgenic lines show diverse fatty acid profiles. To augment our transgenic approach, next generation in-depth 454 sequencing was done on developing seeds from two different Cuphea species. Our lab has created a camelina biotech pipeline; from lab to greenhouse to field. As such, over 40 camelina expression constructs have been generated to date, with an end goal of screening lines for the best jet fuel-like oil properties.

Enhanced production in yeast and substrate specificities of four variants of type 1 diacylglycerol acyltransferase from *Brassica napus*

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Diacylglycerol acyltransferase (DGAT) 1 catalyzes the acyl-CoA-dependent acylation of *sn-1*, 2 diacylglycerol to produce triacylglycerol (TAG). The level of DGAT activity during seed development in *Brassica napus* may have a substantial effect on the flow of carbon into seed oil. Four *DGAT1* genes were isolated from the *B. napus* (DH12075) genome. The polymorphisms of these genes are predominantly located in their first exon encoding a relatively hydrophilic cytosol facing N-terminal domain that is non-essential for catalysis. Based upon homology, these genes can be divided into two sub-groups. Expression of the coding sequences of the DGAT1s in the TAG-deficient quadruple knock-out yeast (*Saccharomyces cerevisiae*) strain H1246 revealed that the enzymes of one sub-group produced twice the quantity of TAG in comparison to the other sub-group. Further investigation using chimeric DGAT1s demonstrated that high and low TAG- producing phenotypes could be interchanged by replacing the native N-terminal region from one sub-group with the N-terminal region from the other sub-group. Western blotting of microsomal proteins from cultures expressing the DGAT1s showed that the high and low TAG-producing phenotypes result from differential accumulation of the enzymes. Analysis of DGAT1s with mutations in their second amino acid residue suggests the altered accumulation of the enzymes results from different turnover rates, dictated by the “N-end rule”. Placement of an N-terminal epitope at the 5’ end of the DGAT1 coding sequences resulted in more stable production of the enzymes irrespective of original high or low TAG- producing phenotype. The enrichment of the DGAT1 polypeptides in yeast microsomes resulted in relatively high specific activities based on *in vitro* assays with [1-14C]acyl-CoA. The four forms of recombinant DGAT1 shared similar specificity patterns for acyl-CoA utilization, but two of the enzyme forms exhibited significantly increased specificity towards linoleoyl-CoA relative to the two other enzymes forms.

Engineering "Super DGATs": targeted mutations in type-2 diacylglycerol acyltransferase lead to enhanced performance in multiple transgenic systems.

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The seed oils of many exotic plant species contain high amounts of unusual fatty acids that contain useful functionalities such as epoxy and hydroxyl groups, or acetylenic and conjugated double bonds. These oils can serve as raw materials for the production of inks, dyes, coatings, and a variety of other bio-based products. Modern biotechnology has provided many basic tools with which to engineer temperate oilseed crops to produce novel value-added oils, and many modest successes have been achieved through expression of single thioesterase or diverged fatty acid desaturase genes. Diacylglycerol acyltransferase (DGAT) shows great promise in the quest to enhance unusual fatty acid production in transgenic systems. DGAT2 enzymes, in particular, appear to play a prominent role in this process. In the current study, we have analyzed DGAT2 from tung tree (*Vernicia fordii*) with regard to its ability to enhance production of oils containing alpha-eleostearic acid, a conjugated trienoic fatty acid. Mutational analysis of a unique region of tung *DGAT2* revealed forms of the enzyme that significantly outperform the native enzyme in transgenic plants and microbes. We are currently investigating the basis for this improved performance. These findings, and a discussion of the insights they provide into the evolution of *DGAT2* genes will be presented.

Metabolic engineering of *yarrowia lipolytica* for the production of omega 3 fatty acids: the role of different acyltransferases in influencing fatty acid desaturation, elongation, and lipid production

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Omega-3 fatty acids, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), mainly found in fish oil, have proven health benefits for both human and animals. Concerns over the quality and sustainability of the fish oil supply have generated interest in alternative sources for production of EPA and DHA. We report the development of a sustainable and land based source of omega-3 fatty acids by fermentation, using a metabolically engineered strain of the oleaginous yeast, *Yarrowia lipolytica*. Disruption of *PEX3* gene and overexpression of different acyltransferease (DAG ATs, LPAATs, and LPCATs) have major effect on lipid content and EPA level in the engineered strains. Our results pointed to several different ways to increase lipid production while reducing by-product formation. The yeast triacylglyceride oil has a unique fatty acid profile with less than 5% as saturated fatty acids and more than 55% as EPA. The use of the EPA-rich biomass in the fish feed resulted in the use of 75% fewer feeder fish in the diets of VerlassoTM salmon while maintaining their omega-3 requirements for a healthy diet.

Metabolic engineering plant seeds with fish oil-like levels of DHA

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Omega-3 long chain (=C20) polyunsaturated fatty acid (ω3 LC-PUFA) have critical roles in human health and development with studies indicating that deficiencies in these fatty acids can increase the risk or severity of cardiovascular and inflammatory diseases in particular. These fatty acids are predominantly sourced from fish and algal oils but wild-harvest marine fish stocks are widely recognised to be under threat. In order to meet the increasing demand for these oils there is an urgent need for an alternative and sustainable source of EPA and DHA. This talk will discuss recent progress in the production of the ω3 LC-PUFA DHA, in plant seeds. Groups have reported good progress in engineering the C20 EPA with seed fatty acid levels similar to that observed in bulk fish oil (~18%) although undesirable ω6 PUFA levels have also remained high. The conversion of EPA to the particularly important C22 DHA, however, has been problematic with many attempts resulting in the accumulation of EPA/DPA but only a few percent of DHA. This presentation will describe the production of 15% of the C22 fatty acid DHA in *Arabidopsis thaliana* seed oil with a high omega-3/omega-6 ratio. This amount exceeds the 12% level at which DHA is generally found in bulk fish oil. This result was achieved using a transgenic pathway where ALA production was first increased for subsequent conversion to DHA by a microalgal ω6-desaturase pathway. We consider this to be a breakthrough in the development of sustainable alternative sources of DHA.

The molecular basis of high and super-high oleic safflower seed oils

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Oleic acid is found in all plant-based food oils, but does not naturally reach the very high levels that are needed for expanding its use as a petrochemical replacement in production of polymers, lubricants and cosmetics. We have addressed this limitation by using genetic engineering to produce safflower seed oil with over 92% oleic acid content, with reduced levels of palmitic acid and minimal linoleic acid. In Australia, safflower is considered an excellent crop platform for industrial oil products because it is only a minor food crop and has favourable reproductive biology for transgenic trait containment. Deep sequencing of safflower DNA and RNA combined with biochemical characterisation efforts were used to determine the genes most likely to contribute to linoleic acid production in safflower seed. Surprisingly, safflower has an expanded family of *FAD2* genes, comprising at least 11 members, with only some functioning as oleic acid desaturases in developing embryos. Safflower also possesses at least three *FATB* genes, with two members expressed in developing embryos. Long fragments of the seed-expressed *FAD2* and *FATB* genes were used to design an RNAi silencing construct, driven by a seed-specific promoter, to ablate *FAD2* and *FATB* activities in safflower seeds to produce very high levels of oleic acid with no noticeable phenotypic abnormalities. We also developed a perfect molecular marker for the *ol/ol* locus in safflower, a naturally occurring mutation that raises safflower oleic acid content from 20% to 80%. Mutations in one *FAD2* gene highly expressed during *OL/OL* seed development render it inactive and this ol/ol locus is detectable using PCR-based techniques. Oleic acid is also the starting substrate for a range of other industrial fatty acids, including ricinoleic acid. The super-high levels of oleic acid that we have obtained will also be favourable for maximising in planta production of ricinoleic acid that require further metabolic engineering of safflower.

Plant lipids in 2030.

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To introduce the session on “Future developments” we will briefly outline some recent advances and speculate on how the plant lipid field might evolve over the next 20 years.

Improved soybean seed quality traits for food, feed, fuel and industrial applications.

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Soybean seeds, which contain approximately 40% protein and 20% oil, are an important feed ingredient and as a source of vegetable oil in many food applications. Our overall goal is to generate soybean seed with improved quality traits that provide added value for food, feed, fuel and industrial applications. The first generation of soybean quality trait research was based upon improving the oxidative stability of soybean oil, which resulted in the development and commercialization of high oleic soybeans (Plenish). This new oil can address a wide range of food and industrial applications. More recently our research efforts have been directed at varying the composition of soybean for increased oil content and improved soybean meal quality. The pathways and genes targeted as well as the outcome in controlled environment- and field-produced seed will be discussed.

Comparison of transcriptome changes associated to oil accumulation in oil palm mesocarp and in oil seeds

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The mesocarp of oil palm (*Elaeis guineensis* Jacq.) accumulates up to 90% triacylglycerol. We have analyzed transcriptome and metabolite changes that occur in oil palm mesocarp during fruit ripening when the oil accumulates. A similar study was conducted with date palm (*Phoenix dactylifera*) mesocarp, which stores soluble sugars but no oil. In addition, oil palm kernel and leaf transcriptomes were analyzed. Several million ESTs were generated from RNA extracted from five developmental stages that span oil accumulation in oil palm mesocarp. ESTs were assembled into contigs that were identified based on their similarity to Arabidopsis proteins using BLASTX. Major polar metabolites were quantified by proton NMR, including recent data on kernel.
The analysis of results showed that oil accumulation is associated with high levels of transcripts coding for all fatty acid synthesis enzymes, plastid sugar transporters, select glycolytic enzymes and DGAT2. Surprisingly, more changes were noted for transcripts coding for select enzymes involved in phospholipid metabolism than for triacylglycerol assembly enzymes. The transcription factor *WRINKLED1* was expressed 60-fold higher than in date. As in oil seeds, all known targets of *WRINKLED1* were up-regulated, implicating its key role in oil accumulation in a non-seed tissue. The most important difference with oil seeds is the strict correlation of oil-related transcript and triacylglycerol levels in oil palm mesocarp while these transcripts peaked well before the end of oil accumulation in oil seeds. Also, ATP availability might be an important factor to synthesize higher levels of oil as oil palm mesocarp showed, contrarily to oil seeds, a preferential up-regulation of ATP-dependent enzymes rather than PPi -dependent enzymes for sucrose breakdown and early glycolysis. These differences might help explain how oil palm mesocarp makes more oil than oil seeds.

RDR1 and SGS3, components of RNA-mediated gene silencing, are required for regulation of cuticular wax biosynthesis in developing inflorescence stems of Arabidopsis

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The aerial surfaces of plants are covered with a lipidic layer called the cuticle that serves to protect the plant from desiccation and the environment. The cuticle is synthesized by epidermal cells and is composed of a cutin polyester matrix that is embedded and covered with cuticular waxes. Recently, we have discovered a novel regulatory mechanism of cuticular wax biosynthesis which involves the CER7 ribonuclease, a core subunit of the exosome. We hypothesized that at the onset of wax production, the CER7 ribonuclease degrades an mRNA specifying a repressor of CER3, a wax biosynthetic gene whose protein product is required for wax formation via the decarbonylation pathway (Hooker et al., 2007). In the absence of this repressor, CER3 is expressed, leading to wax production. To identify the putative repressor of CER3 and to unravel the mechanism of CER7 mediated regulation of wax production, we performed a screen for suppressors of the *cer7* mutant. Our screen resulted in the isolation of components of the RNA silencing machinery, RDR1 and SGS3, indicating that the control of cuticular wax deposition during inflorescence stem development in Arabidopsis is mediated by RNA silencing.

Transgenic nutritional enhancement: the production of omega-3 long chain polyunsaturated fatty acids in plants

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There is now considerable evidence as to the importance of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs) in human health and nutrition. Unfortunately, current sources are either in severe decline (fish oils) or expensive (via microbial fermentation), leading to the search for an alternative source. We have been evaluating the possibility of producing omega-3 LC-PUFAs in transgenic plants, to provide a sustainable source of these important nutrients, since no native higher plant species synthesise these fatty acids. Attempts to metabolically engineer plants with the primary biosynthetic pathway for LC-PUFAs has been carried out in both model plants and crop species, allowing insights into factors constraining the accumulation of these fatty acids. Specifically, a generic bottleneck resides within the primary LC-PUFA biosynthetic pathway as a result of the “substrate dichotomy” between the lipid-dependent desaturases and the acyl-CoA-dependent elongases which catalyze the primary reactions. This bottleneck can be overcome through the use of acyl-CoA dependent desaturase, though not without impact on phospholipid composition. The use of lipidomic analyses have also allowed us to start to model the flux of acyl-chains into triacylglycerols, pointing the way for further interventions in this pathway.

POSTERS

Oil extraction without oxygen

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In cooperation between Contec Maschinenbau Klocke Gmbh and R&D institute PPM e.V. a new technology to extract high value vegetable oils was developed. In that technology oilseeds are processed without any air contact to avoid oxidation process as far as possible.
The patented technology provides the following advantages:
- no contact between seed or oil with air oxygen during processing,
- avoiding of of oxidation as far as possible,
- improved oil quality and shelf life,
- compiliance with with bio criteria.

Basically the systems is suitable to process all kind of oilseeds. But, it is particularly dedicated for the production of oils with high contents of polyunsaturated fatty acis, susceptible for oxidation, like e.g. flax oil.

Fatty acid composition of various plant species randomly sampled on contaminated soils can be used to attribute them a “quality bio-value”.

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Soil is essential for human societies and ecosystems. Because soil is a non-renewable resource and is under increasing critical pressure (agricultural, urban and industrial), it is imperative to ensure its protection. In this context, a French national research Program « Bioindicators of soil quality » has been set up to develop numerous biological indicators able to evaluate global environmental stresses and to further determine their impacts on ecosystems. These parameters were assessed on 13 sites in all the France which differ in terms of land use and agricultural practices (e.g. pasture rotation, tillage impact, management) but also on soil types, contamination origins (PAH or metal) and pollution levels.Hence, as part of this program, we use the plant leaf fatty acid composition as a reliable tool to diagnose soil contamination in situ. We randomly sampled several wild plant species growing on the different areas studied in the program and we determined the leaf fatty acid composition from each species on each area. The results obtained from samples collected on contaminated and uncontaminated soils highlight that the leaf fatty acid composition can be used to calculate a bio-value and to rank the areas of all these sites. These bio-values reflect the adverse effects of contaminated soil on plants. Moreover, our results show that this biological indicator respond to both metal and organic contaminations.This study allowed to highlight that the fatty acid composition of plants harvested from various soil sites, can be used as a biological parameter in the monitoring, characterization and risk assessment of soils.

Increasing the accumulation of medium chain fatty acids in transgenic plants

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Most plant oils are composed of fatty acids with16-20 carbons attached to glycerol. Plant species from the genus Cuphea accumulate 'unusual' oils with medium chain (C8 to C14) fatty acids in their seed oil. The key enzymes that are responsible for production of C8 and C10 in Cuphea species are FatB acyl-acyl carrier protein (ACP) thioesterases that are specific for medium chain acyl-ACP. Previously, genes encoding Cuphea FatB enzymes have been used to genetically engineer oilseed crops to produce C8 and C10 fatty acids. However, so far, expression of these FatB enzymes in transgenic plants results in some medium chain accumulation but to levels substantially lower than in the native plant. Several reasons for this have been proposed/investigated such as beta-oxidation, and acyltransferase specificity. Here we investigate an additional limitation to the accumulation of C8 and C10 fatty acids in transgenic plants. We present evidence that expression of *Cuphea pulcherrrima* FatB in Arabidopsis mutants that are disrupted in two genes of fatty acid metabolism resulted in an 80% increase (from 8 % to 15%) in 8:0 and 10:0 fatty acids in TAG compared to expression in wild-type Arabidopsis

Cloning and molecular characterization of ß-hydroxiacyl-ACP dehydratase genes from *Helianthus annuus* L.

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A rational approach to the biotechnology of seed oil modification is dependent on a detailed knowledge of the biochemistry of seed oil biosynthesis. Accordingly, a molecular understanding of the first steps of fatty acid biosynthesis is of interest. The intraplastidic fatty acid biosynthetic pathway in plants is catalysed by type II fatty acid synthase (FAS), a complex formed by monofunctional enzymes. This complex of four enzymes generates as final products, palmitoyl-acyl carrier protein (ACP) and stearoyl-ACP, using acetyl-CoA and malonyl-ACP as initial substrates. Thereafter, two carbons are added from malonyl-ACP in each elongation cycle which consists of the sequential actions of the FAS complex enzymes. These are a condensation, followed by reduction, dehydration and a second reduction. The dehydration is carried out by ß-hydroxyacyl-ACP dehydratase (HAAD), which dehydrates the reduced product of ß-ketoacyl-ACP reductase, generating 2-enoylacyl-ACP.In sunflower (*Helianthus annuus*) seeds, two ß-hydroxyacyl-ACP dehydratase genes, *HaHAAD1* and *HaHAAD2*, have been identified and cloned from developing seeds whose sequences are 75% identical. Phylogenetic studies and structural models of these genes will be presented. Quantitative PCR analysis disclosed a differential pattern in the expression levels of both genes in seeds and vegetative tissues, with *HaHAAD2* as the main transcript during early stages in seed development and *HaHAAD1* increasing its expression during this developmental process.Moreover, the heterologous expression of the genes in *Escherichia coli* resulted not only in purification of the enzymes and consequentially their biochemical characterization, but also revealed the effect of these Helianthus HAAD isozymes in *E. coli* on growth rate of the bacteria and fatty acid synthesis.

A new metabolic pathway engineering to produce the branched-chain fatty acids in *Saccharomyces cerevisiae*.

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Branched-chain Fatty Acids (BcFA) is one of the most attractive FA structures used for the manufacturing of high-grade lubricants thanks to their excellent viscosity, oxidative resistance as well as hydrolytic and thermal stability. Moreover, the BcFA from vegetal is a biodegradable and recyclable source, who presents no toxicity for human and its environment. In this work, we have successfully developed yeast cells for BcFA production by introducing a newly complete metabolic pathway in *Saccaromyces cerevisiae*. Based on the integration of methyl groups into the carbon chain of fatty acids to synthesize BcFA approach, two heterologous genes from bacteria with their codons modified were cloned and simultaneous expressed in yeast : the *RtmatB* gene from *Rhizobium trifollie* was used to convert the Methylmalonate to the Methylmalonyl-CoA and the KS-AT domains of the Mycocerosic Acid Synthase (MAS) code by the *mas* gene from *Mycobacterium tuberculosis* was used to integrate the MethylMalonyl-CoA into the carbon chain for the BcFA synthesis. Results obtained by radioactivity assays on the TLC plate showed that the KS and AT domains of the MAS protein were able to produce Methyl BcFA (Me-C14:0, Me-C16:0 and Me-C18:0) when expressed in yeast microsomes. More interestingly, data obtained by GC/MS and by LC/MS QTOF confirmed the presence of these Methyl BcFAs (2Me-C14:0, 2Me-C16:0 and 2,4 diMe-C16:0) when the recombinant yeast strain was grown in medium supplied with Methylmalonate. So a newly pathway to produce the BcFA has been validated in yeast, as expected. However, the quantity of these BcFA remains very low compare to the habitual Fatty Acids. The weak quantity of the BcFA could be explained by the heterologous expression system in yeast: the post-translation problem to a mature protein, the low activity of these enzymes caused by substrate competition of Methylmalonyl-CoA with malonyl-CoA (an abundant substrate in cells) for their active sites; or the bad cooperation of KS-AT domains with others of the elongase complex … The localization approach using epitope tag technique was investigated to demonstrate the location of these domains and the fusion KS-KCR is being constructed.

Dissection of *dictyostelium discoideum* fatty acid biosynthesis by tracking of isotopically-labeled metabolites.

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Dictyostelium discoideum, a cellular slime mould, possesses an unusual fatty acid profile with points of unsaturation at Δ5, Δ7, and Δ11 positions in C16 and C18 fatty acids. In order to probe the biosynthetic pathways to these fatty acids, a number of deuterium-labeled precursors were synthesized, fed to *D. discoideum* in liquid culture, and incorporation into endogenous fatty acids tracked by gas chromatography/mass spectrometry. These studies provide clear evidence for the production of 18:2(Δ5,11) directly from stearic acid and describe a network of biosynthetic steps to the production of C16 to C22 saturated, monounsaturated, and polyunsaturated fatty acids.

Cloning and biochemical characterization of two long chain acyl-CoA synthetases from sunflower (*Helianthus annuus* L.)

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Oilseed plants accumulate fatty acids in the form of triacylglycerols (TAG) in their seed to feed the embryo during the germinations of is. Fatty acids synthesis takes place in the plastid by fatty acid synthase complex (FAS) to produce acyl-ACP (acyl carrier protein). The last step in intraplastidial synthesis is carried out by acyl-ACP thioesterases, which hydrolyzing the bond between the acyl group and ACP producing free fatty acids. These fatty acids are transported out of the plastid and activated by long chain fatty acid-CoA synthetase which bounds the free fatty acid to coenzyme A (CoA). In the present work, we show results obtained for two genes which were cloned from developing sunflower kernel and showed LACS activity when were heterologously expressed in bacteria. These genes were called *HaLACS1* and *HaLACS2*. Informatics analysis of their sequence indicated that both of them were membrane bound enzymes, but with different localization: *HaLACS1* was predicted to be localized in plastid membrane, while *HaLACS2* was predicted to be an endoplasmic reticulum membrane. These enzymes were expressed in LACS deficient K27 strain of *E. coli*, and biochemistry characterization was carried out using the membrane fraction, showing a differential role of both enzymes in sunflower lipid metabolism. In addition, we have confirmed the subcellular localization of both enzymes using green fluorescent protein (GFP) on transformed tobacco suspension cells (BY-2).

Amino acids important for chain-length substrate specificities of arabidopsis alcohol-forming Fatty Acyl Reductases FAR5 and FAR8.

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Long-chain (C16 and C18) and very-long-chain (>C18) primary fatty alcohols are found in plants either as free alcohols or in a combined state. Fatty alcohols and derivatives are common components of plant extracellular lipid barriers, such as cuticle, suberin, and likely sporopollenin. Alcohol-forming fatty acyl reductases (FARs) are responsible for the NADPH-dependent reduction of fatty acyl-coenzymeA (CoA) or fatty acyl-acyl carrier protein (ACP) to primary fatty alcohols via an unreleased fatty aldehyde intermediate. FAR enzymes have distinct substrate specificities with regard to chain length and degree of acyl-chain saturation. The genome of *Arabidopsis thaliana* contains eight genes encoding FAR enzymes (*FAR1-FAR8*). The Arabidopsis FAR protein family produce primary fatty alcohols ranging from C16:0 to C30:0, with each FAR having a distinct chain length specificity. *FAR5* and *FAR8* are located in tandem on the Arabidopsis genome and encode proteins that are 85% identical. *FAR5* and *FAR8* were found to have almost strict specificity for C18:0 and C16:0 acyl chain length, respectively. A serine to proline substitution at position 363 of FAR8 was found to greatly increase its ability to produce C16:0 primary fatty alcohol when expressed in yeast. Domain swaps of FAR5 and FAR8 were characterized and a 72 amino acid region between residues 312 and 383 was found to be important for dictating C16:0 versus C18:0 chain length specificity. This study represents an important step forward into characterizing the chain-length substrate binding site of the FAR enzymes.

Dissecting the role of very long chain fatty acids in Arabidopsis growth and development

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Very long chain fatty acids (VLCFAs) have chain lengths greater than 18 carbons. They are the building blocks of sphingolipids, glycerophospholipids, triacylglycerols, surface polyesters (cutin/suberin) and wax esters. These five major lipid pools suggest that VLCFAs have a variety of functions in Arabidopsis depending on their cellular and tissue location. VLCFAs are synthesized by the microsomal fatty acid elongase (FAE), a membrane bound fatty acid elongation system that catalyses four consecutive enzymatic reaction (KCS,KCR,HCD,ECR).Mutations in the last three enzymes of the core elongase complex result in different combinations of root and shoot phenotypes [1,2,3]. Similar phenotypes can also be induced using a range of different chemicals. Herbicides known to affect VLCFAs synthesis induce abnormal developmental and morphological differences in both roots and shoots. Myricion, fumonisin and PDMP, all inhibit different stages of sphingolipid biosynthesis and lead to a build-up of intermediates within the pathway. These compounds affect root development but do not show any obvious shoot phenotype. Using this combination of genetically and chemically induced phenotypes we are investigating the mechanisms responsible for the phenotypic differences observed as a means to improve our understanding of the role of VLCFAs in Arabidopsis growth and development. Lipidomic profiling of the roots and shoots of elongase mutants and chemically treated plants should provide data explaining the phenotypic differences observed. This analysis should reveal whether when VLCFAs levels are reduced these compounds are preferentially channelled to specific lipid classes and whether there is a build-up of toxic intermediates.References:1. Beaudoinn, F. et al. (2009) Functional Characterization of the Arabidopsis ß-Ketoacyl-Coenzyme A Reductase Candidates of the Fatty Acid Elongase.
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Acyl chain biosynthesis for the alkamides of *Echinacea* purpurea

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Alkamides (or alkylamides) are widely distributed in Astereaceae. The coneflower *Echinacea purpurea*, with its long history of use by native peoples and as herbal remedies, contains a range of branched-amine-derived alkamides. To better define the specific biosynthetic processes leading to the C11 to C16 acyl chains of the alkamides, we herein report a range of MS and NMR stable-isotope labeling experiments. By culturing *E. purpurea* seedlings with either [^{13}C] acetate or glucose and analyzing positional labeling within alkamides, the data supports that the acyl chains within alkamides are formed through iterative fatty acid biosynthesis. To further probe the biosynthetic origins of alkamides, labeled fatty acids ([U-^{13}C] oleate and octanoate) were fed to *E. purpurea* seedlings. This provided labeling patterns that we believe arise specifically from mitochondrial fatty acid biosynthesis. Utilizing inhibitors of the cytosolic multifunctional ACCase with either [U-^{13}C] acetate or glucose, labeling patterns present within alkamides and very long chain fatty acids are significantly decreased but labeling within chloroplast-derived fatty acids remained relatively constant. Furthermore, [U-^{13}C]malonate feeding experiments produced similar labeling patterns within alkamides and palmitate, but not in other chloroplast-based fatty acids. Coupled with additional data, we now postulate that the acyl chains of alkamides are produced from mitochondrial fatty acid biosynthesis.

Why do plants make unusual fatty acids? Exploring the curious cases of acetylenic and conjugated fatty acids

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Unusual fatty acids occur in high levels in seeds of species throughout the plant kingdom. The functional significance and evolutionary origin of the biosynthetic pathways for these fatty acids is largely unknown. In some cases, unusual fatty acids occur in seeds of most species of a given plant family. For example, petroselinic acid occurs widely in seeds of *Apiaceae* and *Araliace* species. In most cases, however, unusual fatty acids are found in selected species within plant families. As genomic information increases, new insights emerge for the evolutionary origins of unusual fatty acids, including acetylenic and conjugated fatty acids, the focus of our current study Acetylenic fatty acids occur almost exclusively in high levels in seed oils of Crepis species in the *Asteraceae* family. Despite this, we have previously demonstrated that *FAD2*-related acetylenase genes occur in plants throughout the *Asteraceae*, *Apiaceae*, and *Araliacaeae* families, and their expression is induced by fungal pathogenesis. With the recent availability of sequence information from *Solanaceae* species, including genome sequences for potato and tomato, we have discovered that acetylenase-like genes also occur widely in species of this family, despite the fact that no *Solanaceae* species produces seed oils enriched in acetylenic fatty acids. Using a yeast expression system, we have confirmed that acetylenase-like genes from tomato, pepper, potato, and eggplant are functional acetylenases. In the tomato and potato genomes, acetylenase genes occur in tandem with typical *FAD2* genes. A common feature of *Asteraceae*, *Apiaciae*, *Araliaceae*, and *Solanaceae* species is that they produce polyacetylenic defense compounds such as falcarinol that require acetylenases for their biosynthesis. These findings suggest that in the case of acetylenic fatty acids, a widely-occurring secondary metabolic pathway is the origin of acetylenic acid-rich seed oils of Crepis species. A similar story is emerging for conjugated fatty acid synthesis in the *Cucurbitaceae* family that will also be presented.

The multigene family of lysophosphatidate acyltransferase (LPAT)-related enzymes in *Ricinus communis*. Cloning and molecular characterization of two *LPAT* genes expressed in the castor bean.

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The multigene family encoding proteins related to lysophosphatidyl-acyltransferases (LPAT) has been analyzed in the castor plant *Ricinus communis*. Cladistic analysis reveals the existence of six well supported clusters containing diverse acyltransferases previously described in other plants. Among them, two genes designated *RcLPAT2* and *RcLPATB* that are significantly expressed in the castor bean, have been cloned and characterized in some detail. Both genes are able to rescue the *plsC* mutation in *E. coli* and, accordingly, they encode proteins with LPAT activity when assayed *in vitro*. RcLPAT2 groups with characterized members of the so-called A-class LPATs and, as expected, it shows preference for monounsaturated acyl-CoA substrates besides a generalized expression pattern along the plant. RcLPATB groups with B-class LPAT enzymes, like those from Limnanthes and Cocos, which are seed specific and selective for unusual fatty acids. However, RcLPATB exhibit a broad specificity on the acyl-CoAs, with saturated fatty acids (12:0 to 16:0) being the preferred substrates. Moreover, although RcLPATB is upregulated coinciding with seed TAG accumulation, its expression is not restricted to the seed. Both RcLPAT2 and RcLPATB catalyze the incorporation of ricinoleoyl-CoA at a significant rate, when using oleoyl-LPA as acyl aceptor, although it is not the preferred substrate, thus suggesting that LPAT selectivity might not be determinant in the channelling of ricinoleate into TAG. Experiments are being also conducted with ricinoleoyl-LPA to further contrast this hypothesis. These results will be discussed in the light of a possible role for the two LPAT isoenzymes in the castor bean.

Refined isotopomer labeling analysis of lipid-linked epimerization and desaturation of glyceroglycolipids in cyanobacteria.

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In cyanobacteria, MGDG is synthesized from GlcDG as a precursor, which is initially synthesized as an entirely saturated molecular species. The epimerization of the glucose moiety to galactose, and the desaturation of palmitate and stearate are believed to proceed on the lipid, namely, without replacement of these parts. However, acyl exchange is also known to operate in cyanobacteria. The palmitate at C-2 of MGDG was demonstrated to be essentially desaturated by lipid-linked mechanism using a method involving ^{13}C labeling *in vivo* (Sato et al. 1986), but the exact mechanism of conversion of the saturated GlcDG over to various polyunsaturated MGDG remained uncertain, mainly because all the reactions can be realized only within intact cells. The enzyme catalyzing the epimerization remained uncharacterized.

I recently developed a more refined computational method of analyzing isotopomer (molecules differing in the number of ^{13}C) distribution of lipids after photosynthetic assimilation of ^{13}C, using both Synechocystis and Anabaena. The isotopomer distribution is characterized by the abundance (enrichment) 'p' of ^{13}C in the labeled population. The p-value (typically about 0.90) remained unchanged in all molecular species of GlcDG and MGDG during the conversion of glucose to galactose and the desaturation of fatty acids during labeling and chase experiments. MS/MS analysis also supported this result. This suggests that all these processes proceed by the 'lipid-linked' mechanism, namely, without replacing the parts.

Localization of plastidial *At*FAD6 and *At*FAD7 desaturases in the chloroplast envelope from Arabidopsis

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Plastid desaturases, as many enzymes participating in plant lipid biosynthesis, have been localized in chloroplast envelopes either by chloroplast peptide prediction or proteomic studies (Ferro et al, 2003). However, very little is known about their *in vivo* distribution and organization in the plastid lipid membranes. We obtained stable transgenic lines of *Arabidopsis thaliana* (Col0) in which the plastidial desaturases AtFAD6 and AtFAD7 were fused at the C-terminus to a fluorescent protein (CFP and YFP, respectively) using pEarleyGate vectors. Confocal fluorescence microscopy analysis (Leica SP2 confocal microscope) of fresh leaf tissue from different transgenic T2 lines showed that the FAD7:YFP or FAD6:CFP signals were found associated with the chloroplast and localized in foci (concrete spots on the plastid envelope membrane). These results suggest the existence of concrete desaturation areas whithin the complex chloroplast envelope membrane structure in Arabidopsis.

Differencial contribution of the microsomal and plastidial linoleate desaturases to the α-linolenic acid content in olive oil

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Olive oil is one of the world's major edible oils and is highly enriched in oleic acid (55-83%), whereas linoleic acid accounts for 3.5-21% and α-linolenic acid for less than 1%. The linoleic and α-linolenic acids ratio in the olive oil affects the nutritional properties and has also important consequences in the technological properties, such as its oxidative stability. The desaturation of linoleic acid to α-linolenic acid is carried out by two different .ω-3 fatty acid desaturases, located in the endoplasmic reticulum (FAD3) and in plastidial membranes (FAD7/8). In this work, we have isolated two microsomal (*FAD3-A* and *FAD3-B*) and two plastidial (*FAD7-1* and *FAD7-2*) linoleate desaturase genes from olive. The correlation between their expression levels and the α-linolenic acid content in different tissues of the olive fruit will help us to elucidate the physiological role of each ω-3 fatty acid desaturase, including the specific contribution of each gene to the α-linolenic acid content of the olive oil.

Molecular characterization and functional analysis of four *FAD2* genes from *Brassica napus* L.

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Fatty acid desaturase 2 (FAD2), where exists in endoplasmic reticulum (ER), plays an role in producing linoleic acid (18:2) through desaturation forming double bond of Δ12 position by using oleic acid (18:1) as precursor. FAD2 is the first step enzyme involved in producing polyunsaturated fatty acids composed in glycerolipids of cell membrane and triacylglycerols in seed. We have identified four *FAD2* genes from allotetraploid *Brassica napus* genome by PCR amplification, which divided into two groups: type I (*FAD2-1, FAD2-2*) shows ubiquitous and type II (*FAD2-3, FAD2-4*) is seed-abundant expression. Four *FAD2* genes of *B. napus* were originated from crossing in diploid *B. rapa* and *B. oleracea*. Each *B. rapa* and *B. oleracea*, respectively has two genes corresponding with type I and type II from *B. napus.* The *FAD2-3* of *B. napus*, nonfunctional gene derived from multiple deletions and insertions in nucleotides, was originated from *B. rapa.* Three FAD2 proteins except for FAD2-3 were localized on ER, but nonfunctional FAD2-3 protein leads to localize on nucleus. The function of four BnFAD2s was confirmed by analyses of fatty acid from budding yeast (*Saccharomyces cerevisiae*) introduced pYES vector carrying four *BnFAD2* genes, respectively. Budding yeast transformed by *BnFAD2-1, -2, -4* can synthesize 18:2 as well as 16:2. However, wild type yeast and transformed by blank vector cannot synthesize any polyunsaturated fatty acid.

Functionality of the soybean microsomal omega-3 desaturases *GmFAD3A*, *GmFAD3A-T*, *GmFAD3B* and *GmFAD3C* examined by heterologous expression in yeast

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Three genes, named *GmFAD3A*, *GmFAD3B* and *GmFAD3C*, encoding members of the omega-3 microsomal fatty acid desaturase family are present in the soybean genome. Exposure of soybean plants to cold temperatures (5ºC) resulted in an increase of the *GmFAD3A* transcripts without changes of *GmFAD3B* or *GmFAD3C* expression levels. This increase was reversible and accompanied by the accumulation of an mRNA encoding a truncated form of *Gm*FAD3A (*Gm*FAD3A-T), which was originated from alternative splicing of the *GmFAD3A* gene in response to cold. The functionality of *Gm*FAD3A, *Gm*FAD3A-T, GmFAD3B and GmFAD3C was examined by heterologous expression in yeast (*Sacharomyces cerevisiae*). No activity was detected with *Gm*FAD3A-T, consistent with the absence of one of the His boxes necessary for the desaturase activity. Linolenic acid content of yeast cells over-expressing *Gm*FAD3A, *Gm*FAD3B or *Gm*FAD3C was higher when the cultures were incubated at cooler temperatures, suggesting that reticular desaturases of the *Gm*FAD3 family, and more specifically *Gm*FAD3A, may play a role in the cold response, even in leaves. Data obtained from *in vitro* assays of microsomal linoleate desaturase activity and biochemical characterization of these enzymes will be discused.

Functional analysis of a delta 6-desaturase gene from the spike eel (*Muraenesox cinereusa*).

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Delta-6 desaturase1 (D6DES1) is an enzyme that catalyzes the formation of double bond at C6 of fatty acid. It is a key enzyme in biosynthesis of gamma-linolenic acid (GLA, 18:3n-6) which is a precursor of prostaglandin that is essential to the proper functioning of each cell, and stearidonic acid (STA, 18:4n-3) that is the significant intermediate on the biosynthesis of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). *McD6DES1* cDNA (1,335 bp) has been isolated from the liver of spike eel (*Muraenesox cinereusa*), cloned into pGEM-T easy vector, sequenced and confirmed by similarity at amino acid level. A recombinant vector expressing McD6DES1 (pYES-*McD6DES1*) was subsequently constructed and transformed into *Saccharomyces cerevisiae*, which was cultured by feeding linoleic acid (LA, 18:2n-6) or a-linolenic acid (ALA, 18:3n-3) in each media. After that, the fatty acid composition of the cell membrane was analyzed by gas chromatography (GC). The results showed that LA (18:2.9,12) and ALA (18:3.9,12,15) were converted into GLA (18:3.6,9,12) and STA (18:4.6,9,12,15), respectively. The conversion rate was 14% for LA, 46% for ALA. The *Mc*D6DES1 preferentially catalyses double bond formation at delta-6 carbon of n-3 fatty acid rather than that of n-6 fatty acid. In future, *Mc*D6DES1 will be applied to metabolic engineering for development of transgenic oil plants producing GLA, EPA and DHA.

LEAFY COTYLEDON1 regulates the .-3 polyunsaturated fatty acid content of Arabidopsis seed oil by [LEC1-LIKE:NF-YC2:bZIP67]-directed transactivation of FATTY ACID DESATURASE3.

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Arabidopsis seed maturation is associated with the deposition of storage oil, rich in the essential ω-3 polyunsaturated fatty acid a-linolenic acid (ALA). The synthesis of ALA is highly responsive to the level of *FATTY ACID DESATURASE3* (*FAD3*) expression, which is up-regulated during late embryogenesis. By screening mutants in *LEAFY COTYLEDON1* (*LEC1*)-inducible transcription factors using fatty acid profiling, we identified two mutants (*lec1-like* and *bzip67*) that have not previously been reported to have a seed lipid phenotype. Both mutants share a reduction in seed ALA content. Microarray and Q-PCR analysis performed on developing *bzip67* seeds showed that *FAD3* is down-regulated. Using a combination of *in vivo* and *in vitro* assays we show that bZIP67 binds G-boxes in the *FAD3* promoter and enhances *FAD3* expression, but that activation is conditional on bZIP67 association with L1L and NF-YC2. Although FUSCA3 and ABSCISIC ACID INSENSITIVE3 are required for LEC1 induction of L1L and bZIP67 expression, they do not participate directly in transactivation of *FAD3*. Our data suggests that an 'enhancersome' complex containing L1L, NF-YC2 and bZIP67 is induced by LEC1 during embryogenesis and specifies high levels of ALA production by activating *FAD3* expression.

Developmental and temperature regulation of omega-3 fatty acid desaturases from soybean

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In soybean, omega-3 fatty acid desaturases from the endoplasmic reticulum and the plastid are grouped into multigenic families. Thus, differently to other plant species, two *GmFAD7* and two *GmFAD8* genes encoding plastidial omega-3 desaturases are found in the soybean genome. We investigated the regulation of omega-3 fatty acid desaturases during plant development and in response to cold. In seeds, the low presence of 18:3 fatty acids together with the high transcript levels of all reticular and plastidial desaturases pointed to a post-transcriptional control of the omega-3 desaturase activity in this developmental stage. Leaf maturation produced an increase in 18:3 levels concomitant with a decrease of both *GmFAD3A* and *GmFAD3B* transcripts as well as an increase of the *GmFAD8-2* transcript. No changes were detected in *GmFAD7* transcripts. These results suggested the existence of transcriptional and post-transcriptional mechanisms operating for the control of the plastidial and reticular omega-3 desaturase activity during leaf maturation. Exposure to cold resulted in an increase of *GmFAD3A* and *GmFAD7-2* transcripts and the accumulation of a cold-induced splice variant of the *GmFAD3A* gene. Our data point to a complex regulatory mechanism of omega-3 fatty acid desaturases in soybean affecting specific isoforms in both the plastid and the endoplasmic reticulum, acting at the transcriptional and post-transcriptional levels to maintain appropriate levels of 18:3 upon plant development or low temperature conditions.

How does lipid desaturation affect the architecture of photosynthetic apparatus?

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DesA and *desD* genes of *Synechocystis* sp. PCC6803 cyanobacterium encode acyl-lipid desaturases those introduce double bonds at the delta12 and delta6 positions of C18 fatty acids esterified to the glycerol backbone of membrane glycerolipids. Previously, the mutation of each of these genes by insertion of an antibiotic resistance gene cartridge was carried out. This gene manipulation completely eliminated the corresponding desaturation reaction. Comparisons of the various mutant cells revealed that the replacement of all polyunsaturated fatty acids by monounsaturated fatty acids suppressed growth of the cells at low temperature and, moreover, it decreased the tolerance of the cells to photoinhibition at low temperature by suppressing recovery of the photosystem II (PSII) protein complex from photoinhibitory damage.For the structural characterization of photosynthetic machinery, we used differential scanning calorimetry (DSC) as a technique capable of identifying protein complexes on the basis of their calorimetric transitions. Annotation of thermal transitions was carried out with thylakoid membranes isolated from various photosynthetic mutants of *Synechocystis* sp. PCC6803. Heat sorption curves, analyzed by a subsequent annealing procedure, exhibited seven major DSC bands between 40 and 85 C.We compared the thermograms of thylakoids isolated from wild type and *desA-/desD-* mutants grown at 25 and 35º C. In the thylakoid membrane of wild type cells, regardless from the growth temperature, we could detect a thermal transition at 68-70º C that relates to the denaturation of PSII sorrounded by other proteins of the photosynthetic complexes, and another DSC band at 81-84º C corresponds to the PSI complexes. In the *desA-/desD-* mutant cells a further DSC band was observed around 50º C, resulting from the high enthalpy change that corresponds to non-interacting complex of PSII that was identified using a PSI-less mutant. The appearance of thermal transition of non-interacting PSII complexes suggests that in the lipid-desaturase mutant cells the balance between PSII and PSI is affected by the mutation and it can explain changes in photosynthetic processes.

Morphological changes and fatty acid characterization of *Camelina sativa* developing seeds

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Nowadays there is an increasing demand for biolubricants, environmentally friendly lubricants mostly based on vegetable oils such as rapeseed or sunflower. The problem is that those oils are mainly used by the food industry, so there is a need to use different species not competing with food applications. *Camelina sativa* is a good alternative for the production of biolubricants because is a non-food oilseed crop. Its high oil content, productivity and good agronomic characteristics make this species desirable for this purpose. In this work, we had characterised the lipid content and fatty acid composition during seed development as well as of other vegetative tissues of a germplasm collection of *Camelina sativa*. Camelina is an annual crop that produces small seeds rich in oil, about 30% fresh weight. These are characterized by high levels of polyunsaturated fatty acids, such as linoleic and linolenic acids (about 51.2% in total). Despite these excellent levels of omega-3 and omega-6 fatty acid, Camelina oil is outside the food circuits by contain different anti-nutritional compounds, being this oil used for different industrial applications. In this paper we analyzed the composition of 40 different accession of *Camelina sativa* oil grown in Córdoba, Spain. This oil has a mean composition of 7.02% palmitic acid, 2.52% stearic acid, 6.92% oleic acid, 14.52% linoleic acid, 41.02% linolenic acid, 10.87% gondonic acid and 3.50% erucic acid. The best accession of *Camelina sativa* to produce an oil with good thermo-oxidative properties for industry, which is, those with low PUFA/MUFA index (1.40, CAS-CS16), minimal value of C20-C24/C16-C18 ratio (0.25, CAS-CS13) and the highest content in oil (41.7% of CAS-CS38).To maximize the utility of Camelina oil in industrial application is necessary to study the fatty acid content of seed during the maturation. The development of *Camelina sativa* seed can be divided into three distinct stages, growth phase (until 18 DAF), accumulation phase (from 18 DAF to 24 DAF) and desiccation phase (from 24 DAF to completed maturation). In the first phase, the fatty acid profile is different to mature seed, in this stage the seed present high level of palmitic, stearic and oleic acids. In the second step, Camelina seed possesses a fatty acid profile similar to the mature seed, and consequently, the seed reaches its maximum fresh weight, although this will decrease in the final stage due the water loss. In the desiccation phase, the content of fatty acid is stabilized.

Metabolic interactions in the functions of plastids isolated from soybean somatic embryos

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Soybean (*Glycine max* L.) somatic embryos and their plastids are being used as a model system to study the developmental relationships and metabolic interactions involved in plastid function in this important seed crop. Plastids isolated from exponentially growing embryos are actively engaged in *de novo* fatty acid biosynthesis. Although soybean somatic embryos and their plastids are green, the plastids appear to function like heterotrophic plastids. In preliminary experiments, light has essentially no effect on fatty acid biosynthesis, while ATP, coenzyme A and bicarbonate are all essential. Acetate is the preferred precursor at low (1 mM or less) concentrations while pyruvate is greatly preferred at higher concentrations. Isolated plastids can also utilize glucose-6-phosphate as a precursor for fatty acid biosynthesis, indicating that embryo plastids likely have a complete glycolytic pathway that may be able to provide metabolic resources for fatty acid biosynthesis. Precursor radioactivity is recovered in palmitic and oleic acids esterified in typical plastid lipids, but especially phosphatidylcholine and triacylglycerol. Embryos and plastids also show high rates of activities for key enzymes of nitrogen assimilation, including glutamine-oxoglutarate amino transferase (GOGAT), glutamine synthetase (GS) and aspartate aminotransferase. Similarly, zygotic and somatic embryos express homologs of GOGAT and GS. These observations suggest that developing embryos and their plastids are involved in nitrogen assimilation. The addition of selected intermediates of glycolytic metabolism and nitrogen assimilation to plastids engaged in fatty acid biosynthesis has various effects on fatty acid biosynthetic activity, indicating that these metabolic processes may interact with fatty acid biosynthesis. This research was supported by projects 8233 and 1233 from the United Soybean Board.

Cloning, heterologous expression and biochemical characterization of plastidial *sn*-glycerol-3-phosphate acyltransferase from *Helianthus annuus*

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Sunflower (*Helianthus annuus* L.) is a highly valuable oil crop, which oil is not only being used for human consumption, it is also being tested as a source of biofuel. Efforts in uncovering the lipid biosynthetic pathway in this crop are being made. In this regard, the enzyme *sn*-1-glycerol-3-phosphate acyltransferase (GPAT, EC 2.3.1.15) catalyzes the acylation of a molecule of glycerol-3-phosphate in the position *sn*-1 to yield 1-acylglycerol-3-phosphate. This is an essential activity in the synthesis of triacylglycerol and other glycerolipids in oil seeds. There are many isoforms for this enzyme, most of them are integral membrane proteins, although a soluble form is found inside the chloroplasts taking part into the biosynthetic pathway of plastidial phospholipids, sulpholipids and galactolipids. This plastidial isoform uses acyl-acyl carrier protein (ACP) as acyl donor, whereas membrane-bound GPATs use acyl-CoA as the source of acyl moieties. The main substrates used by the plastidial GPAT are oleic (18:1) and palmitic (16:0) acids. After studying the specificity of this enzyme in different plants classified for their chilling tolerance, it was shown that chilling-sensitive plants have a non-specific form of the GPAT whereas chilling-resistant plants have plastidial GPAT showing higher affinity for 18:1-ACP. As these lipids form part of the plastidial membrane, the level of unsaturation in their fatty acids determines the membrane fluidity and, thus, the tolerance to varying environmental temperatures.In the present work, the plastidial GPAT from sunflower (*HaPlsB*) was identified, sequenced and cloned in a heterologous production system (*Escherichia coli*). The purified enzyme was biochemically characterized for different substrates and pHs values. Lipids from sunflower leaves were extracted and analyzed to investigate the role of GPAT activity in the assembly of plastidial lipids. Correlation between this composition and the kinetic parameters will be discussed.

Agroinfiltration of *Nicotiana benthamiana*, an efficient system for evaluation of candidate genes for short and medium chain triacylglycerol synthesis

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Functional characterization of genes using stable transformation of plants can be a time- and labor consuming process. Agroinfiltaration of *Nicotiana benthamiana* (Nb) leaves has been shown to be a very efficient system for fast functional screening of novel genes. Furthermore, with transient expression via agroinfiltration, it's possible to combine many genes to find the right gene combination for a specific trait. Short and medium chain fatty acids have a favorable structure for production of jet fuel, a renewable product that in the future can replace some of the fossil oil used today. Engineering industrial oil crops, like for example *Camelina sativa*, to produce short and medium chain fatty acids is of great interest. Cuphea is a plant family accumulating high amounts of the short and medium chain fatty acids C8, C10, C12 and C14 in their seed oil. Next generation in-depth 454 sequencing has been done on developing seeds from two different Cuphea species, which has generated a number of candidate genes involved in short and medium chain triacylglycerol biosynthesis. Agroinfiltration of a *WRINKLED1* (*WRI1*) gene from *Arabidopsis thaliana*, a transcription factor activating genes in fatty acid biosynthesis, increases the triacylglycerol (TAG) synthesis in *Nb* leaves several fold. In this study we have combined the *WRI1* gene with different short and medium chain acyl-ACP thioesterases and acyltransferase candidates from the Cuphea family. Analysis of resulting fatty acid composition changes in TAG confirmed that agroinfiltration is a promising system for verification of gene involved in short and medium chain TAG biosynthesis.

Molecular characterization of lipase gene

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Oilseeds are characterized by relatively high fat reserves (between 30 and 50% dry weight). These reserves of lipid are usually triacylglycerol (TAG). Plant cells produce fatty acids to supply the needs of membrane biogenesis. The fatty acids released by lipolysis are further metabolized in glyoxysomes to provide energy for embryonic growth and development. In seeds, specific lipases control triacylglycerol hydrolysis, and may regulate germination. These lipases are absent from ungerminated seeds, but appear in the post germination stage when their activity increases. Lipases are responsible of the triglycerides catabolism into fatty acids and glycerol. In many organisms, this reaction plays a major role in the fat and lipid metabolism. In addition, lipases are also able to hydrolyse phospholipids and cholesterol esters. In organic solvent, lipases could catalyse reactions such as esterifications, acidolysis or alcoolysis with enantio-, regio- and chimioselectivity. Lipases constitute the most important group of biocatalysts for biotechnological applications. The high-level production of plant lipases requires not only the efficient overexpression of the corresponding genes but also a detailed understanding of the molecular mechanisms governing their folding and secretion. All those properties led to the development of many applications in the food and chemical industries but also in the medical and therapeutic field.
Our study is to obtain the lipase gene by the methods of molecular biology. DNA extracted was amplified by PCR and we have obtained a good quality of product. The only primers F1wupper and lower gave this results, it is a single band well identified on gel agarose. The product amplification was sequencing and bioinformatics tools we could show that this is the lipase gene.

Lysophosphatidylcholine acyltransferase 2 (LPCAT2) is responsible for assisting in PDAT1-catalyzed TAG synthesis in Arabidopsis

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Most plants accumulate storage oil in the form of triacylglycerols (TAG) that are produced either via an acyl CoA-dependent or -independent pathway. In the former, *sn*-1,2- diacylglycerol acyltransferase 1 (DGAT1) produces TAG by acylating diacylglycerols, while in the latter TAG is generated by the action of phospholipid:diacylglycerol acyltransferase (PDAT1) which transfers a fatty acid from a phospholipid (e.g. PC) to DAG. Their importance for TAG production was recently shown when it was reported that knocking out both *DGAT1* and *PDAT1* reduces seed oil content to 20-30% of that of Wt.TAG generation by PDAT1 generates lyso-phosphatidylcholine (LPC), which needs to be reacylated in order to avoid ensure that PDAT1 does not run out of substrate. Two lysophospholipid acyltransferases with preferences for LPC, LPCAT1 and LPCAT2, exist in Arabidopsis. In this study through biochemical, metabolic and gene expression studies we have investigated the effect of crossing the *DGAT1* knock-out mutant *AS11* with either *lpcat1* or *lpcat2* knock-out mutants. Seed oil content in the *AS11* mutant is reduced to about 75% that of Wt plants. The combined effect from the *AS11* and *lpcat1* mutants results in a further 5% reduction of the TAG content (to about 70% of Wt). In contrast, crossing *lpcat2* mutant with *AS11* drastically reduces the seed oil content to 35% that of Wt plants. This study show convincingly that it is through the action of LPCAT2 and not LPCAT1, that LPC is reacylated to PC, thereby providing PDAT1 with the necessary substrate for TAG production.

Involvement of a caleosin in storage lipid biosynthesis and mobilization during olive (*Olea europaea* L.) pollen development

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The olive (*Olea europaea* L.) tree is an oil-storing plant of great agronomical importance in the Mediterranean basin. The anther tapetal cells contains several lipidic structures, such as pro-orbicules and plastoglobuli, while mature pollen grains store neutral lipids in the form of sphere-shaped structures called oil bodies. However, little is known about the molecular mechanisms involved in the biogenesis and mobilization of these organelles. Caleosins are oil body-associated proteins involved in storage lipid mobilization during seed germination. In this work, a 30 kDa caleosin, which is able to bind calcium *in vitro*, was identified in both the tapetal and germ line cells, with its synthesis independently regulated. Thus, the protein was firstly localized in the tapetum, the exine of developing microspores and the surrounding locular fluid. As anthers developed, tapetal cells showed the presence of structures constituted by caleosin-containing lipid droplets closely packed and enclosed by ER-derived cisternae and vesicles. After its degradation, the caleosin-containing remnants of the tapetum were released to the anther locule and filled the cavities of the mature pollen exine, forming the pollen coat. During pollen maturation, the caleosin also accumulated in the vegetative cell, concurrently with the number of oil bodies. Within the pollen grain, the protein was mainly attached to the boundaries of oil bodies, displaying a structural conformation similar to seed oil body-associated caleosins. During pollen germination, the protein level progressively decreased, coincidentally with a decline in the number of OBs. Within the growing pollen tube, the protein was mainly localized on the surface of OBs, but a pool of caleosins was also visualized in the cytoplasm at the subapical region. All these findings strongly suggest that this caleosin might have a function in oil body biogenesis and mobilization during anther development.This work was supported by ERDF co-financed projects AGL2008-00517/AGR (MICINN) and P06-AGR-01791 (Junta de Andalucía).

Transparent testa 16 plays multiple roles in plant development and is involved in seed oil biosynthesis in *Brassica napus*.

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Transparent testa16 (TT16), a transcript regulator belonging to the Bsister MADS-box proteins, regulates proper endothelial differentiation and proanthocyanidin accumulation in the seed coat. Our understanding of its other physiological roles, however, is limited. In this study, four TT16 homologs were identified from an important oil crop, *Brassica napus*. These genes shared a high sequence similarity but displayed different genome origins and expression patterns. Subsequently, the physiological and developmental roles of BnTT16s were dissected by a loss-of-function approach. RNAi mediated down-regulation of *tt16* in *B. napus* caused dwarf phenotypes with a decrease in the number of inflorescences, flowers, siliques and seeds. Fluorescence microscopy revealed that the *tt16* deficiency affects pollen tube guidance, resulting in reduced fertility and negatively impacting embryo and seed development. Moreover, *Bntt16* RNAi plants had reduced oil content and altered fatty acid composition. Transmission electron microscopy showed that the seeds of the RNAi plants had fewer oil bodies than the wild type. Further analysis by microarray showed that tt16 down-regulation alters the expression of genes involved in lipid metabolism, gynoecium development, and embryo development. The broad regulatory function of TT16 at the transcriptional level may explain the altered phenotypes observed in the transgenic lines. Overall, the results uncovered important biological roles of TT16 in plant development, especially in seed oil biosynthesis.

Production of wax esters by FAR-WS fusion proteins

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Wax esters (WE) consist of a long chain alcohol esterified with long chain fatty acid. The biosynthesis of WE from fatty acyl-CoAs requires only two enzymatic reactions: in a first step, a fatty acyl-CoA is reduced to a fatty alcohol by a fatty acid reductase (FAR) and, in a second step, a wax ester synthase (WS) combines another fatty acyl-CoA with the fatty alcohol in an ester linkage. WE are extremely stable at elevated temperature and resistant to oxidation. In addition, their linearity grants high viscosity, so that they represent a perfect additive to lubricating oils. In all species so far studied, the proteins catalyzing each step of WE biosynthesis have been found to be encoded by separated genes. We recently characterized a protein from *Tetrahymena thermophyla* involved in ether lipids biosynthesis that displays both Fatty Acid Reductase and Acyl Transferase (AT) activities. In order to evaluate the feasibility to produce WE from acyl-CoAs with a single bifunctional protein, different FAR and WS coding sequences were selected to generate various FAR-WS fusion constructs using the Tetrahymena protein as model.Heterologous expression of these fusion proteins in the yeast strain H1246 that is devoid of storage and ester lipids (*Mat a ura3 - lro1::TRP1 - dga1::kanMX4 - are1::HIS3 - are2::LEU2*) resulted in both fatty acid reduction and esterification. The fact that most of the produced fatty alcohols were found in WE indicates that optimal metabolic channeling of fatty alcohols into wax esters could be achieved by single peptide carrying both FAR and WS activities. Using different FAR and WS genes for generating these fusions allowed the production of WE mixtures with various compositions.

Regulatory mechanisms underlying oil palm mesocarp maturation and functional specialization in lipid and carotenoid metabolism

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The lipid-rich fleshy mesocarp tissue of the oil palm (*Elaeis guineensis*) fruit is not only the main source of edible oil for the world, but it is also the richest dietary source of provitamin A. This study examines the transcriptional basis of these two outstanding metabolic characters in the oil palm mesocarp. Morphological, cellular, biochemical, and hormonal features defined key phases of mesocarp development. A 454 pyrosequencing derived transcriptome was then assembled for the developmental phases preceding and during maturation, when high rates of lipid and carotenoid biosynthesis occur. A total of 2,629 contigs with differential representation revealed coordination of metabolic and regulatory components. Further analysis focused on the fatty acid and triacylglycerol assembly pathways and during carotenogenesis. Notably, a contig similar to the Arabidopsis (*Arabidopsis thaliana*) seed oil transcription factor *WRINKLED1* was identified with a transcript profile coordinated with those of several fatty acid biosynthetic genes and the high rates of lipid accumulation, suggesting some common regulatory features between seeds and fruits.

Studying the genetic control of seed oil content and composition using the Arabidopsis 'MAGIC' population.

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The Arabidopis 'MAGIC' population is a large multi-parent recombinant inbred mapping population made up of 19 parental accessions (Kover *et al*., [2009] PLoS Genetics 5: 71). Arabidopsis accessions exhibit significant genetic variation in seed oil content and fatty acid composition. To understand the molecular basis of this variation we have grown ~500 MAGIC RILs in a replicated random block design experiment, measured seed size, % oil content and fatty acid composition and performed statistical analysis of the data using REML. Calculated heritability for each of the traits was between 0.72 and 0.89. Using QTL mapping methods developed for the population, one or more QTLs were identified for each trait (i.e. seed size, % oil content and the proportion of each fatty acid species). For several major QTLs the 90% confidence intervals were <1 Mb. As an example, further dissection of one QTL (assisted by a parallel genome-wide association study) suggests that allelic variation at the *LYSOPHOSPHATIDYLCHOLINE ACYLTRANSFERASE2* locus is primarily responsible for determining seed very-long-chain fatty acid (20:1n9) content within this population.

Arabidopsis CGI-58 interacts with the peroxisomal transport protein PXA1

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CGI-58 (Comparative Gene Identification-58) is a soluble a/ß hydrolase-type protein that plays a key role in regulating triacylglycerol (TAG) homeostasis in plants and animals. In mammals, CGI-58 is conditionally localized to the surface of lipid droplets (LDs) via protein-protein interaction with an LD-associated protein called perilipin. Upon adrenergic stimulation, perilipin becomes phosphorylated, which releases CGI-58 from perilipin and allows it to interact with and stimulate the activity of adipose triglyceride lipase, which subsequently degrades TAG to produce FAs. Loss of CGI-58 activity is therefore thought to result in aberrant TAG turnover, resulting in enhanced TAG accumulation in LDs. In plants, loss-of-CGI-58 function similarly results in accumulation of TAG in cell types that do not normally accumulate lipid, suggesting that some aspects of CGI-58 function are evolutionarily conserved. Unlike mammals, however, plants lack any apparent homologs to perilipin, and relatively little is known about how CGI-58 functions in plants in general. To begin to address this issue, we used CGI-58 as bait to screen an Arabidopsis cDNA library using the yeast two-hybrid assay, and identified the peroxisomal membrane-bound ABC-transporter 1 (PXA1) as an interacting protein. Specifically, we showed that CGI-58 interacts with the cytosolic facing, C-terminal region of PXA1 in both yeast two-hybrid and in planta nuclear relocalization assays, as well as *in vitro* via co-immunoprecipitation assays. Mutations in the C-terminus of PXA1 that are known to modulate its transport activity *in planta* also modulated the ability of the protein to interact with CGI-58. Consistent with these data we showed also that CGI-58 is localized primarily to the cytosolic surface of the peroxisomal boundary membrane when transiently expressed in Agrobacterium-infiltrated leaf cells. The implications of these results in terms CGI-58's ability to regulate PXA1 functioning in both general and specific aspects of plant lipid metabolism, particularly in non-lipid-storing cell types, as well as the possibility that human CGI-58 might similarly interact with peroxisomal ABC-type transport proteins to facilitate the breakdown of various lipid substrates in mammals, are discussed.

Oil accumulation in stem tissues of aspen (*Populus tremula*) is induced by short day treatment

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There is a global need to develop new oil crops to meet the increased demand for plant oils as renewable alternatives to fossil oil in the future. In Sweden, the largest quantity of plant fatty acids available is actually from wood tissues processed in the pulp industry. The concentration of fatty acids in this tissue is very low, however, the vast amount of wood processed every year results in large production quantities. Even a small increase in triacylglycerol concentration in tree tissue would therefore result in a significant increase in oil productivity. By studying different aspects of oil synthesis in aspen (*Populus tremula*) as a model species we want to explore the potential for increasing oil content in stem tissues of trees. Lipid analyses of hybrid aspen (*P. tremula x P. tremuloides*) showed that massive accumulation of oil occurs in stem tissues during transition to dormancy after short day treatment in controlled climate chambers. Oil accumulation in stem seedlings was shown to be associated with an increased expression of a *WRINKLED1* (*WRI1*) homolog in aspen. The *WRI1* homolog from aspen was cloned and functionally characterized using Agrobacterium-mediated transient expression in *Nicotiana benthamiana* leaves. Moreover, the natural variation of lipid content in mature stems of aspen is screened in a collection of 120 genotypes growing in a common garden experiment in south of Sweden. Preliminary data show significant genotype variation which allows for the identification of candidate genes affecting lipid content. Taken together, these results demonstrate that aspen trees are metabolically capable to synthesize significant amounts of oil.

WRI1 homologs from non-embryonic storage tissues of aspen, oat, and nutsedge induce a massive increase of oil in tobacco leaves

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To meet future demands for a sustainable and increased plant oil production, new oil crops needs to be developed. We are exploring the possibility to transform high-yielding starch or sugar crops into oil crops by genetic engineering of carbon partitioning. By studying model species with unique abilities, as compared to their close relatives, to accumulate high amount of oil in non-embryonic storage tissues, key genetic factors involved in channeling carbon into oil synthesis can be identified. In this work we have focused on the transcription factor *WRINKLED1* (*WRI1*) that in several plant species is known to divert carbon flow into fatty acid synthesis. *WRI1* homologs from the non-embryonic tissues of oat (*Avena sativa*) grain endosperm, aspen (*Populus tremula x P. tremuloides*) stem tissues, and nutsedge (*Cyperus esculentus*) tuber parenchyma were cloned and functionally characterized by Agrobacterium-mediated transient expression in *Nicotiana benthamiana* leaves. Arabidopsis and potato (*Solanum tuberosum*) embryonic WRI variants were also included in the study. All homologs were shown to induce a massive increase in accumulation of oil in the leaves, up to 4 % by dry weight. Fatty acid profiles as well as concentrations of lipids from *N. benthamiana* leaves expressing the different *WRI1* homologs showed significant differences. This data suggests that the studied *WRI1* homologs cause a differential transcriptional regulation.

Comparative transcriptome analysis of two oil accumulating tissues from olive fruit

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Olive is the second most important oil fruit crop cultivated worldwide. Most olive production is destined for olive oil, ranking sixth in the world production of vegetable oils. The development of new cultivars with increased oil content in the olive fruit mesocarp is one of the more important aims of the olive breeding programs. However, understanding of the molecular basis of this trait requires the generation of sequence information and genomic tools, which are still scarce in olive.
Within the project OLEAGEN, three non-normalized cDNA collections corresponding to seeds and mesocarp tissue at two different developmental stages from Picual cultivar have been synthesized and sequenced using 454 pyrosequencing technology. After ESTs assembly, the resulting contigs have been annotated and incorporated to the olive sequence database generated within the project. *In silico* expression analysis has been performed, with special emphasis in those unigenes that codify enzymes of the metabolic pathways for fatty acid biosynthesis, triacylglycerol assembly, biosynthesis of plastidial lipids and carbon supply for fatty acid and glycerolipid biosynthesis. Genes with differential expression between the studied tissues have been identified, and its involvement in the regulation of the mentioned metabolic pathways will be discussed.

Biochemical characterization of substrate specificity of lipases from germinating seeds of jojoba (*Simmondsia chinensis*)

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Jojoba (*Simmondsia chinensis* Link, Buxaceae) is a perennial shrub that grows naturally in the deserts of Arizona and western Mexico. It is the only plant which accumulates wax esters in the seeds. These esters have numerous industrial applications, e.g. can be used as compounds of lubricants or cosmetics,. Still, it is very expensive to obtain high amount of wax esters from commercial jojoba farms. This is why several laboratories try to obtain, using tools of genetic engineering, other oilseed crops, which produce wax esters and store them in its seeds. Such seeds need to be viable and able to germinate. To do so, elucidation of wax esters catabolic pathway is needed. The aim of this study was to determine substrate specificity of lipases from germinating jojoba seeds. Jojoba seeds were germinated in growth chamber at 28º C with 16-hours photoperiod. The seeds were collected at various stages of germination. Microsomal fractions for *in vitro* assays were prepared from the cotyledons. Radiolabelled substrates: triacylglycerols, diacylglycerols, monoacylglycerols, wax esters and polar lipids were implemented to the assays and the reaction products were visualised and quantified by electronic autoradiography. At very early stages of germination the lipase activity of investigated seeds towards tested substrate (tri-[^{14}C]18:1-TAG & [^{14}C]18:1-18:1 wax ester) was very low. However it increased considerably during the germination. For substrate specificity experiments microsomal fractions with the highest lipase activity were used. It appears that TAG's, DAG's and MAG's are preferable substrates. Wax esters were also hydrolysed by lipases from germinating jojoba seeds, but with slightly lower intensity. No activity against polar lipids was detected. This work was supported by the European Commission through the FP7 ICON project

Senescence-inducible *LEC2* differently modulates genes involved in fatty acid and triacylglycerol synthesis to accumulate TAG in Arabidopsis leaf

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LEAFY COTYLEDON2 (*LEC2*) is a master regulator for seed maturation and trigger oil accumulation in seeds. The ectopic *LEC2* expression causes somatic embryogenesis or defects in seedling growth. Arabidopsis leaf is not physiologically coupled with triacylglycerol (TAG) synthesis even though the machinery for fatty acids and glycerolipids synthesis exists in such as seeds. Here, we tested if senescence-inducible *LEC2* expression could produce significantly amount of TAG in leaf without negative effect in plant growth. *LEC2* expression under control of Arabidopsis NAC promoter, TAG was accumulated in senescence leaves in transgenic Arabidopsis. TAG was increased according to senescence and decreased in completely dried leaf. Amount of total fatty acids were reached to 48μg mg^{-1} in dry weight of leaves, which is a threefold increase of wild-type. During senescence in leaf of *LEC2* transgenic plants, composition of fatty acids were more transited from status of plastidial fatty acids to those of endoplasmic reticulum (ER), and appeared seed-type fatty acids. *LEC2* in senescence leaf specifically modulated genes of certain steps in fatty acid and TAG synthesis metabolic pathways to accumulate TAG. Genes encoding enzymes involved in synthesis of building blocks for fatty acid condensation in plastid and glycerol 3-phospate backbone for glycerolipid synthesis in ER were up-regulated. Two plastidial long-chain acyl-CoA synthase (LACS) genes, *LACS8* and *LACS9* for increasing fatty acid flux from plastid to ER and five seed-type oleosin genes for oil-body formation was highly induced by *LEC2*. Especially seed-specific *FAE1* gene was highly up-regulated for synthesis C20:1 and C22:1. Senescence-inducible *LEC2* provides a new insight into where are key metabolic steps to increase TAG accumulation and a network for transcriptional factors in seed development. [This research was supported by a grant from Agenda Program (PJ006715) and the Next-Generation BioGreen 21 Program (SSAC, grant No:PJ00810) from Rural Development Administration, Republic of Korea]

Protein structure modeling, expression patterns, subcellular localization, and enzymatic analysis of monoacylglycerol lipase (*MAGL*) gene family in *Arabidopsis thaliana*.

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Monoacylglycerol lipase (MAGL) catabolizes monoacylglycerols (MAG) to produce fatty acids and glycerol and completed the hydrolysis of triacylglycerols (TAG). To understand the roles of MAGL in plants, 16 members of the Arabidopsis MAGL family were characterized. Predicted protein structures of all *At*MAGLs have a cap domain, catalytic amino acid triad (Ser119, Asp239 and His269 of *At*MAGL8) and GXSXG consensus sequence, which are conserved in MAGLs from mammals and microorganisms. The activity of *At*MAGL enzymes and most *At*MAGL transcripts were ubiquitously detected in various organs, germinated seedlings and senescing leaves, but *AtMAGL10* and *11* transcripts were specifically expressed in pollen. The levels of expression of all *AtMAGLs* were not significantly changed during leaf senescence and no *AtMAGL* genes were significantly induced by salt and dehydration stress, nor by ABA hormone. When *AtMAGLs:eYFP* gene fusion constructs were introduced into tobacco epidermis or protoplasts, the fluorescent signals were observed in the cytosol (*At*MAGL10, 12, and 15), the chloroplast (*At*MAGL2, 4, and 5) and the ER and Golgi network (*At*MAGL1, 3, 6, 7, 8, 11, 13, 14, and 16). The recombinant *At*MAGL proteins purified from *Escherichia coli* all had lipolytic activity in an alkaline condition (pH 8 ~ 10), except *At*MAGL14 and 16 which showed optimal activity at pH 6.5 ~ 7.5. When the recombinant AtMAGL proteins were incubated with various glycerolipid substrates such as MAG, DAG, TAG, LPE, LPC, MGDG, and DGDG containing an acyl group at the *sn*-1 position, *At*MAGL1, 3, 6, 7, 8, 10, 14, and 16 exhibited preference for MAG substrates. *At*MAGL2, 4, 5, 9, and 15 proteins displayed a strong preference for MAG and also relatively high activity for LPC and/or LPE. *At*MAGL11protein showed preference for MAG and TAG substrates. *At*MAGL1, 2, 4, 5, 10 and 14 preferred *sn*-1 MAG over *sn*-2 MAG, but *At*MAGL3 preferred *sn*-2 MAG. The other *At*MAGLs showed similar regiospecific activity on both *sn*-1 and *sn*-2 MAGs. When 16:0, 16:1, 18:0, 18:1, and 18:2 fatty acids were used most of the *At*MAGLs proteins showed specificity for unsaturated fatty acids over saturated fatty acids, with the exceptions of *At*MAGL6, 7, 8, and 15 which showed strong preference for 16:0 as well as unsaturated fatty acids. Taken together, this study suggests that *At*MAGL is a family of housekeeping enzymes that plays essential roles in metabolic pathways for growth and development in plants.

SUCROSE TRANSPORTER 5 supplies Arabidopsis embryos with biotin and affects triacylglycerol accumulation

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Biosynthesis and storage of triacylglycerols (TAGs) in the developing seed is driven by the interplay of numerous factors. One limiting factor is the efficient translocation of photoassimilates through the phloem stream and loading of these building blocks from the mother plant into the embryo. Here we show that the embryo also requires biotin as cofactor for both the cytosolic and the plastidic isoform of ACCase, which is the rate limiting enzyme in fatty acid synthesis. In plants, the embryo is isolated from the surrounding maternal tissues, endosperm and testa via apoplastic barriers. Import of sucrose and biotin into the embryo is mediated in Arabidopsis by the transporter SUC5. Its gene is expressed specifically at the embryo epidermis and in the endosperm, demonstrating the role of SUC5 in substrate transport across these barriers. We analyzed whether altered expression of *SUC5* or other transport proteins under control of the *AtSUC5* promoter would influence directional transport of these substrates accurately into the embryo and the endosperm. *suc5* single knock-out plants and biotin synthesis mutants *bio1* and *bio2* show that reduced biotin transport and synthesis activities result in a significant reduction of the seed TAG content. On the other hand, Arabidopsis plants overexpressing *SUC5* exhibited a higher transport activity for both, biotin and sucrose resulting in elevated TAG content. This strongly suggests that a directed import of the cofactor also promotes higher fatty acid biosynthesis.

Exploring the diversity of triacylglycerol-estolides in seed oils using MALDI-TOF mass spectrometry.

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The majority of seed oils are composed primarily of triacylglycerol, three fatty acids esterified to a glycerol “backbone”. A small number of plant species have been reported that store acylglycerols with more than 3 fatty acids. These plants generally produce fatty acids with hydroxyl groups. The hydroxyl moiety enables the attachment, by ester linkage, of additional fatty acids secondary to the glycerol backbone. The ester linked fatty acids are generally referred to as fatty acid estolides (FA-estolides), and when attached to a glycerol backbone the molecule is termed a triacylglycerol estolide (TAG-estolide). We have surveyed a number of seed oils using MALDI-TOF MS to identify the presence of these unusual acylglycerols. A glimpse into the weird world of TAG-estolide oils will be presented.

The tomato (*S. lycopersicum*) GDSL-lipase SlGDSL1 is required forcutin deposition in fruit cuticle

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Plant cuticle consists of cutin, a polyester of glycerol, hydroxy and epoxy fatty acids, covered and filled by waxes. While the biosynthesis of cutin building blocks is well documented, the mechanisms underlining their extracellular deposition remain unknown. Among the proteins extracted from dewaxed tomato peels, we identified SlGDSL1, a member of the GDSL esterase/acylhydrolase family of plant proteins, whose gene is strongly expressed in the epidermis of growing fruit. In *SlGDSL1*-silenced tomato lines, we observed a significant reduction of the fruit cuticle thickness and a decrease of cutin monomer content proportionally to the *SlGDSL1* silencing levels. FTIR analysis and determination of polysaccharide contents of isolated cutins revealed a reduction of cutin density in silenced lines. Indeed, FTIR-ATR spectroscopy and AFM imaging showed that a drastic *SlGDSL1* silencing leads to a reduction of the ester bond crosslinks and to the appearance of nanopores in tomato cutins. Furthermore immunolabeling experiments attested that SlGDSL1 is essentially entrapped in the cuticle proper and cuticle layer. These results suggest strongly that SlGDSL1 is specifically involved in the extracellular deposition of the cutin polyester in fruit cuticle.

Bifunctional wax ester synthases in *Arabidopsis thaliana*

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Wax esters consist of a fatty acid esterified to a fatty alcohol. Similar to triacylglycerol, wax esters serve as energy storage reserves in bacteria (e.g. Acinetobacter) and a few plant species such as jojoba. They are components of the cuticle of the aerial plant tissues, and presumably of the suberin layer of roots. For industrial application, wax esters are used as lubricants, pharmaceuticals and as food ingredients. Arabidopsis contains 11 genes (*WSD*, for wax ester synthase/diacylglycerol acyltransferase) with sequence similarity to the Acinetobacter gene *ADP1* encoding a bifunctional acyltransferase with capacity to synthesize wax esters and triacylglycerol. *WSD1* harbors wax ester synthesis activity and is involved in wax ester production for Arabidopsis stems (Li, Wu, Lam, Bird, Zheng, Samuels, Jetter, Kunst, 2008, Plant Physiol. 148: 97-107). *WSD3* presumably encodes a pseudogene. *WSD2* to *WSD11* are still uncharacterized. Arabidopsis *WSD* genes were heterologously expressed in the yeast acyltransferase mutant H1246. Feeding of palmitic acid and oleyl alcohol resulted in the accumulation of a wax ester band as revealed by thin-layer chromatography for the strains expressing *WSD2, WSD4, WSD6, WSD7* or *WSD8*. Interestingly, triacylglycerol accumulated in the *WSD4* expressing strain, but not in the others, indicating that WSD4 harbors wax ester/DGAT activity. Furthermore, homozygous T-DNA insertion mutants were obtained for the different Arabidopsis *WSD* genes. We will present the results on the wax ester measurements by Q-TOF mass spectrometry in leaves, stems and roots of wild type and different *wsd* mutant lines.

The feruloyl transferase FHT: cellular localization and potato tuber development and maturation

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Previous work from other groups and ours showed that BAHD feruloyl transferases are involved in suberin and associated wax biosynthesis. Silencing of *FHT* (Fatty ω-hydroxyacid/fatty alcohol Hydroxycinnamoyl Transferase) in potato periderm results in a significant decrease in ferulate esters of suberin and waxes and concurrently it also produces a large increase in the periderm water permeability (Serra *et al*., 2010). To get further insight on the function of this gene we studied the promoter activity and the protein accumulation in different tissues, developmental stages and under wound-healing and ABA treatment. Besides, immunolocalization and subcellular fractionation analyses confirmed the localization predicted "*in silico*".Results demonstrated that *FHT* is induced and the protein specifically accumulated in tissues that undergo cell wall suberization such as native periderm, root endodermis and exodermis and in wound-healing tissue. The time course analysis of *FHT* during tuber development and storage showed that *FHT* is expressed in the late stages of tuber development, reaching its maximum during the post-harvest maturation and then progressively decaying. Concerning gene inducing factors, wound-healing triggers promoter activity and *FHT* accumulation. This capacity was observed in leaf, stem and in potato discs. Moreover, ABA, a hormone involved in suberization-based wound-healing processes, induces protein accumulation in healing potato discs. Detailed histological observations showed that GUS activity driven by *FHT* promoter was limited to a single layer of phellem derivative cells underlying the mature (dead) phellem cells. GUS activity and *FHT* accumulation was also specific of the exodermis and endodermis of the primary root. In healing tissues, *FHT* expression was restricted to cells beneath the dead wound closing layer with walls not yet fully suberized.The subcellular fractionation was performed from native periderm, wound-healing periderm and root tissue. In all these tissues the FHT protein was mainly found in the cytoplasmic fraction in agreement with the pattern displayed by the cytosolic marker UDP-glucose pyrophosphorylase. Accordingly, confocal microscopy of root exodermal cells labelled with FHT antibodies corroborated the FHT cytoplasmic localization.Altogether our results confirm the specificity of FHT for suberized tissues, suggest that feruloylation of fatty acid derivatives takes place in the cytosol and provide new insights into the role of FHT in native and healing periderm. Results obtained through development, maturation and post-harvest storage of potato tubers may be important for potato manipulation and conservation during storage.Serra et al. (2010) Plant J 62:277-290.

Secretion of wax and cell wall components share common components in *Arabidopsis thaliana*.

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The primary aerial surfaces of plants are covered by a lipidic cuticle, which protects against non-stomatal water loss, serves as a barrier to pathogen invasion and prevents organ fusion. The cuticle consists of cutin and cuticular waxes, which are made in the epidermal cells. The biosynthesis of cuticular waxes has been studied for years and the major biosynthetic pathways are established. However, little is known about the export of wax molecules to the plant surface. Recently, we discovered that one of the wax-deficient mutants, *wax10*, has a reduced wax load on the stem surface compared to the wild type due to a defect in the secretory pathway. In addition, the *wax10* mutant also has a defect in seed coat mucilage release during hydration, and exhibits a delay in the production of the secondary cell wall in the seed coat epidermal cells. These data suggest that WAX10 is involved in the transport of wax molecules, or of the wax transporters, or both, to the plasma membrane, as well as the transport of components required for cell wall biosynthesis in the seed coat epidermis. Here we present preliminary phenotypic characterisation of the wax and seed coat defects. In addition, we outline future work to determine the subcellular localization of wax transporters, cell wall biosynthetic and mucilage modifying enzymes, in the *wax10* mutant compared to the wild type, which should allow the role of WAX10 in the secretion of these extracellular components to be more precisely determined.

Phenotypic changes in knockout mutants of lipid transfer proteins in *Arabidopsis thaliana*

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The plant non-specific lipid transfer proteins (nsLTPs) have the ability to transfer different lipids between bilayers *in vitro*. The *in vivo* function is not clear, but they are thought to be involved in plant stress resistance. This hypothesis is further confirmed by different studies pointing their function toward cuticle development, long distance signalling or pathogen defence, but also by our previous study where we have shown that nsLTPs are abundant in all land plants but not present in red or green algae. In order to better understand the *in vivo* function of plant nsLTPs, we have studied several knockout mutants in *Arabidopsis thaliana.* At the conference I will present some of the phenotypic changes we could observe in these mutants.

The role of *OsABCG31* in the formation of the epidermal extracellular matrix in rice

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A hydrophobic extracellular matrix layer that is called cuticle covers the epidermis of plant organs in primary growth stage, such as leaves and fruits. The cuticle plays important roles in protecting the plant against biotic and abiotic aggressions as well as during plant development. A important structural component of the cuticle is the polyester cutin formed by oxygenated fatty acids, glycerol and minor amounts of phenolic acids. Waxes that impregnate the cuticle and shape its surface are mainly composed of very long chain fatty acids, alcohols, aldehydes, ketones and alkanes. Transporters of the ABCG subfamily of ABC transporters are hypothesized to mediate transport of wax molecules as well as cutin intermediates. Characterization of the *pec1* mutant in Arabidopsis and the *eibi* mutant in barley lead to the identification of *AtABCG32* and its homologues *HvABCG31/OsABCG31* in cutin formation.Here we present the characterization of *OsABCG31*, the homologue of *AtABCG32* in rice. *OsABCG31* shares 71% and 92% identity to the Arabidopsis and barley homologues, respectively. *Osabcg31* knockout mutants have a permeable cuticle. In addition, *osabcg31* knockout mutants display a severe dwarf phenotype that had not been seen in *pec1* and *eibi* mutants. Cutin analysis shows a 50% reduction of hydroxylated fatty acids in parallel to an increase in unsubstituted fatty acids confirming a role of OsABCG31 in cutin formation. Transmission electron microscopy gives evidence to a broad range of structural alterations in the cuticle/cell wall continuum in the *osabcg31* knockout mutant. Scanning electron microscopy demonstrates also perturbations in epidermal cell development as well as a strong reduction in wax load. The lack of OsABCG31 leads thus to a much broader impact on the formation of the epidermal extracellular matrix than its homologues in Arabidopsis and barley that may be also the cause of the phenotypes in epidermal development and plant growth.

The Arabidopsis *cer26* mutant is specifically affected in VLCFA elongation process.

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Plant aerial organs are covered by cuticular waxes, a hydrophobic crystal layer which serves as a waterproof barrier. Cuticular wax is a complex mixture of very long chain aliphatic lipids with predominant chain lengths from 26 to 32 carbons. To learn more about wax biosynthesis in *Arabidopsis thaliana,* we characterized the *CER26* gene, a closed homologue of *CER2*. The analysis of *CER26* and *CER2* expression patterns in the different organs of Arabidopsis showed that both genes are differentially expressed in an organ and tissue-specific manner. Using *cer26* and *cer2* TDNA insertional mutants together with a *cer2cer26* double mutant we characterized the impact of the inactivation of both genes on cuticular waxes. Therefore we showed that *cer26* accumulates, specifically in leaves, wax components with chain length with less than 30 carbons whereas *cer2* accumulates, specifically in stems, wax components with chain length with less than 28 carbons. The analysis of the acyl-CoA pool in the different tissues of the different lines confirmed that the inactivation of both genes affects the VLCFA elongation process at specific stages. In addition, CER26 overexpression in *cer26* background rescued correct wax biosynthesis in leaves. Strikingly the stems of the complemented *CER26R* line showed a large increase of the C31 and C33 alkane amounts suggesting that ectopic expression of *CER26* leads to production of wax components with chain length with more than 30 carbons. Our results demonstrate that CER26 and CER2 regulate the activity of the acyl-CoA elongase with high tissular and substrate specificity.

Arabidopsis 3-keto acyl-CoA synthase 9 is involved in the synthesis of tetracosanoic acids, which are essential precursors for the biosynthesis of cuticular waxes and suberin polyesters

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The plant surface is surrounded by a lipophilic cuticle layer that plays roles in protection from environmental stresses. The cuticle is composed of a top layer covered with wax, a middle layer containing cutin embedded in wax, and a lower layer formed of cutin and wax mixed with the cell wall carbohydrates. Very long chain fatty acids (VLCFAs) with chain lengths from 20 up to 34 carbons are essential precursors for the biosynthesis of cuticular waxes, cutin and suberin polyesters, sphingolipids, and storage oils. The first step in VLCFAs biosynthesis is condensation of two carbons to an acyl-CoA, which is processed by 3-keto acyl-CoA synthase (KCS) on the ER membrane. Arabidopsis genome harbors 21 *KCSs* that might have different substrate specificity. In this study, *KCS9* gene showing higher expression in stem epidermal peels than in stems was isolated. When spatial and temporal expression of *KCS9* gene was analyzed using the promoter::GUS reporter system in Arabidopsis, GUS expression was detected in the aerial portions of young seedlings, meristematic and elongation zones of roots, leaves and stems including epidermis, silique walls, sepals, the upper portion of the styles, seed coats, but not in developing embryos. The fluorescent signals of *KCS9::eYFP* construct were merged with those of *BrFAD2::RFP*, which is a ER marker in tobacco epidermal cells. In fatty acid analysis, the amount of C20 and C22 fatty acids was increased by 10% in roots, silique walls and flowers of *kcs9* knock-out mutant, but the amount of C24 fatty acids was decreased in *kcs9* roots compared with wild-type. Also C20 and C22 fatty acids of leaves, stems and aerial parts of young seedlings were increased by over 30%. In cuticular wax analysis, the amount of C24, C26, and C30 fatty acids in leaves, C26 and C28 primary alcohols in stems, and C26 and C28 fatty acids in seed coats was also significantly decreased by approximately 30%, 20%, and 40%, respectively, in *kcs9* relative to wild-type. Root and seed polyester analysis revealed that the amount of C20 and C22 fatty acids was increased by 10%, but the amount of C24 fatty acids was reduced in *kcs9* compared with wild-type. The chemical phenotypes of *kcs9* mutant were restored to those of the wild-type by the expression of *KCS9* gene fused with *eYFP* gene under the control of *CaMV35S* promoter. Taken together, KCS9 is involved in the elongation of C22 to C24 fatty acids, which are essential precursors for the biosynthesis of cuticular wax and suberin polyesters.

Characterization of cutin mutants of Arabidopsis by Fourier Transform Infrared-Microspectroscopy

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The epidermis of several organs of the shoot, such as leaves and flowers, has a specialized extracellular matrix since the cuticle consisting of soluble waxes and cutin, a polyester of mainly oxygenated fatty acids and glycerol, forms the interface between the plant and its aerial environment. The cuticle covers and protrudes deeply into the primary cellwall and its formation is tightly regulated during cell expansion as the expansion of the primary cellwall itself. The tri-dimensional structural of cutin however as well as its structural modifications during plant growth and development are still elusive as well as its potential covalent linkage to the cellwall.Fourier transform infrared spectroscopy (FTIR) is a method that has been used for the molecular characterization of cellwall polymers, cutin and proteins and maybe therefore useful for the simulataneous characterization of all three classes of compounds present in the plant extracellular matrix in muro. Here we characterize the distribution of spectral features associated with the different classes of extracellular matrix components in Arabidopsis by FTIR-Microspectroscopy. While in cotyledons, only veins show distinct spectral features, petals exhibit a specific chemical zoning dividing the petal in three regions, the blade, the stalk and the hinge region, being rich in aliphatic esters, carbohydrates and amides, respectively. Mutants having lesions in cutin formation, such as *lacs2* and *pec1* attributed the peaks of aliphatic esters to cutin. The blade of petals gives thus a unique opportunity to gain information on the cuticle-cellwall continuum in Arabidopsis because of their high ratio between epidermal to internal cell wall quantity.

Characterization of Bayberry surface lipid production provides evidence for a new pathway to produce triacylglycerol in plants

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Bayberry (*Myrica pensylvanica*) is a deciduous shrub that accumulates very large amounts of “wax” on the surface of its fruits (approximately 20% of total fruit mass). This is one of the largest known accumulations of surface wax in nature. The wax is produced and secreted from large multicellular structures that protrude from the fruit exocarp (called knobs). Bayberry wax is composed entirely of saturated acylglycerols, mostly in the form of di- and triacylglycerols (DAG and TAG) with palmitic and myristic acids. Plants that contain large quantities of DAG and TAG in their wax are very unusual and there is no literature describing the possible mechanisms for the potentially valuable property of triacylglycerol secretion from cells. We have used a combination of transcript, biochemical and ultrastructural analyses to characterize Bayberry wax production and secretion. Preliminary RNA-SEQ results from wax producing knob tissue reveal that the most abundantly expressed lipid-related genes are those known to be involved in surface lipid biosynthesis such as *sn*-2 glycerol-3-phosphate acyltransferases, ABC transporters, and lipid transfer proteins. Furthermore, there is a substantial accumulation (up to 5% of total surface wax) of monoacylglycerol (MAG), with the acyl-chain on the 2 position of glycerol (2-MAG or ß-MAG). Previously we proposed that 2-MAG is an intermediate in the biosynthesis of surface lipid polyesters cutin and suberin. Because the major lipids in Bayberry wax are saturated acylglycerols, we speculate that enzymes involved in surface lipid biosynthesis may be producing DAG and TAG Bayberry waxes. Such a pathway to synthesize DAG and TAG has not previously been described in plants.

Identification and characterization of a cytochrome P450 involved in the synthesis of cutin hydroxyacids in *Arabidopsis thaliana*

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Cutin is a lipid polymer forming the framework of the plant cuticle. The elementary components of the cutin polymer (monomers) are C16 and C18 hydroxyacids, epoxyacids and diacids that are esterified to each other and to glycerol. We have previously identified an in-chain fatty acid hydroxylase responsible for the synthesis of 10,16-dihydroxypalmitate, a major member of the C16 monomer family (Li-Beisson et al. PNAS 106:22008). Using a reverse genetic approach in *Arabidopsis thaliana*, we identify here a cytochrome P450 involved in the synthesis of hydroxyacids from the C18 family of cutin monomers. The poster presents the characterization of the gene/protein and the knockout mutants.

Structure and biological functions of dihydroxylated metabolites synthesized from alpha-linolenate by soybean lipoxygenase.

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Soybean lipoxygenase oxidizes polyunsaturated fatty acids (PUFAs) into mono- and di-hydroxylated derivatives. Recently, we have described an isomer of protectin D1, named protectin DX (Chen P. et al., FEBS Lett., 2009), which derives from docosahexaenoic acid via oxygenation by soybean lipoxygenase (sLOX). Such a compound possesses potent anti-aggregatory properties (Chen P. et al., FASB J., 2011). Taking into account the increasing interest of n-3 PUFAs for their health benefits, we have investigated the sLOX oxygenation of alpha-linolenic acid which is also consumed in large amount by beta-oxidation.
We report here that 18:3n-3 is converted by soybean lipoxygenase mainly into 13(S)-OH-18:3n-3 and also into four dihydroxylated isomers. Their complete characterization was established using high field NMR and GC-MS. The following structures were detected: 9(S),16(S)-dihydroxy-octadeca-10E,12E,14E-trienoic, 9(S),16(R)-dihydroxy-octadeca-10E,12E,14E-trienoic , 9(S),16(S)-dihydroxy-octadeca-10E,12Z,14E-trienoic and 9(S),16(R)-dihydroxy-octadeca-10E,12Z,14E-trienoic acids.

These molecules were then tested on platelets isolated from human blood. We observed that only molecules having a conjugated E,Z,E triene inhibited platelet aggregation induced by collagen as well as an antagonist of the thromboxane A2 receptor. We also found that such E,Z,E compounds inhibited the sheep cyclooxygenase-1, in agreement with the inhibition of collagen-induced platelet aggregation. The 5-lipoxygenase pathway is a relevant one to promote inflammation through arachidonic metabolites. Those metabolites are LTB4 and its geometrical and stereochemical isomers (LTB4 isomers). Measuring 5-HETE as well allows having a complete view on the 5-lipoxygenase pathway acting upon arachidonic acid. We observed that the E,Z,E compounds weakly inhibited the 5-lipoxygensae pathway by around 9%. Moreover the recombinant cyclooxygenase-2 was also inhibited by around 30%. Taken together, our results suggest that alpha-linolenic acid could exhibit anti-inflammatory potential via its lipoxygenase products, and may be protective in chronic inflammatory diseases.

In conclusion dihydroxylated derivatives from 18:3n-3 which inhibit cyclooxygenase activities would be protective against inflammation and might be used for prevention and treatment of various inflammatory diseases, such as inflammatory bowel disease, rheumatoid arthritis and coronary heart disease.

Spectral shift and partitioning of activities between allene oxide synthase and hydroperoxide lyase by binding of type II ligands and point mutations at F92 and P430 in rice allene oxide synthase-1

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Allene oxide synthase (AOS) and hydroperoxide lyase (HPL) are a member of cytochrome P450 subfamily 74 (CYP 74) which is classified as a non-classical cytochrome P450 since they do not require molecular oxygen or NADPH-dependent P450 reductase for their activities. UV-Visible spectral analysis of wild type OsAOS1 revealed that a Soret maximum at 393 nm was shifted to 424 nm. The magnitude of spectral shift induced by imidazole binding was linearly co-related with the partial inhibition of OsAOS1activity of using HPODE as a substrate. The spectral shift indicates that imidazole may coordinate to ferric heme iron, triggering a heme-iron transition from high spin state to low spin state. In order to clarify relationship between spectral shift and relative partitioning of activities between allene oxide synthase and hydroperoxide lyase, F92 and P430 of OsAOS1were selected for point mutations. Partition ratio for HPL activity was increased by F92L mutation with the partial shift of Soret maximum toward 424 nm. P430A mutation also shifted the Soret maximum toward 424 nm. Double mutation (F92L/P430A) of OsAOS1 increased the partition ratio for HPL activity with the expected spectral shift. Therefore, the structural variations of OsAOS1 by binding of Type II ligands and point mutations at F92 and P430 manipulated the partition of OsAOS1 between for AOS and HPL activities. Our results imply that the spin state of OsAOS1 is an important determinant to functionally separate AOS from HPL pathway of OsAOS1 reaction or CYP74 in general, requiring high spin for AOS activity and low spin for HPL activity, respectively.

Dependence of oligomeric state of dual positional substrate specific rice allene oxide synthase-1 on detergent micelle

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Allene oxide synthase (AOS) is a key enzyme in jasmonic acid (JA) biosynthesis and plays an important role in plant host defense. Kinetic analysis showed that OsAOS1 is dual positional substrate specific with preference for the 13-positional isomer over the 9-positional isomer. HPLC and GCMS analysis of OsAOS1 reaction products indicated that OsAOS1 is specific for (cis,trans)-configurational isomers of 13(S)- and 9(S)-hydroperoxyoctadecadienoic acid. Oligomeric state of OsAOS1 was characterized in the absence or the presence of detergent micelle. Native gel analysis showed that OsAOS1is in multimeric form in the absence of detergent micelle. Cross-linking experiment detected multimeric form of OsAOS1 in the absence of detergent micelle, and monomeric form in the presence of detergent micelle. MALDI-TOF analysis indicated that OsAOS1 is in monomeric form in the presence of detergent. Gel-filtration analysis revealed that the oligomeric state of OsAOS1strongly depends on concentration of detergents and a monomeric form is favored in the presence of detergent micelle. Therefore, OsAOS1 is in equilibrium between multimeric and monomeric states and its oligomeric structure strongly depends on detergents favoring monomeric state in detergent micelle.

Membrane Binding and Activation of 11R-Lipoxygenase: The activating capability of Ca^{2+} is strongly influenced by the nature of membrane

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Activation of lipoxygenases (LOX) is often related to the selective membrane binding upon cell stimulation. In this study, a systematic analysis of the effect of the lipid composition to the membrane binding efficiency, Ca^{2+} affinity and enzymatic activity of 11R-LOX was performed. *In vitro* membrane binding analysis by fluorometric and surface plasmon resonance measurements showed an exclusive binding of 11R-LOX to the anionic phospholipids (phosphatidylinositol < phosphatidylglycerol ≈phosphatidylserine) containing model membranes in a Ca^{2+}-independent manner. The presence of Ca^{2+} enhanced the rate of interaction and influenced its mode. The modulation of the activity and Ca^{2+} binding affinity of 11R-LOX indicated that (i) the Ca^{2+} binding is a prerequisite for a productive membrane association; (ii) the reaction of 11R-LOX with arachidonic acid coincided with and was driven by its Ca^{2+}-mediated membrane association; (iii) phosphatidylethanolamine and anionic phospholipids had a synergistic effect on the Ca^{2+} affinity, in line with a target-activated messenger affinity mechanism. *In vivo*, 11R-LOX accumulated in the cytoplasm of resting COS-7 cells where it was associated with internal membranes or cytosolic vesicular structures. No translocation was detected upon ionophore stimulation. Truncation of the N-terminal C2-like domain abolished the Ca^{2+}-dependent targeting of 11R-LOX to the cellular membranes but did not significantly affect the Ca^{2+}-independent membrane association. According to the mechanism proposed in this report 11R-LOX can bind to the membranes in two different modes and the efficiency of the productive membrane binding is determined by a concerted association of Ca^{2+} and lipid headgroups.

Functional analysis of a JA responsive gene *CHJ*

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Jasmonates (JAs) are plant hormones which have crucial roles in regulating stress responses and development. JAs regulate a broad spectrum of physiological events such as plant growth, defense response against wounding and pathogen infection, and developmental process including root elongation, senescence, tuber formation. In JA signaling cascade, JAZ family proteins are repressors of MYC2, a JA responsive transcription factor, and targets of SCFCOI1. Upon jasmonate perception, JAZ repressors are polyubiquitinated and degraded by 26S proteasome, releasing MYC2 and allowing the activation of JA responses. However, the molecular details of signaling process after expression of JA responsive transcription factors remain largely unknown. Here we report the identification of *CONSTITUTIVELY HYPERSENSITIVE TO JASMONATE* (*CHJ*) in *Arabidopsis thaliana*. *CHJ* is co-regulated with JA responsive genes like *JAZs* and *MYC2*. *chj* mutants treated with MeJA turned yellow in 4 weeks after germination, whereas WT plant remained green. It suggested that *chj* mutant is hypersensitive to jasmonate. Next, we analyzed histochemical localization of GUS activity in transgenic plants. Our result suggested that *CHJ* is expressed in cotyledon after MeJA treatment. Moreover, the expression of a defensive gene *PDF1.2*, which is regulated by both JA and ET signaling pathways, is up-regulated in *chj* mutant. Therefore, CHJ negatively regulates the signal pathway after JA perception in JA signaling.

Regulation of oxidative stress-induced cell death via sphingolipid metabolism and remodeling of plasma membrane microdomain.

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We have studied molecular functions of Bax inhibitor-1 (BI-1) that is an endoplasmic reticulum (ER)-localized cell death suppressor conserved in plants and animals. Recent advances revealed that BI-1 modulates sphingolipid metabolism via interaction with cytochrome b5, an electron donor for ER-localized lipid metabolizing enzymes. Implication of sphingolipid metabolism in BI-1-mediated cell death regulation is supported by the observations that sphingolipids accumulate in plants overexpressing BI-1 and that several sphingolipid-metabolizing enzymes are indispensable for BI-1 activity to suppress Bax lethality in yeast. In mammalian cells sphingolipids are known as a key molecule for membrane microdomain, a platform for various cellular signaling events including apoptosis. To evaluate whether BI-1-mediated sphingolipid alteration affects components and functions of membrane microdomain in plants, we prepared detergent-resistant membrane (DRM) as a microdomain-enriched fraction from rice cells overexpressing BI-1. Lipid analysis revealed that glucosyl ceramide, one major membrane sphingolipid, increased in total cell extracts and DRM fractions prepared from BI-1-overexpressing cells compared to WT cells. Comparative proteomics indicated that several cell death-associated factors and microdomain-structural proteins significantly decreased in BI-1-overexpressing cells. Western blot, RT-PCR and confocal microscopy analyses demonstrated the plasma membrane microdomain-specific localization of the identified DRM proteins, whose protein levels were drastically decreased in BI-1 overexpressing cells without transcriptional changes. Insertional mutation and RNAi-mediated downregulation of the altered DRM proteins attenuated oxidative stress-induced cell death in rice cells. Our observations suggest that plasma membrane microdomains intermediate signal transduction in oxidative stress-induced cell death, which is negatively regulated by BI-1 via remodeling of lipid and protein profiles in microdomains.

56-Amino acid small subunits of serine palmitoyltransferase stimulate sphingolipid synthesis, impact mycotoxin sensitivity and are essential for pollen viability in Arabidopsis

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Sphingolipid synthesis in plants is tightly regulated to provide sufficient amounts of these lipids for growth and endomembrane function and to mediate levels of sphingolipid metabolites that control basic cellular processes such as program cell death (PCD). Increasing evidence points to serine palmitoyltransfersase (SPT), the first enzyme in sphingolipid long-chain base synthesis, as the key regulated step in eukaryotic sphingolipid synthesis. We have previously shown that the core Arabidopsis SPT consists of a heterodimer of AtLCB1 and AtLCB2 subunits that are able to weakly rescue long-chain base auxotrophy when co-expressed in a yeast *lcb1lcb2* mutant. Through homology searches using amino acid sequences for mammalian small subunits of SPT (ssSPT), two Arabidopsis genes designated *AtssSPTa* and *AtssSPTb* were identified that encode 56 amino acid polypeptides. Co-expression of *AtssSPTa* or *AtssSPTb* was shown to enhance SPT activity of the AtLCB1-AtLCB2 heterodimer by ~100-fold in the yeast *lcb1lcb2* mutant. Consistent with the subcellular localization of AtLCB1-AtLCB2, AtssSPTa and AtssSPTb were found to be ER-associated. Though both genes are constitutively expressed, *AtssSPTa* transcripts were >400-fold more abundant in pollen than those for *AtssSPTb*. Consistent with this, homozygous *AtssSPTa* T-DNA lines were not recoverable, and 50% pollen lethality was detected in heterozygous *AtssSPTa* T-DNA mutants, which was rescued by expression of the wild-type *AtssSPTa* gene. In addition, over-expression lines of *AtssSPTa* displayed enhanced sensitivity to the PCD-inducing mycotoxin fumonisin B1 (FB1), whereas *AtssSPTa* RNAi lines showed enhanced resistance to FB1. These results show that AtssSPT polypeptides play a critical role in sphingolipid synthesis and cellular functions in Arabidopsis through their ability to stimulate SPT activity. The larger role of AtssSPTs as components of the intricate sphingolipid SPT regulatory network that is responsive to sphingolipid homeostasis will be discussed.

Unraveling the metabolic and biological importance of sphingolipid long-chain base Δ4 unsaturation in plants

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Long-chain bases (LCBs) are unique structural components of the sphingolipid ceramide backbone and also function in their free and phosphorylated forms as regulators of cellular processes, such as programmed cell death. A portion of the immense sphingolipid structural diversity arises from the C-4 hydroxylation and Δ4 and Δ8 desaturation of LCBs. In these studies, we are attempting to address why most tissues of Arabidopsis are deficient in LCB Δ4 unsaturation, a structural modification typically limited to the glucosylceramide (GluCer) sphingolipid class, while tomatoes are highly enriched in LCB Δ4 unsaturation. To this end, we generated Arabidopsis lines with constitutive up-regulation of the LCB Δ4 desaturase from Arabidopsis and tomato, and tomato RNAi lines with suppression of the native LCB Δ4 desaturase. Arabidopsis lines with up-regulated Δ4 desaturase expression accumulated the diunsaturated LCB d18:2Δ4,8 to levels of >40% in GluCer, and displayed >2-fold increase in total GluCer content. Of the two sources of LCB Δ4 desaturase genes tested, the strongest chemical phenotypes observed were achieved with the tomato gene, and these lines also displayed defects in flower and pollen development. Conversely, RNAi silencing of the LCB Δ4 desaturase in tomato leaves resulted in a reduction of d18:2.Δ4,8 to ~5% of the GluCer LCBs, a nearly 15-fold reduction compared to leaves of non-transgenic plants. Most notably, the total GluCer content of leaves of RNAi lines was only 10% of that of wild-type leaves, but had little impact on levels of inositolphosphoryl ceramides. Despite this dramatic reduction of GluCer content, no phenotypic differences were observed in the growth and development of the tomato RNAi lines. Overall, these results demonstrate the importance of LCB Δ4 unsaturation to ceramide channeling for GluCer synthesis, and suggest that high levels of LCB Δ4 unsaturation can have deleterious effects on Arabidopsis reproductive capacity. The mechanism for this phenotype is currently under investigation.

Analysis of glucosylceramides and steryl glucosides in plant samples by LC–MS/MS

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Glucosylceramides and steryl glucosides are major structural components in plasma membrane and tonoplast. These lipids are of particular interest in microdomain formation and/or function. We will investigate putative functions of glucosylceramides and steryl glucosides in rose petals. Here we demonstrated that HPLC–ESI–MS/MS is an efficient method for characterizing plant glucosylceramide species having the cis-8 and trans-8 isomers of sphingoid bases. A complete baseline separation was achieved using a high carbon content octadecylsilyl (ODS) column and a simple binary gradient. A similar method was employed for the analysis of steryl glucosides, which allows direct measurement of sitosterol, campesterol or stigmasterol glucosides.

Functional analysis of a gene for long-chain base kinase in Arabidopsis

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Sphingoid long-chain base (LCB) kinase catalyzes the phosphorylation of LCBs to form LCB 1-phosphates. Based on sequence identity to murine sphingosine kinase (*mSPHK1*), we cloned and characterized a LCB kinase gene from *Arabidopsis thaliana* (*LCBK1*). Using recombinant LCBK1 protein from *Escherichia coli* cells, we confirmed that the enzyme specifically phosphorylated D-erythro-dihydrosphingosine (DHS), but not N-acetyl-DHS or D-threo-DHS. LCBK1 also phosphorylated D-erythro-sphingosine, trans-4, trans-8-sphingadienine and phytosphingosine. RT-PCR data showed that *LCBK1* mRNA was expressed highly in flowers. (Plant Cell Physiol. 46: 375-380, 2005). To understand the physiological role of LCBK1 by loss-of-function strategies, we tried to characterize Arabidopsis mutants, in which the *LCBK1* gene is knocked out by T-DNA insertion. However, we were not able to obtain the homozygous mutants. The expression signals of the β-glucuronidase (*GUS*) gene under the regulation of a 1.0 kb genomic sequence upstream of the transcription initiation site on *LCBK1* were observed at some anthers, suggesting that *LCBK1* expression is involved in anther ontogeny.

Preliminary characterization studies on Sacha Inchi (*Plukenetia volubilis* L.) seeds and oils grown in San Martín, Peru.

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An approach to the chemical composition of seeds and oils of Sacha Inchi (*Plukenetia volubilis* L.) native ecotypes Pinto Recodo (EC1, EC2) and Ashaninka (EC3) belonging to the agro-ecological zone of San Martin, Peru, is being undertook. Their proximate analysis shows that they have high oil content (37- 42%) and proteins (25.28 - 26.94%) followed by carbohydrates 10,35-14,51%; fibers (5,96-6,78%) and ashes (3.92-3.83%). The oils, obtained by a solvent-free method, show low acidity values 0,03- 0,06%, saponification values between 181,91 - 193,50, iodine values between 165.74 - 161.41 and low extinction coefficients. Analysis of fatty acids methyl esters stands the high percentages of the linoleic and linolenic acids: 48.8-49.6 and 34.3-35.1 respectively. The Triglycerides analyzed by HPLC showed as major molecular species those with equivalent carbon number (ECN) 36, 38 and 40 with percentages of 13.1-12.8; 23.7 - 24.8% for those ECNs respectively. In spite of their high unsaturation, the oils are stable due to their high Tocopherol contents mainly due to the positional isomers .- and d- with concentrations of: 2755-3878 mg kg^{-1} and 1803-2650 mg Kg^{-1} respectively. Other valuable compounds are the sterols: 2270-2500 mg Kg^{-1}, showing as major components those from the .-5 serie: ß-Sitosterol (58%), Stigmasterol (20%) and Campesterol (8%).

KEY- WOORDS: Sacha Inchi Ecotypes- *Plukenetia volubilis* L. – Oil characterization – Fatty acids - Sterols - Tocopherols – Triglycerides.

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Chemical characterization of a novel vegetable resource of phytosterols: Oil Sacha inchi (*Plukenetia volubilis* L)

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Plukenetia volubilis L. (Euphorbiaceae) is a traditionally cultivated plant in Peru, which has been and still is used as a traditional nutrient and remedy in the Peruvian Andes and by the native communities of the Peruvian Amazon. The Sacha inchi oil shows an interesting fatty acid profile, predominated by unsaturated components, composed mainly by linolenic (C18:3 ω-3) and linoleic (C18:2 ω-6) acids. This oil has also interest because is rich in tocopherols and ß-carotene. The profile of sterols of the Sacha inchi oil has not been studied yet but there is a considerable interest in the study of phytosterols due to their promotion of cardiovascular health, especially through their cholesterol lowering properties.

The objective of this work was to characterize the phytosterols composition of Sacha inchi oil and evaluate the differences in the profiles of phytosterols in oil samples of different origin.

A total of 27 commercial oils of Sacha inchi obtained from two consecutive crops and produced in different areas of Peru were analysed by gas chromatography. The analytical method consisted on saponification, separation of the sterolic fraction by thin layer chromatography, formation of the silyl derivatives of sterols and gas chromatographic separation to obtain the sterolic profile. The peak identification was carried out by calculating the relative retention times to ß-sitosterol, major sterol in this oil, according to the procedure described in the Commission regulation (EU) No 61/2011 and quantification was carried out using 5a-Cholestan-3ß-ol as internal standard.

A total of 12 compounds were identified, being the major components ß-sitosterol, stigmasterol, brassicasterol, campesterol and Δ5-avenasterol.

Chemometric procedures based on cluster analysis, principal components analysis and discriminant analysis were applied to the profiles of phytosterols of the samples. The statistical analysis showed significant differences between samples that were associated with their origin.

Is there protein-assisted long-distance phosphatidic acid signaling in plants?

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Plants cannot move to escape adverse conditions. As a consequence, they evolved mechanisms to detect changes in their environment, communicate these to different organs, and adjust development accordingly. One of these adaptations, the phloem, serves as a major trafficking pathway for assimilates, viruses, RNA, plant hormones, metabolites, and proteins with functions ranging from synthesis to metabolism to signaling. The study of signaling compounds within the phloem is essential for our understanding of plant communication of environmental cues. Determining the nature of signals and the mechanisms of their transport will lead to a more complete understanding of plant development and plant responses to stress. Our analysis of Arabidopsis phloem exudates revealed several lipid-binding proteins as well as lipids. Lipid transport in the phloem has been given little attention until now. In other aqueous systems like the human blood lipids are often transported while bound to proteins. In some cases, they serve as messengers and modulate transcription factor activity. We have shown that one phloem lipid-binding protein (PLAFP) binds one of the phloem lipids, phosphatidic acid (PA). PA is known to play a role in intracellular signaling. We will present data showing the effect of PLAFP on development, phloem lipid profile; lipid-binding properties, expression in response to various stresses, and localization within the plant and on a cellular level.Our data suggest that PLAFP, and with it bound PA, may play a role in long-distance developmental signaling.

A role of phosphatidylinositol 3,5-bisphosphate in vacuolar structure change in guard cells of closing stomata

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Rapid stomatal closing is essential for conservation of water, which is critical for survival of plants under fluctuating environment. To close stoma rapidly, guard cells shed their volume by converting the large central vacuole to highly convoluted vesicular and tubular structure. Such change of vacuole is essential for efficient stomatal closing movement, but the molecules involved and the mechanism of the change is not clearly understood. In this study, we demonstrate that the vacuolar convolution is accompanied by acidification of the vacuolar lumen in guard cells during ABA-induced stomatal closure. In addition, we provide evidence that phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5)P2) plays an important role in these processes from genetic and pharmacological studies of PI3P5K, which generates PtdIns(3,5)P2 from phosphatidylinositol 3-phosphate (PtdIns3P). Arabidopsis mutant plants that do not express AtFAB1B and/or AtFAB1C (*atfab1b, atfab1c, atfab1b/atfab1c*), closed stomata more slowly than the wild type upon ABA treatment. Treatment with an inhibitor of PI3P5K inhibited the vacuolar convolution, vacuole lumenal acidification, and stomatal closure in response to ABA. These results, together with the important role of PtdIns(3,5)P2 in vacuole dynamics in other organisms, suggest the possibility of PtdIns(3,5)P2 regulating vacuole acidification and convolution in plant.

ROF1 and ROF2 affect plant germination under osmotic and salinity stress through a phosphatidylinositol-phosphate related pathway

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Interaction of the *Arabidopsis thaliana* immunophilins ROF1 and ROF2 with PI3P and PI3,5P2 was identified using a phosphatidylinositol-phosphate (PIP) chromatography combined to a mass spectrometry (nano LC ESI-MS/MS) analysis. The first FK domain of ROF1 containing its PPIase activity was shown sufficient for binding to both PIP stereoisomers. The second FK domain that shows high versatility also enabled docking to the PI3P and PI3,5P2. Bioinformatics analysis showed that the *ROF* gene evolved independently to other immunophilins with similar domain organisation, like PAS1, justifying the identified differences in their functions. Two C-terminal alternative spliced isoforms of ROF1 and one isoform of ROF2 were identified. Using real-time PCR we showed that all isoforms rapidly accumulate under salinity stress in young plants and their transcript levels are sustained. ROF1 protein was shown to accumulate in response to high salt treatment in *A. thaliana* young leaves as well as in seedlings germinated on high salt media (0.15 - 0.2 M NaCl) in contrast to ROF2 which remained undetectable. In addition ROF1 was shown to accumulate in both dry and imbibing seeds. Immunofluorescence microscopy in root tips of young *A. thaliana* seedlings, showed ROF1 localization in the elongation zone which expanded to the meristematic zone in plants grown on high salt media. GFP-tagged ROF1 under the control of a 35S promoter was localised in cytoplasmic strands and the cell periphery of both A. *thaliana* and *N. tabacum* leaf explants and following salt treatment in positively labelled vesicular structures of variable sizes. Its vesicular localisation was, however abolished, following wortmannin treatment. ROF1 as well as its interacting homologue ROF2 positively and synergistically affected seedling emergence under osmotic/salinity stress through a PI3K related pathway as it was shown following the application of the PI3K inhibitors wortmannin and LY294002 during the germination step. Our experimental evidence suggests that PIP related trafficking events may be of particular importance in directing chaperonins to vacuolar or autophagosome structures for the quality control/ degradation of misfolded proteins during germination under osmotic/salinity stress.

Role of phosphatidylglycerol in cell division.

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Phospholipids play an important role in formation of cell-membrane structure and they are active participants in membrane related cellular processes. The phosphatidylglycerol (PG) is the only phospholipid component of the cyanobacterial cells. The lack of PG affects the normal cyanobacterial cell fission. PG deprivation results in enlarged *Synechocystis* sp. PCC 6803 and elongated *Synechococcus* sp. PCC 7942 cells. The re-addition of PG to the cell culture restores normal cell division, which indicates that the lack of PG perturbs division process. Several proteins and corresponding genes involved in cell division were identified mainly from *Escherichia coli* and various cyanobacteria. For understanding their roles in cyanobacterial cell fission we investigated *Synechococcus* sp. PCC7942 mutants, in which few of these cell-division proteins were fused with GFP (Green Fluorescent Protein). These studies were done by confocal laser scanning microscope. The *cdsA* gene encoded cytidin-diacylglycerol synthase is one of the key enzymes of PG synthesis. Inactivation of the *cdsA* gene in the fusion protein mutants could make way for understanding the effect of PG depletion on cell division.

Phosphatidylserine is important for vesicle transport during root development in *Arabidopsis thaliana*

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Phosphatidylserine (PS) has many important biological roles, but little is known about its importance in plants. We recently showed that PS is enriched in Arabidopsis flowers and that *PHOSPHATIDYLSERINE SYNTASE1* (*PSS1*) that is essential for PS biosynthesis in this plant is required for pollen maturation (Yamaoka et al., 2011). Because PS is also enriched in Arabidopsis roots, we herein investigated the role of PSS1 in Arabidopsis roots.We found that the primary roots of *pss1-2* seedlings are shorter than that of wild-type seedlings due to reduced cell length and thus develop a higher density of root hairs. Indirect immunofluorescence microscopy for tubulins in root epidermal cells revealed a random network of microtubules in *pss1-2* roots in comparison with parallel arrays of microtubules in wild-type roots, suggesting that cell elongation is inhibited in mutant roots due to microtubule disarrangement. *pss1-2* roots contained no detectable level of PS and a reduced amount of very-long-chain fatty acids (VLCFAs) in phosphatidylethanolamine. In parallel, we found that the proportion of telophase cells in the root meristematic zone is higher in *pss1-2* roots than in wild-type roots, suggesting that *pss1-2* causes a delay in phragmoplast formation in root cells. Because VLCFAs are required for vesicular transportation and phragmoplast formation requires vesicular transportation (Bach et al., 2011, Journal of Cell Science, 124, 3223–3234), we conducted time-laps observation of endocytic vesicle formation in root cells stained with FM4-64. The results showed that endocytic vesicles were aggregated in *pss1-2* root cells within 60 min of FM4-64 staining. In transgenic Arabidopsis expressing the PS biosensor GFP-LactC2, the GFP-LactC2 fluorescence or PS was co-localized in the cytosolic sides of ER, plasma membranes, endocytic vesicles and phragmoplasts. Taken together, our data suggest that PS is required for microtubule dynamism, cell-plate formation and vesicle translocation in Arabidopsis roots.

Subcellular mechanism of plant phospholipase A genes and their functional characteristics in plant growth and development

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The phospholipase A (*PLA*) gene family is catalyzing the hydrolysis of phospholipids at the *sn*1 and/or *sn*2 position of glycerophospholipids to liberate a free fatty acid. Plant PLA2s are classified into two groups, low molecular weight PLA2s (PLA2a, ß, ., and d) and patatin-like PLAs (pPLAs) that have both PLA1 (catalyzes hydrolysis at the *sn*1 position) and PLA2 (catalyzes hydrolysis at the *sn*2 position) activities. Patatin-like *PLA* gene family constitutes 10 genes in Arabidopsis and encodes proteins related to the potato tuber storage protein patatin. Low molecular weight PLA2s are relatively better reported to be involved in cell elongation, auxin response, gravitropism, guard cell movement, defense, and trafficking mechanism of PIN proteins. However the functional characteristics of pPLAs are scarcely studied by elucidating their subcellular localization. Here we want to discuss other unknown functions of low molecular weight PLA2s and recently uncovered features of patatin-like *PLA* gene.

A novel membrane protein in the chloroplast inner envelope involved in fatty acid export

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Plastids harbor many vital biosynthesis functions during growth and development. Thus, plastid localized metabolite synthesis (e.g. amino acids, fatty acids or secondary compounds) requires extensive solute exchange across the outer and inner envelope membranes. Because fatty acid synthesis in plants exclusively takes place in plastids, export for further lipid metabolism is required. However, until now few data indicate the mechanism of fatty acid export from plastids.By means of proteomics analysis of plastid envelope proteins, we selected FAX1(fatty acid export 1), a novel membrane-spanning protein in *Arabidopsis thaliana*. According to GFP-targeting in protoplasts and immunoblot analysis, FAX1 localizes to the plastid inner envelope membrane. T-DNA insertion mutants show reduced biomass, thin inflorescence stems and strongly impaired male fertility. Transcriptomic, metabolic and ultrastructural analysis imply that FAX1 is a key protein for synthesis of secondary metabolites, like cutin and wax , secondary cell walls and pollen exine which all need fatty acid export from plastids. Based on these observations, we propose a function of FAX1 in fatty acid export from plastids.

The role of non-specific phospholipase C in plant response to pathogen attack

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Various adaptive responses to pathogen attack have been described and a large superfamily of phospholipases A, D and phosphatidylinositol-specific phospholipase C (PLA, PLD, PI-PLC) identified as key players in stress signalling. Non-specific phospholipases C (NPCs) were discovered as a novel type of plant phospholipid-cleaving enzymes homologous to bacterial phosphatidylcholine-specific phospholipases C. Six-gene (*NPC1-NPC6*) family was established in Arabidopsis and since then evidence is growing indicating involvement of NPCs to stress responses.In our study we show rapid change of NPC activity in tobacco and Arabidopsis cell suspension cultures treated by salicylic and jasmonic acids, substances involved in plant-pathogen interactions, by heat-killed cells of *Pseudomonas syringae* bacteria, lipopolysaccharides or flagellin-derived peptide flg22. Similar results were obtained using three-month-old tobacco plants. Inoculation with *P. syringae* or flg22 induced changes in *NPC* expression. Level of *NPC1, NPC2* and *NPC6* transcript was significantly reduced and level of *NPC4* and *NPC5* raised at 24 h after treatment.We also report that mutant plants with knocked-out NPCs genes have been shown to exhibit significant decrease in resistance against bacterial attack. The potential role of NPC family in plant defence reactions is discussed. This work was supported by The Ministry of Education, Youth and Sports project no. ME09108.

Cardiolipin plays crucial roles in plant mitochondria and development.

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Cardiolipin (CL) is widely distributed in various prokaryotes and eukaryotes as a membrane phospholipid. In eukaryotes, it is localized in the inner membrane and at the contact sites between the inner and outer membranes of mitochondria. CL is suggested to be involved in many mitochondrial functions. We previously identified the *CLS* gene for CL synthase in *Arabidopsis thaliana* and showed it to be a useful tool for understanding the physiological roles of CL in higher plants. In this study, we isolated homozygous *cls* mutants (*cls-1* and *cls-2*) of *A. thaliana*, in which the *CLS* gene was disrupted by T-DNA insertion, by observing the development of embryos obtained after self-fertilization of heterozygous *cls* mutants. Both embryogenesis and growth of the homozygous cls mutants were substantially retarded relative to wild type, and additional phenotypes were observed, namely slow root growth and a low yield of seed production. The delayed embryogenesis and growth of homozygous *cls* mutants were recovered by introduction of CLS into the mutants. These findings demonstrate that CL plays critical roles in plant development and mitochondria.

Carnitine - a fatty acid carrier in plant Implication of carnitine during normal and affected development, in connection with the lipid metabolism

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Carnitine is widely distributed and plays a major role in lipid metabolism in animals and yeast. It is implicated in the trafficking of activated fatty acids towards the mitochondrial matrix, where they are catabolized by ß-oxidation to form acetate. Additional functions have been ascribed to the carnitine such as coenzyme-A homeostasis, energy storage in the form of acetylcarnitine, secretion of poorly metabolized acyl residues and regulation of hormonal action. However, in plant, there are limited information available in the literature, but evidence from enzyme studies carried out by several research groups, and the identification and quantification of a wide range of acylcarnitines, prove that carnitine is associated with lipid metabolism. One of the aims of our study is to elucidate the role of carnitine in the lipid metabolism and trafficking in higher plants. We investigate the role of carnitine during events which modulate or perturbate the lipid metabolism during plant development. Using tandem Mass Spectrometry coupled to Ultra Performance Liquid Chromatography, we have shown an increase of the free and total esterified carnitine contents in *Arabidopsis thaliana* seedlings specifically aged 2 days post-imbibition, and a decrease across the development. This stage coincides with the shift in heterotrophic to a photoautotrophic metabolism. The identified acylcarnitines correspond, for a great part, to the long-chain fatty acids composing membrane lipids, such as palmitoylcarnitine, stearoylcarnitine and linoleylcarnitine, suggesting that carnitine could participate notably in the membrane synthesis during the differentiation of the plasts, maybe for fatty acid trafficking. Myristoylcarnitine is also quantified at high content in some extracts suggesting an implication of acylcarnitines in post-translational protein modification. Interestingly, the acylcarnitines analyzed represent less than 10% of the total esterified carnitine. These results put forward that carnitine is associated with other organic acids and could contribute to the trafficking and/or the recycling of unexpected molecules. We are currently working at the identification of the carnitine esters in this yet uncharacterized pool of carnitine esters.

Carnitine - a fatty acid carrier in plant Study of the carnitine biosynthesis pathway in *Arabidopis thaliana*

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Carnitine, a non-proteinaceous amino acid that belongs to the betains, is present in almost all living organisms where it plays essential roles in energy metabolism and adaptation to inadequate cellular environment. Its main function in animals is to facilitate intracellular transport of activated fatty acids in the form of acylcarnitines, notably during central carbon metabolism. The discovery by different research groups of carnitine acyltransferase activities, and the identification and quantification of acylcarnitines alongside free carnitine by our group, in several plant genera, have confirmed that carnitine takes part in plant lipid metabolism as in animals and yeast.The elucidation of the carnitine biosynthetic pathway in plants is now of prime importance in order to understand its physiological roles through studies of its regulation and approaches of reverse genetics.Our first analyses on *Arabidopsis thaliana* have confirmed the presence of TML (trimethyllysine) and Y-BB (Y-butyrobetaine), two of the known precursors of carnitine in mammals and yeast. Moreover, we confirmed deuterium incorporation in carnitine extracted from plants fed with deuterated-TML, demonstrating a metabolic link between these two compounds and showing thereby a similarity between the biosynthesis of carnitine in mammals, yeast and plants. Our bioinformatic analyses comparing Arabidopsis, animal and yeast protein sequences have highlighted several plant enzymes that could be involved in carnitine biosynthesis, based on their sequence homology with enzymes from the carnitine biosynthetic pathway in yeast and animals. Two of these mutants (named *KO8-2* and *Shmt1-1*) show a lower content of carnitine and Y-BB as compared to the wild-type genotype. The phenotypic study of these mutants is under way in order to get insight into the role that carnitine plays in plant lipid metabolism, during normal development or in inadequate conditions.

Genetic engineering of *Scenedesmus almeriensis* for biofuel production.

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Worldwide, the increasing cost of fossil fuels and the global warming have become two important issues. The biological CO2 fixation and energy production from photosynthetic organisms is an alternative that is expected to mitigate those economics and environmental problems. With this purpose, oilseed crops have been widely used to provide biofuels, however they can provide only a small fraction of the demanded fuels. Instead, the use of microalgae biomass as a renewable energy source is a promising alternative which present several advantages over higher plants: microalgae have higher oil productivity per area, fast growth rates and they are able of growth in a wide variety of waters sources (fresh, brackish, saline, and wastewater). Despite the potential use of microalgae to produce biofuels, there are many technological and biological advances that need to be achieved before microalgae biomass can be used at a commercial scale. Particularly, genetic engineering (GE) of microalgae is a valuable biotechnological tool that might be used to improve strains increasing the lipid productivity.In the last years microalgal GE has experienced a great advance but mostly dedicated to model microalgae (e.g. *Chlamydomonas reinhardtii*) very useful in the lab but with a poor performance in real culturing conditions. The challenge is to develop GE for industrially promising microalgae.We are working to develop GE tools for transformation of *Scenedesmus almeriensis* which fulfills the requirements to be an industrial microorganism: high growth speed, wide culturing conditions (pH, temperature, etc.) and demonstrated ability to grow in a 4,000 L photobioreactor along a whole year under greenhouse conditions in Almeria (Spain). We carried out the genetic transformation of *S. almeriensis* using *A. tumefaciens* LBA4404. To this aim we have used the pCAMBIA 1305.1 vector which harbors the hpt gene, as selection marker conferring resistance to hygromicin, and the GUSplus gene as a reporter. An optimization experiment was designed using Statgraphics Centurion software working simultaneously with six parameters: pH, temperature, light and period of co-culturing, optical density of the *A. tumefaciens* inoculum, and two post-harvest treatments. We have detected important differences among the different parameter combinations. A similar trend was observed for most parameters, although they were statistically significant only for the pH and temperature. The presence/absence of light during co-culturing showed no effect.Overall, near 70% of the initial putative transformant colonies selected on selective plates containing hygromicin were confirmed to be true transformants by PCR using specific primers for the GUSplus gene. As a conclusion, we have established an easy and efficient method to genetically modify *S. almeriensis*.Currently, we are working to create a vector containing the cDNA of the DGAT1 gene from *Echium pitardii* (*EpDGAT1*) under the control of the Chlamydomonas *Hsp70A:rbcS2* chimeric promoter. Additionally, the first intron of *rbcS2* will be included upstream of *EpDGAT1. EpDGAT1* have been cloned and characterized previously in our lab (Mañas-Fernandez et al., 2009), and heterologous expression in a yeast mutant (H1246) defective in the synthesis of triacylglycerols and sterol esters resulted in increased levels of TAGs. Thus, heterelogous expression of *EpDGAT1* in *S. almeriesis* using an adequate promoter will allow us to evaluate the impact of this activity on microalgal TAGs content.

Effects of inoculum size, light intensity and nitrogen availability on growth and LC-PUFA production by the green microalga *Parietochloris incise*

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The long-chain polyunsaturated fatty acids (LC-PUFA), arachidonic acid (AA, 20:4 ω-6) and dihomo gamma-linolenic acid (DGLA, 20:3 ω-6) have emerging roles in human health and nutrition. In our laboratory we study LC-PUFA production and their accumulation in triacylglycerols (TAG) by the two strains of the oleaginous green microalga *Parietochloris incisa*: the wild type strain and its Δ5 desaturase mutant, the richest green sources of AA and DGLA, respectively. In the present work, we examined growth and LC-PUFA production by both strains of *P. incisa* under combined alterations of initial chlorophylls (Chl) culture content and light intensity on both complete and nitrogen (N)-depleted media.On complete medium, the cultures of both strains could be initiated with very low initial Chl concentration (1 - 2.5 mg/L) showing the highest specific growth rate with increasing light intensity. However, maximum biomass productivity was achieved in the cultures started with Chl 15 mg/L under the highest light intensity level in both strains.Under N starvation, AA and DGLA accounted for more than 50% and 30% of the total fatty acids in WT and the mutant, respectively. Furthermore, increasing light intensity substantially affected the FA composition and ratio of the major LC-PUFA to monounsaturated FA as well as total lipid accumulation. The highest biomass and LC-PUFA productivity were obtained by the dense cultures under highest light intensity level studied. Metabolomic analysis has been initiated on the WT strain to indentify the regulatory processes associated with the production of LC-PUFA- enriched TAG induced by stress conditions.

The role of the plastid enzymes of central carbon metabolism in *de novo* fatty acid and TAG biosynthesis in the green microalga *Parietochloris incisa* under nitrogen starvation.

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In oleaginous microalgae nitrogen (N) deprivation intensifies fatty acids synthesis (FAS) and their accumulation in the form of triacylglycerols (TAG). Modulation of expression or activity of the central carbon metabolism enzymes may give rise to elevated carbon flux towards fatty acid (FA) production in plastids in order to meet the increasing demand for TAG formation.
The present research is focused on the study of the plastid isoforms of pyruvate kinase (PK), pyruvate dehydrogenase complex (PDC) and acetyl-CoA-carboxylase (ACCase), involved in the induction of *de novo* FAS under N-starvation conditions in the green oleaginous microalga *Parietochloris incisa*. Grown at N-deprivation, *P. incisa* is able to store in cytosolic oil globules high content of neutral lipids in the form of TAG, rich in the very-long-chain polyunsaturated fatty acid, arachidonic acid (AA). Our findings can clarify the molecular mechanisms underlying induction and modulation of oil content in this unique microalga.
Genes putatively encoding subunits of plastid isoforms of PK (*PiPKp*), E1α subunit of PDC (*PiE1αPDC*) and biotin carboxylase subunit (*PiBC*) of ACCase were cloned using degenerate PCR, followed by RACE. Plastid localization of the proteins encoded by the cloned genes was confirmed by targeting of GFP fused to N-terminal part of the PiPKp and *PiE1αPDC* in Arabidopsis protoplasts. The interrelationship between total fatty acid (TFA) accumulation and the expression patterns of the genes, encoding the central carbon metabolism enzymes and AA biosynthesis, was elucidated by gas chromatography and qPCR.
Due to current unavailability of a transformation system in *P. incisa*, the easily transformable green microalga *Chlamydomonas reinhardtii* was used as a model organism. Silencing of *C. reinhardtii* homologous genes, followed by complementation/overexpression with *P. incisa* genes, was chosen as an approach to study the significance of the cloned genes for TFA production, under N-replete and -deplete conditions, during photoautotrophic growth. The analysis of the resultant mutants is underway.

Characterization of the first non-animal prostaglandin H synthase from the red alga *Gracilaria vermiculophylla*

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Oxygenated derivatives of fatty acids (oxylipins) are signaling molecules in animals and plants. In animal systems, eicosanoids, formed by the action of lipoxygenases or prostaglandin H synthases (PGHS) regulate cell differentiation, immune responses and homeostasis. In plants, derivatives of C16 and C18 fatty acids are used as developmental or defense hormones. Different from green plants, marine red algae are rich sources of C20 polyunsaturated fatty acids, which are precursors in the biosynthesis of prostaglandins and other eicosanoids. Occurrence of prostaglandins has been reported in the different red algae Gracilaria species. In this work, we report on unique features of the PGHS from *Gracilaria vermiculophylla* (GvPGHS). A comparison of deduced primary structures of GvPGHS with corresponding sequences of different animals, from corals to mammals, reveals 21-27% sequence identity, considerably lower than the average sequence identity between PGHS from different animal phyla, 60-70%. The algal PGHS lacks structural elements identified in all known animal PGHSs, such as epidermal growth factor-like domain, and several amino acid residues shown to be important for substrate binding and coordination (Arg-120, Tyr-355, and Ser-530). However, the key residues of animal PGHS, such as catalytic Tyr-385 and heme liganding His-388, are conserved in the algal enzyme and GvPGHS catalyzes the conversion of arachidonic acid into prostaglandin-endoperoxides, PGG2 and PGH2. Differently from animal PGHSs, GvPGHS easily expresses in *Escherichia coli* as a fully functional enzyme. The recombinant protein was identified as an oligomeric (evidently tetrameric) ferric heme protein. Our data suggest that the red algal PGHS shares a common ancestry with animal PGHS enzymes and evolved differently after the divergence of algal and animal lineages.

Eicosapentaenoic acid production by oleaginous filamentous fungus *Mortierella alpina* breeding

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A filamentous fungus, *Mortierella alpina* 1S-4, belonging to the Zygomycetes, has been isolated from soil as a potent producer of polyunsaturated fatty acids (PUFAs) in our laboratory and used for commercial production of arachidonic acid (AA, 20:4n-6). A host-system for *M. alpina* 1S-4 was developed by molecular breeding for improving and modifying the PUFAs productivity and composition. In this study, we demonstrate the transformation system and application in this fungus. In particular, we evaluated the eicosapentaenoic acid (EPA, 20:5n-3) production, which was an end product of .3 fatty acids synthesized in *M. alpina* 1S-4, by overexpression of desaturase genes.

Altering fatty acid composition in *Crambe abyssinica* by multiple-gene RNAi

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Erucic acid (22:1) is a major feedstock for the oleochemical industry. Development of new oil crops with high 22:1 oils is thus of great commercial interest. *Crambe abyssinica* is a novel oilseed crop that contains 50-60% of 22:1, however, further increase in 22:1 levels will add value to its industrial applications. Through manipulating three genes involved in the fatty acid biosynthesis, we have previously developed transgenic crambe lines with high erucic acid level (over 75% in single seeds). The aim of the current study is, by modifying another three key genes involved in the fatty acid biosynthesis, to further increase the 18:1-CoA, the precursor of 22:1 biosynthesis. Up to now, the three candidate genes have been cloned from crambe and several RNAi constructs with seed-specific napin promoter were prepared. Transgenic lines with these RNAi constructs were recovered through Agrobacterium-mediated transformation and are under evaluation. The preliminary results from the biochemical analysis showed that RNAi constructs targeting the *LPCAT* and *PDAT* genes could significantly change the fatty acid composition in the triacylglcyerols and phosphatidylcholine in the transgenic seeds. Demonstration of relative activities of enzymes in the Kennedy pathway in microsomes prepared from developing seeds of non-transgenic and a transgenic very high erucic acid crambe line indicates the importance of the individual enzyme steps in regulating the oil quality in crambe.

Tapping the Potential of Camelina as a Platform for Metabolic Engineering of Novel Fatty Acid and Oil Compositions

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Camelina sativa (false flax) is an emerging *Brassicaceae* oilseed crop in the Great Plains and Pacific Northwest of the United States. The growing interest in camelina is due largely to its potential for biodiesel production in geographic areas that are not well-suited for soybean cultivation. We are also exploring the use of camelina as a platform for the production of lubricants, biofuels, and high-value industrial oils because it is not widely grown in the US for food use and most importantly, genetic transformation of camelina can be achieved by a simple floral vacuum infiltration of agrobacterium. With this method, metabolic engineering of camelina can be conducted in a rapid and non-labor intensive manner. One major limitation of camelina oil for bio-based lubricants and fuels is its low oxidative stability arising from its high content of linolenic acid. To improve the oxidative stability of camelina oil, we have devised a number of metabolic engineering strategies to generate oils enriched in oleic acid, monounsaturated omega-7 fatty acids, and saturated fatty acids. We have also used camelina as a platform for producing novel fatty acids, including conjugated, hydroxy, and short -/medium-chain fatty acids, for industrial and biofuel applications. In addition, as a component of the ICON project, we have used camelina not only as a production platform for jojoba-type wax esters, but also as a crop model system for testing numerous gene combinations for wax ester synthesis. Through this approach, metabolic bottlenecks that limit wax ester production have been identified.

Development of regeneration and transformation protocol for *Lepidium campestre*

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Nutrient leaching is one of the main problems caused by modern agriculture due to the excess use of nitrogen-based fertilizers and tillage. Planting of biennial or perennial crops could alleviate this problem. However, suitable crop species for such a purpose are very limited. *Lepidium campestre* is a wild biennial oilseed crop. The field trial has shown a high yield potential (5 ton/ha). It is cold-hardy and has good features like upright stature, synchronous flowering, and resistance to logging. It could be a potential catch and oilseed crop. However, the species has some serious problems that need to be solved before it can be of economically viable. One serious problem is that it is prone to pod shatter with seed loss up to 50%. It has also low seed oil content, only around 20% compared to 45% in winter rapeseed. Moreover, its seed oil contains high amounts of erucic acid and linolenic acid that are not suitable for food consumption. Genetic engineering offers a more precise and efficient method for genetically improving oil qualities and quantities and this has been proved to be successful in several oil crop species. For a given species, the availability of efficient regeneration and transformation protocols is prerequisite in successful genetic modification of important traits. However, there is so far no transformation method available for *L. campestre*. In this study, we have established a very efficient regeneration and transformation protocol for this species with close to 100% of regeneration frequency and an average 2-5% of transformation efficiency. The transgenic lines were confirmed by PCR and Southern blot analyses. The development of this transformation protocol opens good opportunities for genetic improvement of the abovementioned traits in lepidium.

Novel Acyltransferases from long chain PUFA producing organisms

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Engineering plants with high levels of omega-3 long chain polyunsaturated fatty acids (LC-PUFAs), such as EPA (eicosapentaenoic acid) and DHA (docosahexanoic acid) would increase the production as well as the sustainability of these important fatty acids. However, since higher plants do not naturally produce LC-PUFAs, the introduction of enzymes enabling efficient PUFA accumulation is required. In the PUFA pathway and in the final accumulation of PUFA rich oil, acyltransferases play an important role in shifting acyl groups between the different acyl pools. Therefore, acyltranferases from LC-PUFA producing organisms are interesting candidate enzymes for engineering plants with high levels of EPA and DHA. In this study, novel acyltransferases from high LC-PUFA producing organisms were cloned into yeast. The enzymes were characterized regarding substrate specificity and selectivity, using all possible intermediate acyl-CoAs in the LC-PUFA pathway. Also the positional specificities of the enzymes were investigated. The enzymes were compared with the corresponding enzymes from Arabidopsis thaliana and *Brassica napus*. Interesting variations in enzyme activity were found with the different substrates and a clear preference for some of the acyl-CoAs could be seen. Moreover, remarkable differences in acyl-CoA specificity and positional specificity between enzymes from LC-PUFA producing organisms and enzymes from higher plants were found.

Phenotypic changes in *A. thaliana* insertion mutated in genes encoding lysophosphatidylethanolamine: acyl-CoA acyltransferases (*LPEATs*) and some biochemical properties of the enzymes.

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Lysophospholipid: acyl-CoA acyltransferases (LPLATs) are a large family of enzymes that participate in transfer of acyl group from acyl-CoA to lysophospholipids synthesising the appropriate phospholipids. Moreover LPLATs are probably involved in the transfer of polyunsaturated fatty acids and uncommon fatty acids from phospholipids to storage lipids (triacylglycerols - TAG). In *A. thaliana* genome 2 genes, *At1g80950* and *At2g45670*, coding for proteins with acyltransferase activity with specificity to lysophosphatidylethanolamine, were characterized (Stålberg et al., 2009). These studies showed that *LPEAT1* and *LPEAT2* have somewhat different biochemical properties and expression profiles. The aim of the present study was to further characterise the substrate specificity and selectivity of LPEATs in microsomal fraction from yeast knockout line *ale1* expressing the LPEATs and to study the phenotypes of *A. thaliana* plants disrupted in the *LPEAT* genes. The biochemical assays showed that both enzymes prefer 16:0-CoA as acyl donor when 18:1-LPE was used as acyl acceptor whereas the acylation rates of 16:0-LPE was similar with 18:1-CoA and 16:0-CoA.When mixing the two acyl donor (16:0-CoA and 18:1-CoA) in the same reaction; 16:0 was acylated at a higher rate than 18:1-CoA by LPEAT1 whereas LPEAT2 utilized 16:0-CoA and 18:1-CoA at about the same rate.To investigate the phenotypic effect of knockout of LPEAT gene, *A. thaliana* (Col-0) T-DNA insertion mutants were obtained. Plants homozygous for mutation in the *LPEAT1* and *LPEAT2* genes and the absence of their intact m-RNA were confirmed by PCR. Phenotypic analysis of these mutants showed that they have an abnormal growth phenotype., The obtained results will be discussed on poster.

References:1. K. Stålberg, U. Ståhl, S. Stymne, and J. Ohlrogge, (2009) Characterization of two *Arabidopsis thaliana* acyltransferases with preference for lysophosphatidylethanolamine. BMC Plant Biology, doi: 10.1186/1471-2229-9-60

Characterisation of the *FAD2* gene family from *Hiptage benghalensis*, a ricinoleic acid accumulating plant

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We have cloned and characterised the *FAD2* gene family from *Hiptage benghalensis*, a tropical plant that accumulates high levels of ricinoleic acid in its seeds. Functional characterization of the five full-length clones out of six *FAD2* family members in yeast showed that two of them were capable of functioning as delta-12 hydroxylases while the other members were confirmed to be delta-12 desaturases. The two delta-12 hydroxylases acted on both C16:1 and C18:1 fatty acids substrates and as for the other delta-12 hydroxylases previously cloned from plants *Ricinus communis* (castor) and *Lesquerella fendleri,* and from the fungus *Claviceps purpurea*, also showed some lower level delta-12 desaturase activity. The hydroxylation activity of the two Hiptage hydroxylases was further confirmed by their expression in the Arabidopsis *fad2/fae1* double mutant where they were able to produce equivalent or higher levels of hydroxy fatty acids in the seed oil when compared with the other known delta-12 hydroxylases.

Engineering ricinoleic acid in low-linolenic linseed

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Oil crops have the potential to provide renewable, cost-competitive and environmentally friendly sources of industrial oils as alternatives to those currently derived mainly from non-renewable and increasingly costly petroleum sources. Significant progress has already been made in cloning genes for key enzymes responsible for the introduction of a range of new functionalities (such as acetylenic and conjugated bond systems, hydroxy and epoxy groups) into conventional fatty acids. Linola is the flax mutant with mutations in both *FAD3* genes, resulting in high linoleic acid levels, and is a potential crop platform for producing novel industrial fatty acids. We show here that the transgenic expression of Δ12-hydroxylase from *Ricinus communis* in Linola resulted in the accumulation of ricinoleic acid (RA) in seeds, even though the introduced hydroxylase had to compete with the strong endogenous Δ12-desaturase for oleic acid substrate. LC-MS analysis of distribution of ricinoleic acid on triacylglycerol moieties provides insights into possible approaches for increasing the RA levels in this oil crop.

Wax ester production in Camelina seeds

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Wax esters are natural products composed of primary alcohols and fatty acids, with both moieties being usually long-chain (C16 and C18) or very-long-chain (C20 and longer) carbon structures. In plants, wax esters are most abundant in the cuticle, and in the seed oil of jojoba (*Simmondsia chinensis*). Wax esters have important lubrication properties, however the high cost of production has restricted the use of these compounds to high value products, such as cosmetics, pharmaceuticals, and specialty lubricants. The characterization of the jojoba wax synthase (Lardizabal *et al.* 2000) has allowed the identification of twelve wax synthase candidate genes in Arabidopsis. Our goals are to investigate whether these genes encode functional WS enzymes, and determine their substrate specificities, so that they can then be used for engineering of oilseed crops that synthesize high levels of wax esters in their seed oil. Furthermore, knowledge of the substrate specificity of these WS may allow the production of wax esters with novel chemical compositions specifically targeted to various industrial applications. To achieve these objectives, we have carried out WS enzyme assays after expressing the jojoba-type WS candidates in the yeast strain H1246 (Sandager *et al.* 2002). In addition, we have engineered *Camelina sativa,* an emerging oilseed crop, with the constructs containing the Arabidopsis cDNAs encoding the WS proteins that resulted in the highest wax ester yield in yeast, together with cDNAs encoding the jojoba FAR and KCS. The evaluation of the content and composition of the generated wax esters in transgenic Camelina seeds is currently in progress.

S11 P9

In vitro* substrate specificity of wax synthase from *Marinobacter hydrocarbonoclasticus

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Wax synthases (fatty acyl-CoA:fatty alcohol acyltransferases; WS) are membrane-associated enzymes which catalyze esterification reaction of a fatty acyl-CoA with a long chain fatty alcohol. Wax esters are used in industry as high pressure lubricants, components of pharmaceuticals, cosmetics and inks. As obtaining high amounts of wax esters from existing sources is expensive, possibilities of producing wax esters using plants are currently under investigation. The modification of oilseed crops using genes of enzymes with proper specificities might enable to obtain desired kinds of wax esters.Wax synthase from *Marinobacter hydrocarbonoclasticus* was expressed heterologously in *Saccharomyces cerevisiae*. The *in vitro* substrate specificity was determined in microsomal fractions isolated from yeast expressing wax synthase gene using radiolabelled fatty acyl-CoAs and fatty alcohols.*In vitro* assays using eight [14C] acyl-CoAs in combination with 15 fatty alcohols with different carbon chain lengths as a substrates enabled to determine which of 120 different wax esters are produced by tested enzyme with the highest efficiency. This work was supported by the European Commission through the FP7 ICON project.

AUTHORS INDEX

Analysis of the contribution of the patatin-like protein pPLAIIa to progressive drought stress in Arabidopsis using silenced and overexpressing plants

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Patatin is a vacuolar storage protein present in potato tubers which displays acyl hydrolase activity towards polar glycerolipids. Patatin-like genes have also been cloned from other plant species and expression studies indicate that they are responsive to a variety of biotic and abiotic stresses. We have previously identified drought-responsive patatin-like genes from cowpea and Arabidopsis and investigated their expression and enzymatic activities to evaluate their possible contribution to membrane lipid degradation observed in these plants under water deficit. In the present work we studied the response to progressive drought stress of transformed plants overexpressiong (OE) or silenced (SI) for *pPLAIIa*. Results show that leaf water loss, after suppression of irrigation of soil grown plants, is inversely correlated to *pPLAIIa* expression levels. Results from chlorophyll fluorescence analysis also indicate that Photosynthesis is affected earlier in SI plants comparing to WT and OE. The analysis of the free fatty acid poll present in leaves indicates that, although under control conditions it is mainly composed of saturated molecules (16:0 and 18:0), 16:3 and 18:3 are already present in AS plants. Under water deficit an increase in the relative amount of free linolenic acid is observed for all lines tested but to a further extent in AS plants. Results indicate that altering *pPLAIIa* expression affects the composition of the free fatty acid poll present in leaves and the up-regulation of this gene, previously observed in WT plants, seems to contribute to drought tolerance.

Biosynthesis of phosphatidylglycerol is essential for the development of thylakoid membranes

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Phosphatidylglycerol (PG) is the only major phospholipid in thylakoid membranes of chloroplasts. PG is found in Photosystem I and Photosystem II complexes and is shown to play important roles in photosynthesis. To investigate the function of PG in higher plants, we isolated the *pgp1 pgp2* double mutant of Arabidopsis having mutations in the genes for phosphatidylglycerophosphate synthase, which is the committed enzyme for the biosynthetic pathway of PG. In the isolated *pgp1 pgp2* double mutant, development of embryo was delayed and seeds of the mutant did not germinate. The thylakoid membranes of chloroplasts in embryos of the double mutant did not develop. Biosynthesis of PG was detected only trace in the double mutant. These data indicate that the biosynthesis of PG plays important roles in development of thylakoid membranes and embryogenesis.

Argania spinosa a source of triterpene bioactive compounds

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This work focuses on study different terpenoids, with important biological activity, present in argan tree. The terpenoids of fruits and leaves from *A. spinosa* were studied. In the mesocarp of argan fruits, the triterpenoids detected were ursolic, oleanolic, betulinic and maslinic acid, whereas that in the leaves only ursolic and oleanolic acid were present. Samples collected from two geographical zones, at two different dates were analysed. Terpenic acids content in the leaf were four times higher than in the fruit. Ursolic acid is the major triterpene in the leaf and fruit of argan (0.10–0.44% DW). Our results suggest that the developmental stage of the plant organ is a key factor that modulates the profile and concentrations of the pentacyclic triterpenic acids. At the same ripe stage, no significant differences were found among fruits grown in the two studied production areas. In addition, the argan oil obtained from argan fruit seeds by physical processing presents a very interesting composition of the insaponificable matter, as well as the virgin olive oil. The argan oil composition is of great relevance not only to the economy, but also to the environment, in the argan forest, declared as Biosphere Reserve by UNESCO. Virgin argan oil containing important amounts of sterols (229.3 mg/100g) can be a very interesting natural source of these compounds. The unsaponifiable matter of alimentary and cosmetic argan oil obtained from two geographical zones, by two extraction methods were studied, and the terpene compounds were determined. The two mayor sterols were schotenol and spinasterol with relative percentages up to 48.5 and 41.7 % of total sterols, respectively. This study, clearly demonstrates the importance that argan tree presents as an interesting source of triterpene bioactive compounds, besides of the beneficial effect on human health that argan oil brings; a specific fatty acid balance and high tocopherols level.

Characterization of a new oleaginous microalgal strain isolated form the marshlands of the Odiel river in the southwest of Spain and optimization of its genetic manipulation

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Selection of new autochthon strains is necessary, and for the moment the best strategy, to find robust microalgae well adapted to the local climatological conditions able to produce compounds of biotechnological interest. We describe the isolation and characterization of a new microalgal strain isolated from the marshlands of the Odiel River in the Southwest of Spain. The new microalga belongs to the genus Picochlorum, as deduced from the analysis of its 18S rRNA encoding gene, is able to grow at a high growth rate and thrive with adverse conditions. It has an appreciable constitutive level of lutein (3.5 mg g-1 DW) and zeaxanthin (0.4 mg g-1 DW) which is increased to 1.8 mg g-1 DW at high light intensities. This strain is also characterized by a very low level of linolenic acid (3.8 % of total fatty acids) and no polyunsaturated fatty acids with 4 or more double bonds, which are quite abundant in other green microalgae. The fatty acid composition of Picochlorum´s lipids is similar to that of oleaginous plants typically used for the production of biodiesel. After studying the lipid content in Picochlorum cells cultured at different nutritional and operational conditions, we concluded that the cell age is the main factor to induce the synthesis of lipids, which content was doubled when the culture reached the stationary phase of growth. Using paromomycine as resistance marker, we have established an electroporation based system for the nuclear transformation of Picochlorum and we have evaluated the efficiency of several heterologous promoters, the best results were obtained for the 35S CaMV promoter. The easy genetic manipulation of Picochlorum sp HM1 and its fatty acid profile makes of it a promising candidate for biodiesel production. The high content in the carotenoids lutein and zeaxanthin in this microalgae indicates that it could also be a good source for natural eye vitamin supplements, which could be obtained as a byproduct during biodiesel production.