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Symposium

Plant Lipid Metabolism: From Basic Research to Biotechnology

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Biosynthesis of Phosphatidylglycerol in Plants

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Phosphatidylglycerol (PG) is an anionic membrane lipid found in the different membrane systems of eukaryots as well as in the plasmamembranes of various bacteria. Irrespective of the organism and the subcellular compartments, a phosphatidylglycerophosphate (PGP) synthase and a PGP phosphatase catalyse the synthesis of PG from CDP-diacylglycerol and glycerol-3-phosphate. In contrast to the PGP synthases of bacteria, yeast and mammals, little is known about the plant enzymes and the respective genes.

Data base searches revealed several genes of *Arabidopsis thaliana* the deduced amino acid sequences of which display similarities to known CDP-diacylglycerol dependent enzymes. The open reading frames of these genes were cloned, expressed in *Escherichia coli* or *Saccharomyces cerevisiae* and subcellular fractions of the transgenic cells were used for enzyme assays. In the course of these experiments two PGP synthase genes were identified. Both genes were functional expressed in yeast while only one of them was functionally expressed in *E. coli* as well. To characterise these PGP synthase isozymes and to compare their properties, the recombinant proteins were solubilized from the host membranes and purified by affinity column chromatography.

Monday, July 16, 2001, 9:30 (Session 1 - Lipid Biosynthesis)

Functional Characterization of Sphingolipid Desaturases and Hydroxylases from Higher Plants

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Sphingolipids are essential components of epidermal lipids stabilizing the skin barrier function. Though the isolation of natural ceramides is quite expensive, they are widely used as additives of cosmetics. Further interest in sphingolipids is focussing on their role in signal transduction pathways regulating cell growth, differentiation, apoptosis and pathogenic defense in mammals and yeast, whereas in plants, hardly anything is known about their function. Cerebrosides and phytoglycolipids are generated by the addition of different polar head groups to ceramides which in turn are composed a of a long-chain base (LCB) carrying an *N*-acylated fatty acid. Depending on the source, the basic LCB structure can be modified by differences in chain length, degree of unsaturation, methyl branching and insertion of additional hydroxy groups.

Recently, we identified higher plant genes coding for a stereo-unselective Δ^8-LCB desaturase[1,2] by their expression in *Saccharomyces cerevisiae* leading to plant characteristic Δ^8-*trans*/*cis*-phytosphingenines not present in wildtype cells. We here report the first functional identification of plant C4-LCB hydroxylase genes[3] and of a Δ^4-*trans*-LCB desaturase gene. Heterologous expression of these genes in a yeast *sur2*Δ-null mutant lacking C4-LCB hydroxylation resulted in the formation of D-*ribo*-C_{18}- and -C_{20}-phytosphinganine and of C_{18}- and C_{20}-sphingosine, respectively, not present in mutant cells as shown by HPLC analysis of LCBs as their dinitrophenyl

derivatives. The identity and stereochemical configuration of the isolated LCBs was confirmed by EI-MS, GLC-MS and ^{1}H-NMR. The combined expression of these LCB-modifying genes together with ceramide glucosyltransferases recently cloned in our group enables the production of defined plant-specific cerebrosides not present in yeast cells.

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Monday, July 16, 2001, 10:00 (Session 1 - Lipid Biosynthesis)

Biosynthesis of Digalactosyldiacylglycerol in Plants

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The galactolipid digalctosyldiacylglycerol (DGDG) plays an important role in stabilizing the protein complexes of photosynthesis in chloroplasts. To further our understanding of DGDG biosynthesis in plants, we isolated the cDNAs for the DGDG synthases DGD1 and DGD2 from *Arabidopsis thaliana*, soybean and *Lotus japonicus*. In agreement with its presumed role in chloroplast DGDG synthesis, the *Arabidopsis* DGD1 protein was localized to the outer chloroplast envelope. The two *Arabidopsis* cDNAs were shown to encode active DGDG synthases by co-expression with cucumber MGDG synthase in *E. coli*. In vitro enzyme assays of DGD2 expressed in *E. coli* resulted in synthesis of DGDG from MGDG and ^{14}C-UDP-galactose, but not from MGDG alone. The recombinant DGD2 protein was less active with UDP-glucose as compared to UDP-galactose. Therefore, DGD2 encodes a UDP-galactose dependent galactosyltransferase.

In addition to the high abundance of DGDG in chloroplasts, recent evidence suggested that during phosphate deprivation in the growth medium, phospholipids are substituted for DGDG in extraplastidal membranes. To understand the function of DGDG in extraplastidal membranes in more detail, we decided to analyze the lipid composition in legume nodules which are known to produce high amounts of extraplastidal membranes during symbiotic nitrogen fixation with rhizobial bacteria. The peribacteroid membrane from soybean nodules which surrounds the rhizobial bacteria in the cytosol of the plant cell was found to contain plant derived DGDG, but no MGDG, indicating the existence of DGDG in extraplastidal membranes under normal (phosphate supplied) growth conditions.

Monday, July 16, 2001, 10:30 (Session 1 - Lipid Biosynthesis)

Temperature Regulation of FAD2 Genes in Sunflower

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In plants, there is a general inverse relationship between polyunsaturation of fatty acids and growth temperature, both in membrane and storage lipids. However, the mechanism by which desaturase activities are increased at low temperatures is still unclear.

In order to study the effect of temperature on the *in vivo* oleate desaturation and microsomal oleate desaturase (FAD2) activity in developing sunflower seeds, we have used detached achenes or peeled seeds. Hull removing increased dramatically the FAD2 activity indicating a critical role of the hull as a barrier for oxygen. In addition, a long-term inactivation of the enzyme was observed at high temperatures. Optimal temperature and heat-resistance profile obtained *in vivo* using peeled seeds and *in vitro* with isolated microsomes showed a similar pattern. In peeled seeds subjected to heat or cold shocks only long-term FAD2 activity changes were observed. On the other hand, the effect of different oxygen concentrations on the FAD2 activity in peeled seeds was also studied. Anoxia brought about a rapid decrease in FAD2 activity followed by a long-term increase, recovering initial levels after oxygen reposition.

Recently, we have isolated and characterized three different sunflower *FAD2* genes, and we have functionally expressed the corresponding cDNAs in yeast. We have used these transgenic yeast cells to investigate the temperature regulation on these three isoforms separately.

The possible mechanisms involved in the temperature and oxygen regulation of this desaturase and the different participation of each FAD2 isoform will be discussed.

Monday, July 16, 2001, 14:00 (Session 2 - Lipases and Acyltransferases)

Immunopurification and Characterization of Rapeseed (*Brassica napus L.*) Lipase.

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In oilseeds, storage lipids provide the respiratory fuel for seedling growth. The enzyme responsible for their hydrolysis is lipase (triacylglycerol acylhydrolase: E.C.3.1.1.3). Compared with animal and microbial lipase, little is known about this class of hydrolytic enzymes.

An immunochemical cross-reactivity between rapeseed and PPL (porcine pancreatic lipase) was demonstrated by western-blot technique, using polyclonal antibodies raised in rabbit against PPL (Belguith et al., 2001). These antibodies was found to affect rapeseed lipase activity. Based on these results, an Immuno-affinity column, was used to purify rapeseed lipase from germinating seedling. Rapeseed lipase biochemical and molecular characteristics are discussed.

The method of purification developed in our laboratory is rapid, reproducible and yields highly purified rapeseed lipase. It may therefore be applicable in the purification of a wide variety of plant lipases, which are characterized by a low abundance in plant tissues.

Monday, July 16, 2001, 14:30 (Session 2 - Lipases and Acyltransferases)

Two Conserved Sequence Motifs are Crucial for the Activity of Plant and Bacterial LPAATs

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LPAATs (lysophosphatidic acid acyltransferases) catalyse the acylation of the *sn*-2 position of lysophosphatidic acid yielding phosphatidic acid, the central intermediate in the biosynthesis of various glycerolipids.

Conserved boxes with regard to sequence and spacing were identified by alignment of LPAAT sequences from pro- and eukaryotes. In order to identify the amino acid residues forming the active site and participating in substrate specificity, we performed site directed mutagenesis with LPAATs from *E. coli* and *B. napus*.

Our analyses show that multiple amino acids within these boxes are necessary for acyltransferase activity of both tested LPAATs.

Monday, July 16, 2001, 15:00 (Session 2 - Lipases and Acyltransferases)

Two Isoenzymes of Phospholipase D from Cabbage

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Phospholipase D (PLD; EC 3.1.4.4) occurring in plants, microorganisms and animals catalyzes the hydrolysis of glycerophospholipids at the teminal phosphodiester bond under formation of phosphatidic acid and the free alcoholic head group. In the presence of another suitable alcohol PLD is also able to transfer the phosphatidyl moiety to this alcohol in a so-called transphosphatidylation reaction. In white cabbage, two PLD isoenzymes, PLD1 and PLD2, could be identified and the genes were sequenced. They consist of 3404 and 3614 bp and contain three introns. Due to the genomic structure as well as the amino acid sequence, PLD1 and PLD2 can be assigned to the α-type of plant PLDs. Like in other plant PLDs, two HKD motifs and the C2 domain with a phosphatidylinositol 4,5-bisphosphate (PIP_2) binding motif could be identified. Starting from the cDNA of PLD1, expression studies were performed. While constructs using StrepTactin and Glutathion S-transferase tags yielded no sufficient results, soluble active enzyme was obtained without any tags. PLD2 could be expressed in the same way. Both enzymes were purified by Ca^{2+}-mediated hydrophobic interaction chromatography to high purity. N-terminal sequencing of PLD1 and PLD2 showed that the N-termini of both enzymes correspond to the Met-free complete sequences derived from the coding region of the pld1 and pld2 genes. Both recombinant enzymes showed highest hydrolytic activities at pH 5.5 to 5.6, independent of the Ca^{2+} concentration (10-100 mM). The optimum Ca^{2+} concentration was 45 mM for PLD1 and PLD2. Both enzymes show comparable activities in hydrolysis and transphosphatidylation of phospholipids.

Monday, July 16, 2001, 16:00 (Session 2 - Lipases and Acyltransferases)

Studies on 3-hydroxy-oxylipins, a group of novel eicosanoids produced by fungi

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Oxylipins and especially eicosanoids are oxygenated fatty acid derivatives produced by various enzymes, such as lipoxygenases, cyclooxygenases, cytochrome P-450s and dioxygenases. The present study will focus on 3-hydroxy-oxylipins, a group of novel eicosanoid, which are produced by and/or are active in fungi and mammalian cells and which may be used as a source for the development of novel drugs.

The systematic study of *Dipodascopsis uninucleata* and other members of the family Lipomycetaceae in our groups led to the discovery of two oxylipins α-pentanor $PGF_{2\alpha}$-γ-lactone and 3(R)-hydroxy-eicosatetraenoic acid [3(R)-HETE]. Later, we found that several other fungal strains such as *Mucor* and *Candida* can also produce 3(R)-hydroxy-oxylipins. These 3(R)-hydroxy-oxylipins were found to be microbial growth regulators. Their modulatory role in the vulvovaginal infection by *Candida* in cancer and non-cancer patients is striking. The investigation of biological effects of (3R)-HETE on mammalian cells revealed that it is almost as potent chemotactic and mitogenic as leukotriene B_4. Moreover, it plays a strong immunomodulatory role in inflammation and infection and a regulatory role in tumour cell proliferation and apoptosis.

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Monday, July 16, 2001, 16:30 (Session 3 - Lipid Metabolism in Algae and Fungi)

Lipoxygenase Based Activated Defense in Microalgae

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Diatoms are highly successful unicellular algae occurring in ocean and fresh water phytoplankton, as well as in biofilms on solid substrates. They are exceedingly abundant and among the most important primary producers sustaining the marine food chain. Despite this, little is known about their chemical defence. Two of the few reported defensive secondary metabolites are the aldehydes 2E,4Z-deca-2,4-dienal and 2E,4Z,7Z-deca-2,4,7-trienal from the diatom Thalassiosira rotula, which reduce the hatching success of grazing copepods.[1]

Biosynthetic studies show that, unlike in higher plants, algae do not produce these unsaturated aldehydes from C18-fatty acids. T. rotula, for example, relies on free eicosanoic acids as precursors for its defensive allomones. We show that a new type of hydroperoxide-lyase activity acts on intermediate 11-HPETE or 11-HPEPE to cleave these hydroperoxides into the aldehydes and 5Z,7E-deca-5,7,9-trienoic acid. In strong analogy, lipoxygenase-initiated sequences lead to volatile C8- and C11-hydrocarbons, often detected during diatom blooms of other species. These hydrocarbons are derived from the cleavage of 12- and 9-HPEPE, respectively, resulting in oxo-acid by-products with strong Michael-acceptor properties. The production of hydrocarbons is thus indicative for release of highly reactive oxygenated shorter chain fatty acids that also proved to be active in bioassays.

In all investigated cases the production of α,β,γ,δ-unsaturated dienals is activated seconds after cell disruption and leads to high local concentrations of defensive metabolites only on demand.[2] This strategy allows planktonic diatoms to maintain an efficient chemical defence withouth the costly production of constitutive defensive metabolites. Cellular resources are, moreover, invested in the production of fatty

acids, which are transformed to the aggressive defensive metabolites upon predation. This efficient strategy simultaneously reduces the risk of self-intoxication.

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Monday, July 16, 2001, 17:00 (Session 3 - Lipid Metabolism in Algae and Fungi)

Phaeodactylum Tricornutum as a Source of Genes for Implementation of Polyunsaturated Fatty Acids in Crops.

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Very long chain polyunsaturated fatty acids (PUFAs) such as arachidonic acid (ARA, $20:4^{\Delta5,8,11,14}$), eicosapentaenoic acid (EPA, $20:5^{\Delta5,8,11,14,17}$) or docosahexaenoic acid (DHA, $22:6^{\Delta4,7,10,13,16,19}$) have been shown to display many beneficial effects in human health. Today, the main commercial source of these fatty acids is fish oil which results in overfishing of the marine resources. Since biotechnology may allow the production of specific fatty acids by genetic engeneering of domestic crops, the possibility of producing PUFAs in rapeseed or linseed has lead to the search for the genes encoding the enzyme activities involved in PUFA biosynthesis, i.e. desaturases and elongases.

Phaeodactylum tricornutum is a unicellular silica-less diatom in which the EPA content reaches 30% (1). This marine diatom has been used as a model to study PUFA biosynthesis, and labeling experiments have shown that EPA was synthesized by desaturations and elongation of fatty acids issued from the de novo fatty acid synthase (2). We decided to use this organism for cloning the different genes encoding the enzymes involved in EPA synthesis. Using a combination of mass sequencing, PCR and library screening, we identified the coding sequences of several desaturases. The full length clones were expressed in Saccharomyces cereviseae for functional characterization. This system enabled us to identify four desaturases with different regioselectivities: a Δ5, a Δ6 and two Δ12 desaturases.

Although we could not clone any gene encoding an elongase from Phaeodactylum tricornutum yet, we investigated the possility to produce PUFAs in yeast using the available desaturases and the Δ6-specific elongase from Physcomitrella patens (3).

Results concerning the synthesis of ARA in yeast will be presented and the problems encountered when expressing simultaneously several activitites involved in a single biosynthetic pathway that can compete for substrates will be discussed.

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Monday, July 16, 2001, 17:30 (Session 4 - Lipid Catabolism)

Fatty Acid Breakdown and PHA Synthesis

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Polyhydroxyalkanoates (PHAs) are bacterial polyesters having a wide range of thermoplastic and elastomeric properties. At present, the main limitation to the industrial use of these polymers as commodity plastics is the high cost of bacterial fermentation. Synthesis of PHAs in transgenic plants could thus be used as an alternative to fermentation. Synthesis of polyhydroxybutyrate (PHB) in the plastids was shown to lead to polymer accumulation to nearly 40% dry weight in leaves and 7% in seeds. Unfortunately, PHB is a somewhat stiff and brittle plastic. There is thus considerable interest in engineering plant metabolic pathways for the synthesis of PHA copolymers with better physical properties, such as medium-chain-length PHAs (MCL-PHAs), a group of polymers having properties ranging from flexible plastics to elastomers and rubbers. MCL-PHAs are synthesised in bacteria using intermediates of the ß-oxidation of alkanoic acids. We have shown that Arabidopsis expressing the PHA synthase in the peroxisomes accumulated MCL-PHAs containing saturated and unsaturated 3-hydroxyalkanoic acids ranging from 6 to 16 carbons. A wide range of monomers derived from fatty acid degradation could be included in the plant PHA, such as branched-chain hydroxyalkanoic acids. Furthermore, the nature and quantity of PHA produced in leaves or seeds could be modulated through the co-expression of a peroxisomal PHA synthase along with transgenes involved in the synthesis of unusual fatty acids, such as medium-chain thioesterases, or by blocking triacylglyceride synthesis. Yet, the amount of MCL-PHA synthesised is still low and further engineering is required to increase the amount to commercial levels. In addition to its application in biopolymer synthesis, PHA synthesised from ß-oxidation intermediates can be utilised as a novel tool to study various aspects of fatty acid degradation, including the pathway of degradation of unusual fatty acids.

Monday, July 16, 2001, 18:00 (Session 4 - Lipid Catabolism)

Lipoxygenase Dependent Degradation of Storage Lipids

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The degradation of storage lipids during germination of oilseeds as an important carbon source for the germinating seedling is an essential physiological process. Until now the complexity of lipid degradation during the early steps of germination is only partially clarified.

Beside the well known degradation of lipids via lipases a new pathway of lipid mobilization can be described which depends on a lipid body trilinoleate 13-lipoxygenase (LOX). This enzyme is specific against non-polarized esterified fatty acids as substrates and is induced during the early germination. The lipoxygenase activity leads to transient accumulation of ester lipid hydroperoxides (mainly 13S-HPOD [(13S,9Z,11E)-hydroperoxy-9,11-octadecadienoic acid]) in the storage lipids. After this initial lipid-peroxidation the esterified 13S-HPOD are subsequently hydrolyzed by specific lipid body triacylglycerol lipases (TAG-lipases). Afterwards the free hydroperoxy fatty acids are reduced to their hydroxy derivates which are degraded during ß-oxidation.

Recent data indicate that a membrane degradation or disintegration is a prerequisite for the initial lipid-peroxidation. Therefore, it can be assumed that proteases and/or phospholipases degrade at least particularly the lipid body membrane to ensure an access for the lipid degrading enzymes to their substrates which initiate consequently the degradation of the storage lipids.

Tuesday, July 17, 2001, 9:00 (Session 5 - Lipid Biotechnology)

Biotechnology Activities of the Plant Breeding Company NPZ-Lembke

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Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (NPZ-Lembke) is a family owned company with more than 100 years of experience in plant breeding and with approx. 125 employees in Hohenlieth and Malchow. NPZ focuses on breeding of new varieties of oil-, protein- and fodder-crops and on the production and marketing of certified seeds. NPZ-Lembke is represented by partners and subsidiaries in France and England and has international activities within EU, East-Europe and North America.

In addition to traditional plant breeding, biotech methods (e.g. *in vitro*-tissue culture-techniques, marker-assisted selection or *in vitro* analysis of resistance) are integrated step by step in the breeding process. New breeding material with transgenic traits (e.g. resistance, quality), which is developed in various projects and co-operations, is cultivated in S1 facilities and optimised for classical breeding programmes.

NPZ-Lembke cooperates closely with universities and scientific institutions as well as with other breeding companies and processing industry. Most of the R&D projects are focussed on oilseed rape in order to improve on one hand the agronomy (input traits, e.g. yield, resistance, winter hardiness, hybrid systems) and on the other hand quality characters (output traits, e.g., quality and quantity of major components like oil, fatty acids, protein and of secondary components antioxidants, phenolic components).

Following two major R&D projects are explained in detail:

Following a competition announcement of the Federal Ministry of Research and Education (BMBF) the project “NAPUS 2000 – Functional food from transgenic rapeseed” was worked out and started in autumn 1999. The goal is to improve the nutritional value of the whole oilseed rape kernels (Brassica napus) for human food applications. It is aimed (1) to produce a new rapeseed oil containing LCPUFAs (EPA, DHA, ARA) as health benefit, (2) to improve the content of antioxidants (tocopherol, resveratrol), (3) to use the protein and (4) polar lipids (lecithine). A number of 17 partners from different working areas are carrying out this complex project. New genes of interest must be discovered, characterised, isolated and transferred into the crop, followed by the breeding of well performing varieties with essential agronomic value. Economic studies, food processing research as well as investigations on the nutrional value of the new qualities are part of the project. First results indicate that it is possible to select suitable plant material with certain seed compositions for an efficient implementation of genetic transformation.

September 2000 the project “Technical oils – Evaluation for a new market” has been started, supported by the Federal Ministry of Consumer Protection, Food and Agriculture (BMVEL). It is aimed to optimize the synthesis of specific fatty acids for technical use (Erucic-, Myristin-, Caprin- and Oleic-Acid). Eleven partners (scientific institutes, breeding companies and industrial consumers) are working together on the improvement of gene constructs, the transformation of oilseedrape, the cultivation of new transgenic varieties, the production of oil samples and the evaluation of these new oil qualities for technical use.

Tuesday, July 17, 2001, 9:30 (Session 5 - Lipid Biotechnology)

Increasing the Seed Oil Content by Modulating the ABA Level During Seed Development

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The phytohormone abscisic acid (ABA) is an important regulator during seed development. ABA is directly involved in the development of seed dormancy, in regulating biosynthesis of storage proteins and in development of desiccation tolerance. In order to study these phenomenons we generated transgenic plants, which specifically accumulated a single-chain antibody (scFv) against ABA within the endoplasmic reticulum of developing seeds. Here, the antibody was expressed to high amounts even before the deposition of storage compounds started and was kept at that level till seed ripening processes are coming to an end. To achieve this goal, the scFv coding sequences were expressed under the control of the USP promotor from *Vicia faba*. Seeds who accumulated high levels of more than 1 % of total seed protein of the antibody turned out to be viviparous, storage globulins were near the detection limit in the embryo and the number of oil bodies was dramatically reduced. Moreover, oil bodies turned out to fuse to one large vacuole-type organell. Analysis of the seed oil content of controls, accumulating an anti-herbicide scFv, of wild type plants, and these transgenic lines showed no differences in the seed oil accumulation of about 40 % of total seed weight. However, when we analyzed 60 independent lines of the T2 generation and of about 30 lines of their T3 progenies in more detail, it turned out that intermediate phenotypes expressing the anti-ABA-scFv to levels of below 1 % of total seed protein, showed a dramatic increase within the fatty acid content of up to 80 % of total seed weight. Moreover, these lines were not viviparous and the embryos did not exhibit a viviparous phenotype. However, when we analyzed the ultra structure of these embryos, it turned out that within these transgenes many of the embryonic cells were devoid of protein bodies and the remaining space was filled of with oil bodies. We conclude from these results that immunmodulation of the ABA functions beginning at early stages of seed development causes different influences to single elements of the ripening process depending on the different levels of ABA not bound by the antibody. This may result in a loss of protein body formation and in an increase of the biosynthesis of storage lipids in seeds exhibiting a specific relation of ABA to antibody concentration.

Tuesday, July 17, 2001, 10:00 (Session 5 - Lipid Biotechnology)

Lipase-Catalyzed Preparation of Steryl Esters

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Steryl esters, particularly cholesteryl esters, are widely used for technical applications such as liquid crystal display devices. Moreover, fatty acid esters of sterols and steroids are well known ingredients of cosmetic, nutraceutical and pharmaceutical formulations. Recently, plant steryl and stanyl esters have been found to be effective in lowering plasma cholesterol concentration by inhibiting the absorption of cholesterol from small intestine (Miettinen et al., 1995). They are, therefore, added to special margarines which are commercially available as functional foods with the ability to reduce both total and LDL cholesterol levels. Enzymatic procedures for the preparation of steryl esters requiring organic solvents and molecular sieves or other drying agents are known. We have developed enzymatic methods for the preparation of carboxylic acid esters, particularly fatty acid esters, of sterols, stanols and steroids in high yield by esterification and transesterification of fatty acids and other carboxylic acid esters, respectively, with the 3-hydroxy group of sterols, stanols or steroids in vacuo using immobilized lipases as catalysts. Neither an organic solvent nor a drying reagent is required.

Sterols (sitosterol, cholesterol, stigmasterol, ergosterol and 7-dehydrocholesterol) and sitostanol have been converted in high to near-quantitative yields to the corresponding long-chain acyl esters via esterification with fatty acids or transesterification with methyl esters of fatty acids or triacylglycerols using lipase from *Candida rugosa* as biocatalyst in vacuo (20-40 mbar) at 40°C (Weber et al., 2001). Similarly, steroids such as 5α-pregnan-3β-ol-20-one and 5-pregnen-3β-ol-20-one have been converted to their propionic acid esters via transesterification with tripropionin. In addition plant sterols and stanols have been converted in good to near-quantitatve yields to the corresponding long-chain acyl esters via esterification with carboxylic acids or transesterification with carboxylic acid esters including triacylglycerols using lipases from *Rhizomucor miehei* (Lipozyme IM), *Candida antarctica* (lipase B, Novozym) and papaya (*Carica papaya*) latex as biocatalysts.

Sterols contained in steam distillates resulting from plant oils by conventional deodorization and by physical refining as well as those present in tall oils derived from wood-pulping process have been converted *in situ* to the corresponding long-chain acyl esters via esterification and/or transesterification with fatty acids and/or triacylglycerols using different lipases in vacuo. The steryl esters formed were purified to ≥90% by deacidification, flash chromatography on silica gel and solvent fractionation. – Lipases from porcine pancreas, *Rhizopus arrhizus* and *Chromobacterium viscosum* were rather ineffective as biocatalysts for the esterification of sterols with fatty acids under the above conditions.

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Tuesday, July 17, 2001, 10:30 (Session 5 - Lipid Biotechnology)

Genetic Modification of Saturated Fatty Acids in the Seeds of Oilseed Rape (*Brassica Naupus L.)*

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Oilseed rape (Brassica napus L.) is one of the leading agricultural crops which benefit from the application of genetic engineering through recombinant DNA technology. Current rapeseed (Canola) oil is characterised by a high content of unsaturated C18 fatty acids. In order to improve its industrial usefulness we aim to modify the saturated fatty acid content by developing transgenic *B. napus* that accumulates medium-chain triacylglycerols in its seed oil. In a first step, the intention was to modify the content of oleic acid, which is the major precusor for subsequent fatty acid pathways (desaturation, elongation) in rapeseed. For this purpose we investigated the effect of antisense inhibition of the endogenous 18:0-ACP desaturase on the fatty acid composition of genetically engineered spring canola cultivar 'Drakkar'. The gene of interest was cloned from a rapeseed cDNA library based on mRNA of developing seeds. The first gene construct (pAS*Bn*DES1) harboured a chimeric gene based on a *Cuphea lanceolata* seed-specific promoter (*Cl*FatB4) and the rapeseed desaturase sequence mentioned above. For comparison, two other antisense gene constructs, pAS*Bn*DES2 and pAS*Bn*DES3, were used containing a *B. napus* seed-specific napin promoter and the same gene of interest. Following the characterisation of regenerated plants with NPTII ELISA assays a significant augmentation of stearic acid in T2 seeds of transgenic plants ranging from 5 to 22 % was observed only in the case of the latter two gene constructs. The best transformants will be analysed further by half-seed analyses and the number of integrated transgene copies will be determined by using Southern blots. Finally, the transformed rapeseed plants showing a significant shift to more saturated fatty acids are considered as an ideal

material for further *Agrobacterium*-mediated transformations using relevant genes from the *Lauraceae* family encoding the synthesis of medium-chain fatty acids.

Tuesday, July 17, 2001, 11:30 (Session 6 - Phenolic Lipids)

Occurrence and Biological Activity of Resorcinolic Lipids

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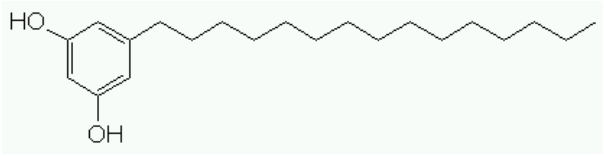
Most of phenolic compounds are of plant origin. Among them, biogenetically derived from the shikimate and acetate (polyketide) pathways, a group of phenolic compounds that are practically insoluble in aqueous solutions attracts increased attention. These compounds are, in most cases, derivatives of mono and dihydroxybenzene (phenol, catechol, resorcinol and hydroquinone) with at least one long aliphatic chain attached to the ring. Due to this feature they are also described as phenolic lipids or long-chain phenols. Some of them are known from their sensitizing and irritating activities (e.g., active principles of some poisonous plants such as poison ivy or poisonous oak) or from their application in the production of Japanese and Chinese lacquer coatings or various resins.

This contribution will focus on one of the group of phenolic lipids, namely resorcinolic lipids that can be considered as long-chain homologues of orcinol (1,3-dihydroxy-5-methylbenzene).



The occurrence of these compounds was initially described in the *Ginkgo biloba* and *Anacardium occidentale* (Cashew tree) but later they were demonstrated in the increasing number of plant and microbial sources. At present their occurrence in over 15 families of the higher and lower plants and several bacterial families has been demonstrated with their contents variability depending on the source. In many cases resorcinolic lipids occur as mixtures of at least several homologues, having a chains of various length (from C9 to C31) and/or degree of unsaturation. In some cereals the presence of numerous saturated and enoic homologues (C13 to C27) has been shown.

Due to amphiphilic charater of the resorcinolic lipid’s molecules they interact very likely with biological membranes and hydrophobic domains of proteins resulting the alteration of their properties and biological activity. It was demonstrated that these compounds exhibit various numerous biological activities such as antimicrobial and antiparasitic, cytotoxic, growth regulating in host-parasite relationship, modulation of enzymatic activities, participation in contact dermatitis, modulation of the barrier properties and the structure of biomembranes and, due to their phenolic nature, inhibition of non-enzymatic and enzymatic lipid oxidation. Interaction with nucleic acids and facilitation of their free radical-dependent destruction, enhancement of the action nucleic acids-specific drugs and enhancement of the properties of liposomal drug carriers make them interesting compounds for future biomedical applications. Additionally, the antifungal properties of cereal resorcinolic lipids together with the correlation between their level, increased production and resistance to the pathogenic fungi upon infection as well as the stimulation of their production by very low concentrations of herbicides suggests their possible application in natural plant protection.

Tuesday, July 17, 2001, 12:00 (Session 6 - Phenolic Lipids)

Resorcinolic Lipids in Liposome Technology

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Liposmes present promissing and versatile drug delivery system. Many drugs when encapsulated in liposomal structure exhibit significant enhancement of their activity and reduced serious side-effects. The effect depends on liposome size, composition and also on the drug/lipid ratio. In the case of water soluble drugs effective drug/lipid ratio depends mostly on liposome volume. The increase of liposome volume will allow delivery of the same amount of drug by lower amount of liposomes. For charged drugs presence of high charged lipids is needed for high drug encapsulation efficiency. The presence of negatively charged lipids in liposome bilayer promotes strong electrostatic interaction with positively charged drugs and high encapsulation efficiency of the drug.

Experiments on liposomes modified with alkylresorcinols showed that these compounds strongly enhance liposome encapsulation of water soluble markers. Physical stability of modified liposomes was also measured. Our data indicated that all studied phenolic lipids (resorcinols) increase the liposomal entrapment volume by 2-6 times. Liposomal formulation containing resorcinols was found to be much more stable than conventional phospholipid liposomes containing cholesterol during long term storage at different temperatures. The distribution of resorcinol modified liposomes in animal organs was similar to the distribution of conventional PC/Chol liposomes.

The synthesis of negatively charged derivative of alkylresorcinols (MSAR) was also made in the laboratory. Liposomes modified with bilayer forming MSAR exhibit high encapsulation efficiency of many anticancer charged drugs, high stability and simplicity of preparation.

Tuesday, July 17, 2001, 12:30 (Session 6 - Phenolic Lipids)

Rapid Gas Chromatographic Method for Quantitative Analysis of Alkylresorcinols in Rye Grains (*Secale cereale L.*)

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Alkylresorcinols (1,3 dihydroxybenzene derivatives) are long chained phenolic compounds found in many plant species including cereals. They are reported in highest levels in rye. Alkylresorcinols have been shown to be bioactive in a range of models, and thus could play a role in the observed health benefits of eating rye. The aim of this study was to establish a rapid methodology for the quantitative analysis of alkylresorcinols using gas chromatography.

The method was developed considering the effects of milling (whole grains verses flour), extraction solvent (ethyl acetate, methanol and acetone), extraction volume per gram of sample, and extraction time. For whole kernels, extraction of 1 gram with 40 mL of ethyl acetate for 24 hours is recommended. The extractability of the alkylresorcinols from whole grains is consistent with the presence of alkylresorcinols only in the outer layers of the kernel.

The extracts were analysed without further purification or derivitisation by gas chromatography, using methyl behenate (C22:0 fatty acid methyl ester) as an internal standard. The extracts were analysed by gas chromatography-mass spectrometry and the major homologues were found to be heptadecylresorcinol (17:0), nonadecylresorcinol (19:0) heneicosylresorcinol (21:0), tricosylresorcinol (23:0) and pentacosylresorcinol (25:0).

The method was then applied to the analysis of alkylresorcinols in 15 rye cultivars grown at two locations in Sweden, and to different milling fractions of one cultivar. Alkylresorcinols are found to be concentrated in the aleurone, and pericarp and testa layers, while the endosperm contains essentially none of these compounds.

Tuesday, July 17, 2001, 13:00 (Session 6 - Phenolic Lipids)

Biophenolic Lipids from the Olive Drupe to the MAC Product

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Eu consumers recently became a demanding bunch of natural products and ingredients for their food, beverage and cosmetics; and olive trees were important source of inspiration for *millennia.*

Due to the particular climate, Mediterranean plants were and still are subjected to prolonged exposure to sunligh, apart from other pathogenic attacks. Thus, olive trees have developed an array of protective biomolecules, than transferred into the food of Mediterranean Aliment Culture (MAC), such as table olive (TO) and extra virgin olive oil (EVOO), for counteracting to the overall damage (1).

Biophenolics (BPs), the only putative defence tool ubiquitous in the plant kingdom, are found in olive drupes, distributed in the mesocarp and the endocarp of the plant cell in the form of souble (*s*), esterifical (*e*) and cell-wall bound entities (2). Olive BPs are often bound to isoprenoid mojeties, secoiridoid (*seco*) unit and fatty acids, thus occurring in different parts of the drupes, localised in cell compartments, such as the hydroxytyrosilmalate, the tyrosiloleate and the *seco*-derivatives, i.e. oleuropein (**1**) and analogues.

Seco-**1**, an intensively bitter *seco*-glycoside found in olive fruits, undergoes a β-glycosidase hydrolysis during the ripening and trasformation processes, with formation of its aglycone and related derivatives, leading to a final rearranged lipidic elenolates; an esterase deactivates **1** *via* two reactive processes during olive maturation, processing and MAC food storage. The pathways reveal effects on EVOO and TOs, influencing the quality descriptors. The hydrolytic conversion, under

abiotic conditions, generates bioactive metabolites. The *seco*-catabolic degradation of **1** to a final lacton shows the Cannizzaro-like behaviour.

The effects on the biomolecular conformations of **1** depend on the environment nature, i.e. hydrophilic or lipophilic ones. The comparison of the biomolecular arrangement, acquired by **1** in different media, has been calculated by biomimetic molecular dynamics, in order to ascertain the partitioning between the experimental environment, during the ripening process from olives to MAC products.

Antioxidant activity, exerted against free radicals and towards metal chelation, taste receptor recognition and enzymatic affinity (3) depend on stable conformations offered by **1**, due to the supramolecular interactions in the biotic and abiotic system. Calculated structure models have been checked by using distance restraints, obtained by ROESY- 2D-NMR spectroscopy (4).

The *seco*-reactivity of **1** was shown to be dependent on the different functionalities, controlled by the experimental conditions, exerted on the olive pulp, and determined by the buffering capacity of the olive mesocarp and by the epicarp molecular components, influencing the reactant peneration into the fruit pulp.

The bioavailability, to sensorial responses into the oral cavity, was shown to be influenced by physicochemical lipidic proprieties of the BP *seco*-molecules, their concentration and the supramolecular interactions with the other components comprised in the MAC matrix, as experimented by NMR biomimesis, with sensorial experiments, performed on single and mixed BPs, for the perception of pungent and bitter tastes.

Work supported by MURST-CNR OEVOCAL Project.

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Wednesday, July 18, 2001, 9:00 (Session 7 - Lipids in Signaling, Stress and Development)

Antiinflammatory Efficacy of the Oxylipin 13-HOTE

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Stinging nettle leaf extracts are registered in Germany for adjuvant therapy of rheumatic diseases. The pharmaceutical efficacy data of Urtica dioica folium is summerized in monographs (BGA, ESCOP) and has been proofed by several postmarketing surveillance studies of nettle extracts. The antiinflammatory active agents in these drugs are unknown until now.

Chronic inflammation is the primary cause of rheumatoid arthritis and related pathology. Due to proinflammatory stimuli, several cytokines are upregulated in response to activation of the transcription factor NF-□B. These cytokines further initiates a cascade of proinflammatory effects such as the expression of cartilage-degrading enzymes.

Here we demonstrate that a novel stinging nettle leaf extract (Hox alpha®) potently decreases stimulated expression of monocyte and T cell derived cytokines in vitro. It is further shown that treatment of different cells types with this extract inhibits NF-□B activation.

We have identified the oxylipin 13-Hydroxyoctadecatrienic acid (13-HOTE) as an acting agent of stinging nettle leaves, which is accumulated by the extraction procedure of Hox alpha®.

It is known that generation of 13-HOTE occurs in plant signal transduction as part of an inducible octadecanoid signaling pathway which is e.g. involved in plant defense mechanisms. 13-HOTE is derived from linolenic acid by 13-lipoxygenase. The function of this oxylipin as mediator in plant signaling has to be elucidated.

Our results suggests that, part of the antiinflammatory efficacy of stinging nettle leaf extract as antirheumatic remedy, may be ascribed to 13-HOTE and its inhibitory effect on NF-□B activation.

Wednesday, July 18, 2001, 9:30 (Session 7 - Lipids in Signaling, Stress and Development)

Oxylipin Signalling in Pathogen-Attacked Plants

Patrick Schweizer

IPK, Gatersleben, Germany

Wednesday, July 18, 2001, 10:00 (Session 7 - Lipids in Signaling, Stress and Development)

Role of oxylipins in the response of potato against pathogens

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Potato reacts to infection with *Phytophthora infestans*, the causal agent of the devastating late blight disease, or to elicitor-treatment with the activation of a variety of defense responses, including the generation of reactive oxygen species, activation of defense gene expression and synthesis of phenolic compounds. In addition, specific oxylipins accumulate which are supposed to act as antimicrobial compounds.

Oxylipin profiling revealed the preferential pathogen- or elicitor-induced accumulation of products of the 9-lipoxygenase pathway. In particular, the 9-lipoxygenase-derived divinylethers colneleic and colnelenic acid are the major oxylipins detectable in suspension-cultured potato cells treated with *P. infestans*-elicitor and in late-blight-diseased potato plants. Furthermore, 9(*S*),10(*S*),11(*R*)-trihydroxy-12(*Z*)-octadecenoic and 9(*S*),10(*S*),11(*R*)-trihydroxy-12(*Z*),15(*Z*)-octadecadienoic acid as well as 9-hydroxy linole(n)ic acid were identified as elicitor-inducible oxylipins in potato. Increases in these compounds correlated with accumulation of transcripts encoding a desaturase, 9-lipoxygenase and divinylether synthase suggesting the transcriptional activation of the pathway in response to pathogen infection of elicitor treatment.

Wednesday, July 18, 2001, 10:30 (Session 7 - Lipids in Signaling, Stress and Development)

Jasmonates and Octadecanoids - Signal in Plant Stress Responses and Development

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Jasmonic acid (JA) and its precursor, 12-oxo-phytodienoic acid (OPDA) are known to be signals in plant development and in responses to various biotic and abiotic stresses. Both of them accumulate stress-induced leading to expression of specific sets of genes. The biosynthesis of jasmonates originates from oxygenation of α-linolenic acid by a 13-lipoxygenase (LOX), followed by activity of an allene oxide synthase (AOS) and an allene oxide cyclase (AOC). All three enzymes were cloned and characterized from barley and other systems. LOX, AOS (1) and AOC (2) were detected immunocytochemically within the chloroplast. Organ- and tissue-specific expression of the AOS gene correlated with elevated levels of jasmonates, e. g. the skutellar nodule of a barley seedling and the leaf base of its primary leaf accumulated AOS mRNA, AOS protein as well as jasmonates. In tomato the AOC was found to be expressed tissue-specifically in parenchymatic cells of vascular bundles and in ovules of young flower buds (3). Upon wounding the main vein of a leaf exhibited up to 10-fold higher levels of jasmonates compared to surrounding leaf areas. Expression of the AOC gene and of jasmonate-responsive genes was analyzed and was compared with quantitative data on levels of octadecanoids and jasmonates. This suggests the role of AOC and jasmonates in amplification of the local wound response and in sink-source relationships. A distinct oxylipin signature of flower organs was found suggesting role in plant defense reactions. With tomato data were recorded on accumulation of AOC mRNA, AOC protein, jasmonates and oxylipins, on *in vitro* AOC activity and on tissue-specific expression of AOC; each of them in non-wounded and wounded leaves of wild-type, *def1* mutant, 35S::*prosystemin*,

35S::AOC*sense* and 35S::AOC*antisense* plants. All data suggest regulation of jasmonate biosynthesis by substrate availability in the AOC-catalyzed step without role of transcriptional up-regulation in the first hours of wounding.

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Wednesday, July 18, 2001, 11:30 (Session 7 - Lipids in Signaling, Stress and Development)

Lipid Binding to PS I (Photosystem I) Protein Subunits

Alfons Radunz

University of Bielefeld, Germany

fällt aus

Wednesday, July 18, 2001, 12:00 (Session 7 - Lipids in Signaling, Stress and Development)

Visualization of Protein Isoprenylation in Tobacco (*Nicotiana tabacum* L.) Bright Yellow-2 cells

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Isoprenylation has been recognized as an important covalent post-translational modification of proteins, either by *S*-farnesylation or by *S*-geranylgeranylation, followed by carboxymethyla-tion, thereby providing a signal for membrane targeting and localization, which is essential for function [1]. Inhibition of cytoplasmic isopentenyl diphosphate (IPP) synthesis by mevinolin, a specific inhibitor of HMG-CoA reductase, led to cell cycle arrest in tobacco BY-2 (TBY-2) cells [2]. Recently, a calmodulin from Petunia (CaM53) carrying a basic C-terminal extension with a CaaL geranylgeranylation motif, was fused to green fluorescent protein (GFP) [3]. We cloned the rice homolog (CaM61), and fused its C-terminal domain carrying the CVIL isoprenylation motif to GFP. We also constructed a GFP fusion protein with the basic domain, but now carrying a CVIM farnesylation motif. 3-d-old TBY-2 cells, but also onion and leek tissue were transformed by particle bombardment. With the CaaM farnesylation motif, cells incorporated the modified GFP mainly into the plasma membrane and the ER, whereas cells transformed with unmodified GFP, showed a diffuse distribution in the cytosolic compartment. When the modified GFP carried the CVIL motif, additional membrane structures became labeled, *i.e.,* the tonoplast. Farnesylation of highly expressed modified GFP is apparently not complete as indicated by labeling of nuclear substructures, similar to mevinolin-induced intracellular translocation of unprenylated CaM53 fusion protein to the nucleus [3]. This also suggests that the capacity for geranylgeranylation of proteins is higher, which corresponds to a higher content of geranylgeranylated proteins in plant cells [*cf.* 1]. Accumulation in small punctate structures of the cytoplasmic network could

indicate trafficking of isoprenylated and carboxymethylated proteins in vesicles, whereas unmethylated, but isoprenylated proteins might be retained to the endomembrane system. Current attempts are directed at cloning of motifs from proteins with highly specific targeting to the plasma membrane and to demonstrate differences between farnesylation and geranylgeranylation of accordingly modified GFP in transiently and/or stably transformed cells. Furthermore, we want to study the effect of farnesol (FOH), which was revealed as inducing apoptosis in TBY-2 cells, but also HMG-CoA reductase activity [4]. FOH could exert two effects, one that may be based on interaction with a signaling cascade, and a second one that may involve interference with formation of a regulatory isoprenic end product. FOH is taken up by plant cells [4] and incorporated into sterols, proteins [5] and mitochondrial ubiquinone-10 [6]. In order to visualize uptake of FOH and its homolog geranylgeraniol (GGOH), we have now applied fluorescent isoprenyl derivatives that mimic both compounds [7]. Both types of analogs (NBD-NH-Ger-OH and NBD-NH-Far-OH, respectively) were readily absorbed by TBY-2 cells and rapidly integrated into membranes (tonoplast, ER, Golgi, plasmalemma), whereas nuclei and vacuoles remained unlabeled. After 3 days of exposure, NBD-NH-Ger-OH induced cell death in the concentration range shown for exogenous F-OH [4], while NBD-NH-Far-OH was about four times less toxic, similar to the difference between the natural F-OH and GG-OH. This observation also suggests that part of the analogs is not only absorbed, but may in fact be used for the synthesis of corresponding diphosphates, which in turn could satisfy structural requirements for their being accepted as substrates for enzymes.

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Wednesday, July 18, 2001, 12:30 (Session 7 - Lipids in Signaling, Stress and Development)

The Calcium-Independent Phospholipase A_2 ($iPLA_2$) Gene Family in Auxin and Plant-Pathogen Signalling

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Previously, it was shown that PLA_2 was activated by auxin or by elicitors within 1-5 min in cultured parsley cells by quantification of fluorescent fatty acids released from fluorescently labelled phosphatidylcholine (Biochem. Biophys. Res. Commun. 163, 111-117; Plant J. 16, 601-11, 1998; Plant Growth Reg. 32, 123-128). This provided a first indication for a function of a PLA_2 in plant signal transduction but the molecular identification of cytosolic plant PLA_2s suited for a function in signal tranduction in plants remained unclear. Data on the isolation of four cDNA sequences and some properties of the respective proteins are presented here, *At*PLAI, *At*PLA IIA, *At*PLA IVA and *At*PLA IVC, which are members of the PLA_2 gene family in Arabidopsis of the patatin or $iPLA_2$ type. The *At*PLAI gene was expressed preferentially in the shoot but also in the flower and the root and very little in the leaves. Genes *At*PLA IIA and *At*PLA IVA were expressed in the root and much weaker in the flowers, shoots, and leaves. The transcriptional avtivity of *At*PLA IVC was found in the root, leaves and flower but was very low in the shoot, as demonstrated by GUS expression and RT-PCR. To demonstrate the compartmentation of *Arabidopsis* $iPLA_2$s three hybrid $iPLA_2$-GFP proteins (*At*PLA IIA, *At*PLA IVA, *At*PLA IVC) were expressed transiently in tobacco leaves and all were clearly not expressed in the vacuoles but in the cytosol. The enzymatic activity of the purified His-tagged protein of gene *At*PLA IVA towards phosphatidylcholine was dependent on Ca^{2+}, being saturated at 0.5 mM, and the pH optimum was at about 7.0. The enzyme showed highest sensitivity towards the PLA_2 inhibitor $PACOCF_3$ (K_i~ 30 nM), $AACOCF_3$ (K_i~ 25 µM), and HELSS (K_i~

200 nM) and was also sensitive to other previously used inhibitors, NDGA (K_i~ 15 µM), and ETYA (K_i~ 3 µM). When the influence of PLA_2 inhibitors on elongation in etiolated *Arabidopsis* seedlings was tested the $iPLA_2$–specific HELSS and ETYA consistently inhibited hypocotyl elongation strongest at concentrations close to the K_i in vitro and NDGA was a weak inhibitor. The same inhibitors also inhibited PLA_2 activation by auxin and elicitors in cultured parsley cells and the biosynthesis of phytoalexin in parsley cells. Because of these data and because of the apparent absence in the Arabidopsis genome of the second type of eukaryotic cytosolic PLA_2, the so-called calcium-dependent **c**PLA_2, it is concluded that the members of the $iPLA_2$ gene family have a function in plant signal transduction.

Poster Session, Monday, July 16, 11:30 - 12:30 and 20:00 - 21:00

Poster No. 1

Studies of Amphiphilic Properties of Phenolic Lipids

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Phenolic lipids, the natural amphiphilic long-chain homologs of orcinol (1,3-dihydroksy-5-methylbenzene) are demonstrated in numerous plant [1] and microbial organisms [2]. They have strong amphiphilic character with the value of octanol/water partition coefficient ($logP_{o/w}$) 7.4 for the 15C homolog [3] and the value of Hydrophilic Lipophilic Balance equal to 4 for the same homolog [4]. The resorcinolic lipids molecules exhibit high affinity for lipid bilayer and biological membranes. The incorporation of these compounds into liposomal and biological membranes induces an increase of their permeability for small nonelectrolytes and cations [5]. This increase of the permeability of membranes may result from formation within the bilayer of the non-bilayer structures, such as reversed micelles or hexagonal phase (H_{II}) [6] often resulting in hemolysis of the cells. For understanding the molecular mechanism of action of resorcinolic lipids in natural membranes it was necessary to determine their CMC and their localization, orientation and effect on the order of membrane lipid molecules.

Critical Micelles Concentration of resorcinolic lipids were investigated by studying changes of surface pressure of aqueous solutions [7,8] and the depth of localization of some homologs were investigated by TMA-DPH and NBD-PE fluorescence polarization measurements.

Resorcinolic lipids show very low values of Critical Micelle Concentrations. The CMC values determined for various homologs are in the micromolar range (from 5 to 27 μM) and depend on lenght of aliphatic chains. These values of CMC indicate that these compounds are localization between phospholipids (CMC 0.006 mM) and other surfactants like Tween-80 (CMC 0.012 mM) or Triton X-100 (CMC 0.024-0.03 mM).

Changes of TMA-DPH and NBD-PE fluorescence polarization shown that the depth of phenolic lipids incorporation into liposomal membrane are nor very high. Polar „heads" of the molecules of investigated compounds are localized on the level of fatty acid's ester bonds in phospholipid molecules.

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Poster No. 2

Biological Monitoring Studies Aiming the Synthesis of Cardol and $^{11}\Delta$-Cardol

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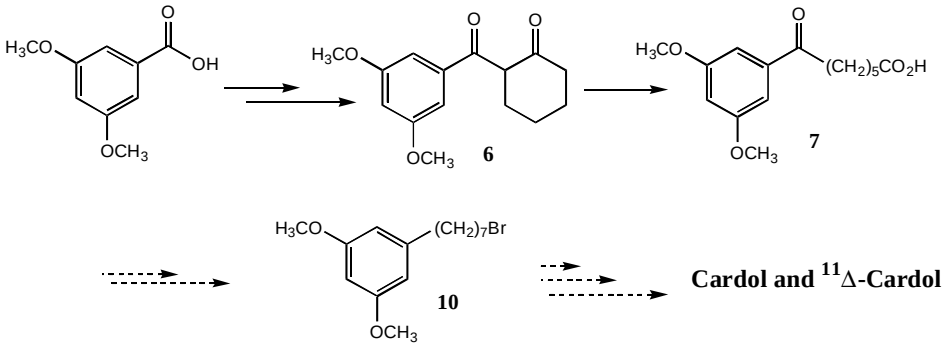
Alkylresorcinols and their derivatives present a great variety of biological activity. Previous studies showed a several biological activities associated to these types of compounds (Matsumoto *et al.*, 1990).

The aim of this work is the biological monitoring synthesis of Cardol and $^{11}\Delta$-Cardol, that are resorcinolic lipids coming from the dihydroxyphenols from the secondary metabolism of plants, and their derivatives, by fungitoxic and allelopathic activity assays. We have proposed a convergent synthesis of Cardol and $^{11}\Delta$-Cardol via the key intermediate 10.The synthetic route for preparation of these compounds is known as related in a recent review (Kosubeck, 1999). However, in our work we have optimised and modified experimental techniques and then improved the yields of reactions. We have also been able to scale up some steps to eventually achieve compound 10.



Compounds 6 and 7 were submitted to allelopathic assays. Compound 6 was not capable to inhibit the germination of lettuce (*Lactuca sativa*) seeds in the all tested concentrations. This compound also did not interfered in the root-growth rate when compared to negative controls (buffer and $CHCl_2$). However, compound 7 presented high allelopathic activity, as on the inhibition of germination as in the negative interference on root growth, when compared to positive control (caffeine) in the concentrations of 5 to 1,0 mg. In low concentrations (0.5 mg, 0.25 mg and 0.1 mg) the effect on germination was moderate. In bioautography assays the compound 6 was efficient on the growth inhibition of the fungus *Cladosporium sphaerospermum* (MIC = 7,8 μg). Compound 7 also showed fungitoxic activity although the minimum-need concentration is four times higher than compound 6 (MIC ~ 30μg). Therefore, compound 6 does not show significant allelopathic activity, but it is a potent inhibitor of fungus growth, as compared to commercial fungicides. In the other hand, compound 7 presents high capability of inhibition of germination and root growth of lettuce seedlings, but demonstrates moderate fungitoxic activity. One can suggest that the observed biological activities are directly related to the structure of these molecules in view of the fact that the synthetic sequence is being conducted to a more amphiphilic in nature compounds.

Acknowledgments: We thank UFMS and Merko Comércio de Produtos Agropecuários Ltda for financial support.

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Poster No. 3

Preparation and Biological Activity of Alkylresorcinols from Lichen Compounds

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Poster No. 4

Resorcinolic Lipids in Seeds of *Ailanthus Alitissima*

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Plant seeds contain numerous chemicals that protect them from external aggression and predators attack. These "preformed" compounds prevent also the germination of fungal spores on the seed surface. However, the composition of those chemicals depends on environmental changes, physiological status of the plant, and other minor factors.

We focused our interest in heaven tree seeds' chemicals that have antimicrobial activities.

The accumulation of resorcinolic lipids was found to be strongly dependent on the climate conditions that prevailed at a period of seed forming. Significant differences in concentration of studied resorcinols were shown between seeds formed under distinct conditions that directly resulted from diverse trees' localisation). Analytical methods for resorcinolic lipids were applied and two homologues with 29 and 31 carbon atoms in the chains were newly identified. Quantitative analysis indicated a concentration range of 13.1-33.9 µg/g, doses being sufficient for the total growth inhibition of certain phytopathogenic fungi.

Long-chain resorcinolic lipids have been identified in heaven-tree seeds and their presence indicates that they may be one of the compounds responsible for the health status of plant seeds.

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Poster No. 5

Purification and Characterization of Sunflower (*Helianthus annuus L.*) Excised Cotyledon Glyocysomes

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Our previous works (Jridi and al. 2000) showed that gibberellic acid is the necessary and sufficient hormone to trigger the transformation of triacylglycerols in sugars by the neoglucogenese way. The particularity of this way is the implication of glyoxysomes that topples acetyl-CoA molecules toward the synthesis of sugars instead of transform them in energy by the Krebs cycle. These glyoxysomes are cytoplasmic cellular corpuscles containing β-oxidization enzymes and those of glyoxylic cycle. In order to study the impact of this hormone on the activity of neoglucogenese enzymes, we had first attend to purify and then to characterize glyoxysomes from germinating sunflower cotyledons compared with those from excised cotyledons.

The method of separation used is the ultracentrifugation in a linear sucrose gradient varying between 20% to 60%( ). To identify glyoxysomal fractions, we had proceeded to *in-vitro* dosage and a specific revelation of two key enzymes of this organites : isocitrate lyase, key enzyme of glyoxylic cycle and the catalase that permit the cell to get rid of electrons formed during glyoxysomal β-oxidization. We tested the activity of malate deshydrogenase to verify if glyoxysomes are either contaminated or not by mitochondria.

Basic on the results of enzymatic activity tests and the specific revelations of these marker enzymes, we have characterized a pure glyoxysomal fraction. This study confirms the role of gibberellic acid in triggering glyoxysomal enzymes.

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Poster No. 6

The Role of Choline-Phosphotransferase in the Synthesis of Triacylglycerols by Sunflower Seeds

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We have previously shown that in developing sunflower seeds phosphatidylcholine (PC) was the major phospholipid synthesised. This has been demonstrated with labelling experiments "in vivo" as well as "in vitro". PC appeared as the site of desaturation of oleate into linoleate ; the latter was sequentially transferred to diacylglycerols (DAG) then to triacylglycerols (TAG).

On the other hand, the CDPcholine:DAG-phosphotransferase (EC.2.7.8.2) has been described as catalysing the formation of PC from CDPcholine and DAG. Recently, we have evidenced the back-reaction giving DAG from PC. So, this enzyme is involved in a rapid turn-over occurring between PC and DAG in maturing sunflower seeds which accumulate polyunsaturated fatty acid rich TAGs.

In this study we have investigated the mechanism of the phosphotransferase action and its subcellular localisation. The microsomal reverse-activity was characterised using [^{14}C]dioleoyl-PC as substrate. An important property of the enzyme was the sigmoid shape of the kinetic which revealed a possible regulating role in the reserve lipid biosynthesis. In plants where only the classical way of progressive acylation of *sn*-3-glycerophosphate, namely Kennedy pathway, is operative, it is the phosphatidate-phosphatase step which is habitually considered to be rate-limiting the TAG formation.

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Poster No. 7

Characterization of the Lipoxygenase Gene Family of *Arabidopsis*

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Lipoxygenases (LOXs) catalyze the hydroperoxidation of polyunsaturated fatty acids, containing an (1*Z*, 4*Z*)-pentadiene double bound system. The resulting hydroperoxides serve as substrates for a multiple set of other enzymes to produce physiological important compounds, like jasmonic acid, traumatic acid, aldehydes, divinyl ethers, and ketols. Numerous reports suggest, that LOXs are involved in plant growth and development, wound response and pathogen resistance, but because of the existence multiple isoforms within one organism and their different tissue specificity, the analysis of the physiological function in plants is complicated.

Since the genome of the model plant *Arabidopsis thaliana* is sequenced the absolute number of six LOX genes could be determined. In order to analyze all LOX isozymes we cloned at least five of these LOXs (AtLOX1-5) from different cDNA libraries by PCR based on sequences of the *Arabidopsis* data base. The recombinant proteins were expressed in *E. coli.* Using the affinity purified proteins, their pH-optima, substrate and regio-specificities were determined. Moreover, their occurrence in different tissues from *Arabidopsis* was analyzed. Due to the involvement of LOX-derived products in signaling processes, we tested the transcriptional regulation of LOXs by sorbitol (leading to an endogenous rise of the prostaglandin homologue jasmonic acid), methyl jasmonate and salicylate.

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Poster No. 8

Isolation of new *CYP74*-Enzymes from Plants and the Moss *Physcomitrella patens*

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So far CYP74-enzymes are unique to plants and belong to the P450-enzyme family. They metabolize lipoxygenase-derived hydroperoxides of polyunsaturated fatty acids. At least three different subfamilies of enzymes are known which form the CYP74-family: (i) Allene oxide synthase (AOS, CYP74A) leading to the formation of α- and γ-ketols as well as to the formation of 12-oxo phytodienoic acid. (ii) Hydroperoxide lyase (HPL, CYP74B + C) leading to formation of volatile aldehydes and ω-oxo fatty acids. These aldehydes are important constituents of fruit flavors. (iii) Divinyl ether synthase (DES, CYP74D + E) leading to formation of divinyl ether polyunsaturated fatty acids. Moreover, these types of enzymes are unique in comparison to other P450-enzyms in that they do not need molecular oxygen and reducing agents as cofactors. All known *CYP74*s show a high homology among each other. Here, we present the isolation of cDNAs by RACE-PCR of new members of the *CYP74*-family from higher plants and from the moss Physcomitrella patens. All enzymes were overexpressed in E. coli utilizing a His-Tag expression vector. After purification by affinity chromatography we characterized them at the biochemical level, for example pH-optima, substrate specificity and analyzed the product specificity by radio HPLC and GC/MS.

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Poster No. 9

Isolation and Characterization of a new Lipoxygenase from *Momordica charantia*

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Lipoxygenases (LOXs) are non heme iron-containing dioxygenases that catalyze the regio- and stereo selective dioxygenation of polyenoic fatty acids with a (1*Z*,4*Z*)-pentadiene system forming hydroperoxy derivatives. LOXs are widespread in higher plants and animals. Their activity has been correlated with diverse processes in development, germination, maturation and senescence in plants.

We cloned a new LOX from *Momordica charantia* via PCR-based approach. This LOX (MoLOX) comprises 880 aa and has 79 % homology to the lipid body LOX of cucumber seedlings (cslbLOX). MoLOX is a 13-linoleate LOX and converts arachidonic acid to 15-HPETE. In contrast to the lipid body LOX from cucumber, trilinoleate is no substrate of MoLOX. Moreover, this specific LOX shows a new pH-dependency of positional specificity. At basic pH (above pH 8.5) MoLOX shows a high positional specificity for formation of 13-HPO(D/T) and of 15-HPETE, respectively. At acid pH the enzyme is even more active, but shows no positional specificity, if arachidonic acid is used as substrate. However, it shows an altered specificity towards 9-lipoxygenation, if linoleic acid or linolenic acid are used as substrate. This unusual feature might be due to an altered active site, which contains at a highly conserved position a glutamine (Q_{599}) instead of a phenylalanine, a methionine or a histidine in case of other plant 13-LOXs.

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Poster No. 10

Isolation and Characterization of a Jasmonate-Induced Lipase from Barley Leaves

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Jasmonic acid is a lipid-derived plant hormone and is involved in the induction of a senescence-like phenotype in leaves. Thereby it induces numerous mRNAs and proteins derived therefrom. In order to study the function of jasmonates during establishment of senescence, we analyzed mRNAs specifically induced by jasmonates in barley leaves by differential display. By that method at least four transcripts have been identified in leaf segments of barley (*Hordeum vulgare* L. cv. *Salome*). One of the putative translation frames was homologous to caffeic acid methyltransferase, one was homologous to chalcone synthase, but there was no significant homology found for the other two. After isolation of the full length cDNA clones, we inspected their ORFs in more detail and one out of the two cDNAs (JRG 12) showed now high sequence homology to acyl lipid hydrolases. This putative enzyme was cloned into a His-Tag expression vector and overexpressed in *E. coli*. After purification by affinity chromatography lipolytically active protein was obtained. The biochemical properties, as well as results of a further, more detailed characterization will be presented and discussed with respect to its putative physiological function.

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Poster No. 11

Triclosan Specifically Inhibits Fatty Acid Biosynthesis in Plants

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Triclosan (2,4,4'-trichloro-2'-hydroxydiphenylether) is a widely used antibiotic in various products such as anti-septic soaps, toothpaste or plastics. In the past it was believed that triclosan might act as non-specific antibiotic, e.g. by membrane perturbation due to the lipophilicity of triclosan. Recently it was shown that triclosan has a specific enzymatic target in bacteria: the enoyl-ACP reductase, a key enzyme in the bacterial fatty acid biosynthesis. In our investigations we could show that triclosan inhibits the growth of plants in a dose-dependent manner. The reason for the growth retardation was not due a non-specific inhibition of photosynthesis but caused by an inhibition of plant *de novo* fatty acid biosynthesis. This was shown by measuring the incorporation rate of different precursors (radiolabelled acetate or hydrogencarbonate) into fatty acids in either isolated chloroplasts or etioplasts from pea. Direct measurement of the enoyl-ACP reductase from crude homogenates indicate that, as in bacteria, this enzyme is a target for triclosan also in plants. It appears that, like in bacteria, the strong inhibition of enoyl-ACP reductase is caused by the formation of a ternary complex of the enoyl-ACP reductase with triclosan and NAD^+ (one of the products of the enzymic reaction). Preliminary results indicate that plants also posseses a second enoyl-ACP reductase activity which appears to be insensitive against triclosan.

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Poster No. 12

The Role of Acyl-CoA and Acyl-ACP in Regulating Plastidial Fatty Acid Biosynthesis

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During plastidial fatty acid biosynthesis (FAS) acyl-ACPs with different chain lengths are produced. Free fatty acids are then released, transported out of the plastids and esterfied to CoenzymeA. Usually long chain acyl-CoAs do not accumulate in plastids but in the cytosol. One hypothesis suggests that long chain acyl-CoAs are involved in regulation of plastidial fatty acid biosynthesis, particularly ACCase, by feedback inhibition. To obtain *in vivo* evidence for the influence of acyl-CoAs on FAS we expressed the bacterial *PHAG* gene and the *ACBP* (acyl-CoA binding protein) cDNA from *Glycine max* with plastidial target sequences in Arabidopsis thaliana. *PHAG* encodes a 3-hydroxylacyl-ACP:CoA-acyltransferase, which supplies the substrates for polyhydroxyalcanoate production in Pseudomonas putida. Transgenic plants expressing *PHAG* show a stunted growth phenotype. No significant change of total fatty acids compared to the wild type was found. Binding of acyl-CoAs by ACBP in the plastids of transgenic plants might affect FAS activity, if acyl-CoAs have a regulatory role on FAS. However, transgenic plants overexpressing *ACBP* also do not show any changes in amount and composition of fatty acids. Taken together, these results do not support an *in vivo* role of acyl-CoAs in regulating FAS activity in plastids.

A second approach to study the regulation of FAS was initiated with the isolation of an oleoyl-ACP thioesterase (fatA) Arabidopsis mutant. This mutant was isolated by screening the University of Wisconsin (M. Sussman) T-DNA transformed Arabidopsis collection. The T-DNA in this mutant line is inserted in the second exon of the *FATA* gene. Characterisation of the fatA mutant is in progress.

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Poster No. 13

Digalalctosyldiacylglycerol in the Extraplastidial Peribacteroid Membrane of Legumes

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The fixation of atmospheric nitrogen in the root nodules of legumes is localized to Rhizobia bacteria which exist inside the cytosol of the plant cell, surrounded by a bacterial membrane and by a plant-derived, so-called peribacteroid membrane. By thin-layer chromatography, we could show that the galactolipid digalacto-syldiacylglycerol DGDG is an abundant lipid in the peribacteroid membrane of soybean (*Glycine max*). This finding demonstrates that DGDG in higher plants is not restricted to plastids and corroborates previous experiments which showed that DGDG accumulates in extraplastidal membranes after phosphate deprivation. We are currently analysing the fatty acid and sugar composition of DGDG from the soybean peribacteroid membrane. To address the function of DGDG for the symbiotic plant-bacteria interaction, we extended our analyses to *Lotus japonicus*, a model plant for the characterization of nitrogen fixation in legumes. We obtained cDNAs with sequence similarities to the Arabidopsis DGDG synthases DGD1 and DGD2 from soybean and Lotus. By Northern hybridisation of non-infected roots and of nodules, we anticipate to further our understanding of the role of these two genes during nodulation.

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Poster No. 14

Enzymatic Characterisation of Prenyltransferases involved in Vitamin E Biosynthesis

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Tocopherols, which are lipid soluble antioxidants collectively known as vitamin E, are synthesised by photosynthetic pro- and eukaryotic organisms. In the course of tocopherol biosynthesis a membrane bound prenyltransferase catalyses the transfer of a phytyl group to homogentisate leading to the formation of 2-methyl-6-phytyl-benzoquinol. This enzymatic activity, which has so far only been investigated in subcellular fractions, might be critical for the levels of tocopherols in cells.

To characterise this membrane bound enzyme we have started to clone sequences from *Synechocystis* and plants which according to database searches might encode prenyltransferases. The identity of the sequences was verified via functional expression studies in *Escherichia coli* lacking vitamin E. Optimized assay conditions and kinetic properties of prenyltransferases will be presented.

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Poster No. 15

Phosphatidylcholin-Galactolipid Fatty Acid Exchange in Transgenic Maize Leaves Engineered with Antisense FAD2 cDNA Evaluated by Chemometrics of Lipid Analysis

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The fatty acid export from lipids synthesized in endoplasmic reticulum to chloroplasts for the biosynthesis of plastid galactolipids was examined in maize leaves transformed with FAD2 cDNA in antisense orientation. For that, individual glycerolipid classes (monogalactosyldiacilglycerol – MGDG, digalactosyldiacylglycerol – DGDG, sulphoquinovosyldiacylglycerol – SQDG and phosphatidylethanolamine – PEA, phosphatidylinisotol – PI, phosphatidylcholine – PC and phosphatidylglycerol – PG) were fractionated by multiple solid phase extraction. Subsequently, the level of unsaturation in each of these seven glycerolipids was evaluated in the palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) fatty acids. The data set from the analysis of six fatty acids in seven individual lipid classes from eighteen leaf samples of FAD2 antisense transformed, plus two control maize plants (in total 840 individual values) was analyzed by multivariate statistical approach. The cluster analysis (CA) and the exploratory application of principal component analysis (PCA) of six fatty acids allowed the partition of the plants into three groups for all glycerolipid classes. The first plant group included the controls and showed glycerolipid composition similar to published values. The second and third groups exhibited increasing deviation from control values and mainly contained increased levels of oleic acid accompanied by decreased levels of linolenic acid. This was true for lipids produced via the “eukaryotic” part of the glycerolipid synthesis pathway (PC, PE, PG and PI) as well as for the lipids produced via the “prokaryotic” part of the pathway (MGDG, DGDG and SQDG). Interestingly, the direct product of the FAD2 (dellta-12 desaturase) catalyzed reaction – linoleic acid – was much less affected (decreased) compared to the decreases of the

linolenic acid. The most significant decrease in the linolenic acid content (8 fold) was detected in MGDG in plants of third group, when compared to the other two groups. This might be explained with the role of PC for the release of the unsaturated fatty acids into the acyl-CoA pool, which is then used for diacylglycerol synthesize with a high levels of unsaurated fatty aids (Williams et al., 2000, *Biochem. J.*, 349, 127-133). This highly unsaturated diacilglycerol may be used as a source for the biosyntyhesis of cellular glycerolipds.

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Poster No. 16

DGD2 – a UDP-galactose dependent digalactosyldiacylglycerol synthase in Arabidopsis thaliana is induced after phosphate deprivation

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The galactolipid digalactoslyldiacylglycerol DGDG is one of the major lipids in plants, it is mainly found in chloroplasts, but also present in extraplastidic membranes under phosphate limitation. At present, two enzymes are known which are involved in DGDG biosynthesis, DGD1 and DGD2. Whilst DGD1 is responsible for 90% of the DGDG synthesis, the in vivo function of DGD2 still remains unclear.

Sequence similarity of the two enzymes, DGD1 and DGD2 to other known glycosyltransferases suggests that they belong to the class of UDP-galactose dependent galactosyltransferases synthesizing DGDG with retention of the α-configuration at the anomeric carbon atom. In vitro enzyme assays with *E.coli* membranes expressing DGD2 using radiolabeled substrates, such as ^{14}C-MGDG and ^{14}C-UDP-galactose were performed to determine the substrate specificity. It could be shown that MGDG is not the only substrate, but that UDP-galactose is required as the galactose-donor for DGDG synthesis. To identify the configuration of the anomeric carbon of the second galactose moiety, DGDG lipid was isolated from E.coli co-expressing either a monogalactosyldiacylglycerol synthase *MGDA* and *DGD1*, or *MGDA* and *DGD2.* Both lipids were analyzed by ^{1}H-NMR and clearly show an α-configuration of the anomeric carbon atom of the second galactose group.

The observed increase of DGDG synthesis in extraplastidic membranes (Härtel, Dörmann, Benning (2000) Proc. Natl. Acad. Sci. 97, 10649-10654) and the induction of DGD2 under phosphate limitation as shown by Northern analysis and promoter

GUS expression, may indicate the in vivo role of DGD2 in DGDG production under specific growth conditions.

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Poster No. 17

Glucosylceramide Synthases: a Gene Family Responsible for the Biosynthesis of Cerebrosides in Animals, Plants, Yeasts, and Fungi

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Glycosylceramides are membrane lipids in most eukaryotic organisms and in a few bacteria. The physiological functions of these glycolipids have only been documented in mammalian cells, whereas very little information is available of their roles in plants, yeasts, fungi, and bacteria. In an attempt to establish appropriate experimental systems to study glycosylceramide functions in these organisms, we performed a systematic functional analysis of a glycosyltransferase gene family with members of animal, plant, fungal, and bacterial origin. Deletion of such putative glycosyltransferase genes in *Candida albicans* and *Pichia pastoris* resulted in the complete loss of glucosylceramides. These knock-out strains were used as host cells for homologous or heterologous expression of candidate glycosyltransferase genes. By this means, five novel glucosylceramide synthases (ceramide glucosyltransferases) were identified from the plant *Gossypium arboreum* (cotton), *Caenorhabditis elegans*, the fungus *Magnaporthe grisea*, and the yeasts *C. albicans* and *P. pastoris*. The glycosyltransferase gene expressions lead to the biosynthesis of

different molecular species of glucosylceramides which contained either C18 or very long chain fatty acids. The latter are usually channelled exclusively into inositol-containing sphingolipids known from *Saccharomyces cerevisiae* and other yeasts. Implications for the biosynthesis, transport, and function of sphingolipids will be discussed.

Poster Session, Monday, July 16, 11:30 - 12:30 and 20:00 - 21:00

Poster No. 18

Approaches to manipulate lipid content in potato tubers

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The exact contribution of the different enzymatic pathways to the incorporation of carbon into lipids (oil) in plant storage tissues is only poorly understood. To investigate the accumulation of storage lipid in plants in more detail, we chose the potato tuber as model system, because due to the negligible amount of endogenous triacylglycerol (TAG), even small changes in their TAG content and lipid precursors can be detected. In addition, the high per hectar yield of potato tubers renders this crop an ideal system for the production of fatty acids and related lipids in transgenic plants. Furthermore, the availability of numerous transgenic lines altered in carbohydrate metabolism opens the way to analyse the effect of alterations in starch accumulation on lipid synthesis. Therefore, the lipid content and composition of four transgenic potato lines, overexpression of yeast invertase and bacterial glucokinase (Trethewey, Geigenberger, Riedel ,Hajirezaei, Sonnewald, Stitt, Riesmeier & Willmitzer (1998) Plant J. 15, 109-118), overexpression of plastidic ATP/ADP-transporter (Tjaden, Möhlmann, Kampfenkel, Henrichs & Neuhaus (1998) Plant J. 16, 531-540), antisense expression of plastidic phosphoglucomutase (Tauberger, Fernie, Emmermann, Renz, Kossmann, Willmizter & Trethewey (2000) Plant J. 23, 43-53), antisense expression of ADP-glucose pyrophosphorylase (Müller-Röber, Sonnewald & Willmitzer (1992) EMBO J. 11, 1229-1238) were analysed in detail. We also generated two new transgenic potato lines overexpressing the acyl-CoA:diacylglycerol-acyltransferase from *Arabidopsis* or the acetyl-CoA carboxylase (Roesler, Shintani, Savage, Boddupalli & Ohlrogge (1997) Plant Physiol. 113, 75-81) to study the effect of an increased production of lipid precursors or increased incorporation of fatty acids into TAG on overall lipid biosynthesis. Via the analyses of lipid composition and fatty acid distribution by thin layer chromatography and gas

chromatography as well as via the analysis of short chain acetyl-CoA and malonyl-CoA by HPLC, we anticipate to obtain novel insights into storage lipid synthesis.

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Poster No. 19

Improving the Production of Unusual Fatty Acids in Oleaginous Plant Species

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Efforts in modifying oleaginous plant species to produce unusual fatty acids have been limited to date. Genes for the diiron non-heme class of fatty acid modifying enzymes that have desaturase, hydroxylase, epoxygenase, and acetylenase actitivites, have successfully been over expressed in species such as *Brassicu napus*. However the levels that these fatty acids have accumulated in the seeds of transgenic plants have been relatively low, in the order of 1-10%. In an effort to try to increase the levels of unusual fatty acid accumulation we are cloning the genes to enzymes of the Kennedy pathway (PLA_2 and DAGAT) in *Ricinus communis.* We intend to coexpress these genes in a single transformed rapeseed line. In addition to these genes of the Kennedy pathway we have identified a cDNA, TargH12, from *Ricinus communis* whose protein product interacts with the delta 12 hydroxylase of the same species. Currently we are examining the activities of the new DAGAT enzyme, in particular with respect to substrate specificity, as well as assessing a possible role for TargH12.

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Poster No. 20

Identification of some Olive Olive Trees Cultivars based on some Chemical Characterisics of Oil

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Multidisciplinary works, undertaken within the framework of the identification of the national olive-growing inheritance since 1984, showed that Tunisian olive plantation is constitute of a multitude of varieties. This situation is the resultant of an uncontrolled propagation during the history of olive grove culture. This variety richness generated a great genetic variability. Studies carried out showed that these cultivars are different each other, as well from the point of view phenotypic as pomological characteristics.

This work represents a contribution to this programme of identification by studying some chemical characteristics of the oil of twelve cultivars planted in the zone of "Sig." at Sfax region.

The olive-trees, object of this study, are planted in the same farm, consequently, they are under the same conditions and they receive the same care of culture. The chemical analyses are carried out on the oil extracted from olives were collected roughly at the same stage of maturity.

The obtained results confirm the heterogeneity observed on the phenotypic and pomological level, stated elsewhere. Thus we notice that four cultivars (S1, S6, S10 and S112) have very high contents of phenolic compounds (> 125 ppm). On the other hand those of S18 and S22 are weakest (< 30 ppm). By another way we observe that S4A, S10 and S112 have the most unsaturated oils (rate of non-saturation > 4.5). As for the acidic composition, although it is different from one

cultivar to another, except S22, all of them have oil with good characteristics. Indeed, this cultivar is characterised by rates very close to the higher limit of the international olive oil marketing norm, recommended by the International Olive Oil Council, for C16:0 (palmitic acid) and of its minimal for C18:1 (oleic acid), however the linoleic acid rate acid exceeds the limit of this norm. This work must be completed by other chemical analyses of other compounds, as tocopherols, triterpenic alcohol's.

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Poster No. 21

Functional identification of a fatty acid elongase component specific for polyunsaturated fatty acids by gene targeting

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We recently described the cloning and functional identification of a cDNA (PSE1) encoding a fatty acid elongase from the moss *Physcomitrella patens*. By heterologous expression in the yeast *Saccharomyces cerevisiae* we showed that the encoded protein (Pse1p) is involved in the elongation of polyunsaturated fatty acids. By feeding experiments we could show that it has a selectivity for Δ^6-C18 polyunsaturated fatty acids and discriminates Δ^9-C18 polyunsaturated fatty acids.

We provide now further evidence for the function of Pse1p as component of the fatty acid elongase complex. We disrupted the PSE1 gene in the moss by heterologous recombination. One mutant plant was obtained that contained significant reduced proportions of C20 polyunsaturated fatty acids (~10% of the wilde-type content) and increasing proportions of γ-linolenic acid, which acts as substrate for the elongase. However, no difference in the appearance of wild-type and mutant plants could be observed, so that possible functions of the polyunsaturated fatty acids arachidonic acid and eicosapentaenoic acid remain to be elucidated.

The PSE1 sequence shares some limited similarities to the ELO1 sequences. e.g. it contains a his box motif normally present in desaturases and related enzymes and a tyrosine box characteristic for the ELO protein family. Interestingly, these sequences

do not share any homology with the beta-ketoacyl-CoA synthases (KCS) isolated from various plant species. It is questionable, whether the ELO protein family catalyzes the same reaction as the KCS protein family since it does not contain a conserved cysteine, which is essential for this activity. Biochemical and phylogenetic implications will be discussed.