Plant Biology

S08-01

DEFENSE REACTIONS OF PLANTS TO PATHOGENIC FUNGI

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Plants respond to infections by fungal pathogens with the activation of a multitude of local and systemic defense reactions. Three major phases can be distinguished: 1. the formation of a physical barrier in the immediate vicinity of the penetrating fungus, often but not necessarily associated with hypersensitive cell death; 2. transient defense gene activation in a locally confined tissue area surrounding the infection site and the consequential accumulation of defense-related proteins/enzymes and their products; and 3. the relatively slow but long-lasting systemic gene activation leading to the accumulation of other defense-related compounds. The first two phases occur more or less concomitantly and will be discussed with respect to the involved signalling and gene activation mechanisms.

S08-02

MOLECULAR CONTROL OF ROOT NODULE ORGANOGENESIS INDUCED BY *RHIZOBIUM*

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Rhizobia are able to induce the formation of nitrogen-fixing nodules on the roots of their leguminous plant hosts. Nodule induction and development are governed by signal exchanges between the two partners. First, specific flavonoids exuded by the roots induce the expression of the *Rhizobium* nodulation genes. Then, the nodulation gene products catalyse the synthesis and excretion of a family of structurally related lipo-chitooligosaccharides, the Nod factors. These molecules evoke plasma membrane depolarization and ion changes in the root hairs and their deformation, induction of early nodulin genes as well as division of root cortical cells leading to nodule formation. The balance of pathways for plant hormones in the roots also controls this organogenetic process. Moreover, turnover of Nod factors by specific plant hydrolytic enzymes seems to be involved in the autoregulation of nodulation.

S08-03

GENETIC ENGINEERING FOR FOOD SECURITY IN THIRD WORLD COUNTRIES. THE CONCEPT: RESCUE HARVESTS LOST TO PESTS.

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The world population increases by 100 mio. per year, predominantly in developing countries. Nearly 50% depend on rice for their basic nutrition. In 25 years 1 billion more rice eaters will need 200 mio. metric tons more of rice harvest. Traditional production systems, plant protection schemes and expansion of agricultural land have reached a plateau. Despite successful resistance breeding and pest control measures, 240 mio. metric tons of rice harvest are lost to biological and physical stress. Genetic engineering offers a chance, to reduce this loss. We develop engineered resistance for pests against which traditional approches have failed. So far, we have achieved sheath blight-resistance (potential 20 mio. tons) and stem borer-resistance (potential 10 mio. tons), we approach Tungro disease-resistance (potential 5 mio. tons). Resistance tests have not only been successful in the lab, but also in the containment greenhouse of our partner, the International Rice Research Institute (IRRI), Philippines. We also approach accumulation of pro-vitamin A in rice seed (134 mio. children suffer from vitamin A-deficiency).

S08-04

INSECT PEST RESISTANCE: YELLOW STEM BORER

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We transformed Indica rice breeding line IR58 with a synthetic version of a truncated *cryIA(b)* gene from *Bacillus thuringiensis* via particle bombardment to immature zygotic embryos. The gene is expressed under control of the CaMV 35S promoter, which allows efficient production of the lepidopteran specific δ-endotoxin in transgenic plants. Stable integration of the transgene could be shown by southern analysis of R_0, R_1 and R_2 generation plants. DNA dot-blot analysis revealed a segregation ratio close to 3:1, indicating the insertion of the *cryIA(b)* gene in a single locus on one chromosome. Protein analysis showed the activity of the transgene in all three generations analyzed so far. The insecticidal effect of the transgenic IR58 plants could be shown by insect bioassays. Feeding studies revealed mortality rates of up to 100% for two of the most destructive insect pests of rice in Asia, the yellow stem borer (*Scirpophaga incertulas*) and the striped stem borer (*Chilo suppressalis*), and feeding inhibition of the two leaffolder species *Cnaphalocrocis medinalis* and *Marasmia patnalis*. Introduction of stem borer resistance into the germplasm of an Indica rice breeding line makes this agronomically important trait available now for conventional rice breeding programs.

S08-05

TOWARDS GENETICALLY ENGINEERED RESISTANCE TO RICE TUNGRO DISEASE

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Rice tungro disease (RTD) is probably the most important viral disease and the major biotic stress for rice in Southeast Asia. No natural resistance against RTD could so far be tranferred to rice breeding lines. We have produced a large number of fertile, transgenic rice plants containing genes which code for rice tungro bacilliform virus (RTBV) proteins, including the viral coat protein, the replicase and a protease. To create mutant proteins that might act as competitive inhibitors for critical viral functions, mutations have been introduced into the coat protein and the reverse transcriptase. In addition, one construct leads to the expression of antisense RNA against the leader sequence of the RTBV pregenomic RNA. For screening the R1-generation of the fertile lines for resistance against RTD, the first 50 lines are currently being tested under natural conditions in the greenhouse at the International rice research institute (IRRI) on the Philippines.

S08-06

TOWARDS GENETICALLY ENGINEERED FUNGUS RESISTANCE FOR RICE

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Fungal diseases of rice cause annual yield losses of about 40 million tons. To supplement the classical methods of fungus control we attempt to increase the defence potential of rice by producing transgenic plants that express one or several proteins with proven or supposed antifungal activity. Rice plants expressing a chitinase already proved to have an increased resistance against *Rhizoctonia solani*, the causative agent of the sheath blight disease [Lin et al., Biotechnology 13, 686-691 (1995)]. We are presently generating more transgenic rice plants plants expressing different chitinases, β-1,3-glucanases, peroxidases, thaumatin-like protein, ribosome inactivating proteins, and others under the control of constitutive and inducible expression signals - either as single genes or as gene combinations. Plants will be tested in the near future for fungus resistance.

S08-07

GENETIC ENGINEERING OF PROVITAMIN A BIOSYNTHESIS IN RICE ENDOSPERM

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Rice is the major food staple for most people in Southeast Asia. Its endosperm, however, completely lacks provitamin A compounds such as β-carotene which are precursors for the production of vitamin A. Thus, many children worldwide, particularly in large areas of Southeast Asia, suffer from a variety of mild to severe health problems resulting from vitamin A deficiency. The goal of our project is to initiate carotenoid biosynthesis in rice endosperm tissue in order to increase the daily provitamin A uptake of those individuals who rely predominantly on rice as a food source. We have produced transgenic rice plants that contain the first of four enzymes necessary to produce β-carotene from a general precursor. We have evidence that these plants are active on molecular and biochemical level and do produce phytoene. Phytoene is the first specific intermediate towards β-carotene, which is not present in untransformed control plants.

S08-08

Modulation by light of GTP-binding on plasma membrane prepared from *Arabidopsis thaliana* leaves.

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Photoperiod and light quality are the most important environmental factors which affect flowering in plants. However, molecular mechanism involved in the floral induction remains unknown. Phytochromes are clearly implicated in this process and they could be linked to some animal like signal transduction system (ie.Inositol phosphate chain).Using an *in vitro* binding assay, a direct effect of red (660 nm) and far-red (730 nm) light on GTP-binding has been tested on purified plasma membrane extracted from *Arabidopsis thaliana* leaves. These two wavelengths modulate the binding in different way. *In vivo* effect of light duration has also been examined, principally during a prolongation of a light period known to induce plants for flowering. The GTP-binding is also clearly modified after 24 h continuous light compared to short day conditions.

S08-09

Ni^{2+} blocks the import of a chimeric precursor protein into chloroplasts of *Chlamydomonas reinhardii*

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Starting with the cloned gene of the precursor of ribulose-1,5-bisphosphate carboxylase (pSS), expression vectors were constructed coding for a fusion protein composed of pSS with the last two amino acids replaced by a C-terminal hexa-histidyl tail ($pSS(His)_6$) and for a fusion protein elongated by dihydrofolate reductase ($pSSDHFR(His)_6$). The import of $pSS(His)_6$ into isolated chloroplasts of *Chlamydomonas reinhardii* could be selectively blocked by the addition of Ni^{2+} and was not processed by chloroplastidial proteases. The protein remained adsorbed at the envelope surface and was completely sensitive to externally added thermolysin. Studies whether the import of $pSSDHFR(His)_6$ can be blocked by Ni^{2+} or by methotrexate and whether import intermediates can be detected that span the chloroplast envelope membranes are in progress.

S08-10

COMPLEMENTATION OF BETALAIN BIOSYNTHESIS IN *P. GRANDIFLORA* BY A FUNGAL DOPA-DIOXYGENASE

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A cDNA encoding Dopa-dioxygenase (Dod) has been isolated from the fungus *Amantia muscaria*. The enzyme catalyses the conversion of DOPA to betalamic acid, the betalain chromophore. Due to its chromogenic properties, the enzyme has many potential applications, including the production of plants with modified flower colours and use as a marker enzyme. To test the feasibility of these applications, the Dod cDNA was cloned in a plant expression vector under the control of a CaMV 35S promoter and a white-flowering variety of the betalain producing plant species *Portulaca grandiflora* was transformed using the particle bombardment technique. Transformation of white petals gave rise to coloured spots that were shown by HPLC to contain naturally occurring betalains, indicating that the fungal DOPA-dioxygenase was expressed and active, and complemented betalain biosynthesis in this plant.

S08-11

TOWARDS GENETICALLY ENGINEERED FUNGUS RESISTANCE FOR WHEAT

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About 250 tons per year of fungicides are consumed in wheat agriculture in Switzerland. This is connected with high costs and a burden for the environment. We attempt to increase the defence potential of wheat against fungi by producing transgenic plants that express one or several proteins with proven or supposed antifungal activity. Similar approaches have been successful with other plants. Transgenic wheat plants expressing combinations of a chitinase and a β-1,3-glucanase or a ribosome inactivating protein or a thaumatin-like protein under the control of constitutive promoters are being generated and will be tested for fungus resistance in the near future.

S08-12

TOPOLOGICAL AND PHOTOSYNTHETIC FUNCTIONAL SIDEDNESS OF PHOSPHOLIPIDS IN SPINACH THYLAKOID MEMBRANE, INSIDE-OUT AND RIGHT-SIDE-OUT VESICLES

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The thylakoid membrane (T) is characterized by a great heterogeneity of its protein and lipid components. The transversal asymmetric distribution of its galactolipids has been determined in intact thylakoids and verified in inside-out vesicles (Siegenthaler et al., FEBS Lett., 228, 94-98, 1988). The aim of this investigation is to verify that the transmembrane distribution of phosphatidylglycerol (PG) in inside-out (I.O.) vesicles is the opposite of that found in T and right-side-out (R.O.) vesicles. To this end, we have isolated I.O. and R.O. thylakoid vesicles by fractionation of thylakoids with the Yeda Press followed by partitioning in an aqueous two-phase system and employed the enzymatic approach to achieve a selective removal of phospholipids, first in the outer and then in the inner monolayer. Depletion was carried out in the presence of phospholipase A_2 from porcine pancreas and of bovine serum albumin (to remove the hydrolysis products). As expected, the molar outer/inner ratios were 66/34 (+/-3) in T, 68/32 (+/-3) in R.O. and 39/61 (+/-5) in I.O. vesicles. Furthermore, the electron flow activity was sustained by the inner PG population in thylakoids and R.O. vesicles and by the outer PG one in I.O. vesicles. As far as the electron flow activity is concerned, our results confirm the occurrence of a topological and functional sidedness of lipids in the thylakoid membrane. Supported by the Swiss National Science Foundation (Nr 31.432 97.95)

S08-13

DIURNAL VARIATIONS IN THE EXPRESSION OF PEROXIDASE GENES OF *SPINACEA OLERACEA*

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Plant peroxidases are reputated to exist as numerous isoforms in a single plant. Molecular cloning of spinach peroxidases using an oligonucleotide probe directed towards a highly conserved domain, has allowed us to characterize eleven different cDNA clones. The spatio-temporal expression of the cloned peroxidases has been investigated by Northern analysis in plants submitted to various physiological conditions (mechanical and hydric stresses, short days vs continuous light). It was observed that at least two isoperoxidases show a diurnal variation at the mRNA level, that can apparently be disturbed by stresses. For both isoperoxidases, the maximum of expression was delayed during the day. Moreover, one of them showed also a transient decrease of expression. We are currently trying to establish whether these variations show circadian rhythmicity, and to what extent they can be perturbed by exogenous factors.

S08-14

GTP BINDING ACTIVITY OF TONOPLAST ENRICHED MEMBRANE FRACTION OF *SPINACIA OLERACERA*

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GTP binding proteins are playing a crucial role as in trafficking membrane processes than in signal transduction in plant. Isolation of tonoplast membrane is realised with sucrose and glycerol gradients. Purity of our membrane extracts are checked with current membrane markers as well as with the presence of phospholipase C, specifically detected on plasmalemma with fluorescent substrate.
Investigations with an antibody raised against the consensus zone of GTP binding, anti-G_{α}Common, show us a positive and specific response with tonoplast membrane. GTP binding assays permit us to characterise global binding of GTP on our membrane preparation. Classical enhancement of this activity with Mas 7 is observed. Furthermore, this activity reveals quantitative and qualitative differences with those from other membrane types.
In vivo light duration effect and *in vitro* hormones actions on GTP binding are investigated.

S08-15

A cDNA CLONE ENCODING AN OSMOTIN-LIKE PROTEIN FROM *ARABIDOPSIS THALIANA*

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Tobacco osmotin and osmotin-like proteins from other plants are believed to play a dual role in osmotic stress and in pathogen defense. Their anti-fungal properties have already been demonstrated *in vitro* an *in planta*. In the frame of a SPP project aiming at the enhancement of potato resistance to late blight, our task is the production of a transgenic line over-expressing the antifungal protein. Due to host-pathogen coevolution, using the native protein in such an approach can be inefficient. We therefore chose to express an osmotin-like protein from a distantly-related species. We report here the isolation and sequence determination of a full-length cDNA clone for an osmotin-like protein from Arabidopsis. The cDNA library was screened with an oligonucleotide probe derived from a conserved region. The encoded peptide shows all the features of the known osmotin-like preproproteins. The highest homology score was found with a soybean protein.

S08-16

PROTEINASE INHIBITORS FOR PRODUCTION OF TRANSGENIC PLANTS TOLERANT TO INSECT PESTS.

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Transformation of potato with the cysteine proteinase inhibitor oryzacystatin I gene (OCI) was considered for the control of the insect pest Colorado potato beetle (CPB). *In vitro* assays showed that 80% of the CPB proteases are of cysteine type and 60% of them are inhibited by oryzacystatins. Complementary studies showed an apparent absence of direct interference between OCs and potato proteases. OCI expressed in potato plant seemed to decrease the CPB growth. But, one problem may come from the high adaptation potential of these insects. The proteolytic pattern in CPB fed with three different diets indicated quantitative and qualitative proteolytic changes in response to the ingested diet. An other problem may come from the potential risks of interference with beneficial insects. *In vitro* assays on digestive proteinases of a natural predator of the CPB, showed a dramatic inhibition by OC. Despite these possible limitations, studies suggest the real potential of PI in insect-tolerant transgenic plants.

S08-17

A *SYNAPTOSOMAL*-ASSOCIATED PROTEIN HOMOLOG INVOLVED IN INTRACELLULAR TRANSPORT IN *ARABIDOPSIS THALIANA* ?

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Proteins to be transported to the plasma membrane through the secretory pathway travel from the ER to the Golgi apparatus before they reach the plasma membrane. The transport of these proteins is mediated by vesicles.

In order to study the mechanisms involved in the targeting of such vesicles in plants, we have searched for plant homologs of SNAREs. SNAREs seem to be responsible for the specific delivery of vesicles to their target membrane.

We have identified an *Arabidopsis thaliana* homolog of SNAP-25, a neuronal plasma membrane t-SNARE. Expression pattern of AtSNAP-25 in *A. thaliana* will be shown.

S08-18

PLASTID ENGINEERING IN CROP PLANTS

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Genetic engineering has become a routine technique for many crops. In view of the paramount importance of cereals as a source for food and feed, it is expected that in the close future the number of field trials with transgenic cereals and the permissions for their commercialization will increase substantially, leading to widespread, unrestricted release of transgenic plants. By then, vertical gene transfer from transgenic crops to related crops or weeds via pollen becomes a major biological safety concern. Traits that provide a selective advantage in an agricultural ecosystem must not be transmitted to related species in order to avoid the creation of new weeds. Research on biosafety of transgenic crops has to be directed towards the bio-containment of the genes encoding such traits. In most agriculturally important species, plastids are transmitted to offspring generations in an uniparental maternal manner, i.e. the plastomes are not transmitted via pollen. As a strategy for the bio-containment of transgenes, we propose to integrate foreign genes into the plastome rather than the nuclear genome of cereal crops, and we started to transfer the plastome engineering technology from tobacco to the monocot crop rice.

S08-19

POTATO GENES ACTIVATED BY AN INFECTION WITH *PHYTOPHTHORA INFESTANS*.

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Three potato genes have been isolated by screening a cDNA library with tobacco clones whose corresponding mRNAs accumulate during the hypersensitive reaction of tobacco leaves infiltrated with an incompatible strain of *Pseudomonas solanacearum*. Those three genes belong to two families. The first gene shows high homologies with cytochrome P-450. The best homology is found with a putative 4-cinamate hydroxylase characterised in Avocado.

The two other genes are 83% similar but they do not show any significant homologies with any gene present in data bases. Nevertheless, the expression of these two genes is different: while the first one is constitutively expressed, the second one exhibits a maximum in induction 14 hours after inoculation with *P.infestans*.

S08-20

RESPONSE OF BARLEY TO POWDERY MILDEW INFECTION : ARE SALICYLIC ACID AND H_2O_2 INVOLVED ?

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Infection of a susceptible barley cultivar with *Erysiphe graminis* f.sp.*hordei* (*Egh*) led to accumulation of extracellular PR proteins. PR proteins also accumulated in barley leaves after infection with a non-pathogen, *Erysiphe graminis* f.sp.*tritici* (*Egt*) after 100mM H_2O_2, but not after 10mM salicylic acid (SA) treatment. Infection with *Egh* did not lead to SA increase. Active oxygen species (AOS) could be generated after infection with *Egh*. A protein of 22KD disappeared after infection with *Egh*, after treatment with 100mM H_2O_2, or after a heat-treatment (HT) of 60 seconds in water at 50°C. HT led to protection of barley against *Egh*. Total glutathione level was increased by H_2O_2 and HT, but was not affected by *Egh* infection. Nevertheless, HT did not induce PR proteins and even delayed their accumulation after infection. The links between protection after HT, PR proteins, AOS and SA will be discussed.

S08-21

DEGRADATION OF PHOSPHORIBULOKINASE: INFLUENCE OF THE ACTIVATION STATUS AND OF ATP

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The light-regulated chloroplast enzyme phosphoribulokinase (PRK) exists in two forms. In darkness this enzyme is present in an oxidized form, which is inactive. It is activated in the light by a thioredoxin-mediated reduction. In extracts PRK can be activated by the artificial reductant dithiothreitol (DTT). The influence of the activation status and of ligands on PRK stability in presence of endogenous (extract from senescing wheat leaves) and exogenous (purified trypsin and chymotrypsin) endopeptidases was detected by immunoblotting and activity measurements. Both methods led to similar conclusions. DTT (artificial reductant) and ATP (substrate) stabilized PRK in wheat leaf extracts as well as partially purified PRK from spinach. The combination of DTT and ATP protected PRK from degradation to a greater extent than either effector alone. Our results suggest that the activation status and substrate concentrations are not only important for the activity of PRK, but are also relevant for the susceptibility of this protein to proteolysis.

S08-22

SYSTEMIC GENE EXPRESSION IN CUCUMBER LEAVES

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After infection by a necrotizing pathogen, often plants develop resistance to pathogens both locally at the site of infection and systemically in tissues distally located from the site of initial infection. An increase in the expression of a number of genes was found to correlate with this induced resistance. However, very little is known about changes that occur early after pathogen attack.
The goal of this work is to look for genes that are expressed early in the induction process leading to induced resistance. This study of gene expression may help to unravel the processes involved in the establishment or maintenance of local and systemic induced resistance. For this analysis, we used polymerase chain reaction (PCR) differential display of mRNAs expressed after infection in the non infected resistant part of an infected cucumber leaf.

S08-23

Biotransformation of sulfonated aromatic compounds by plant cells

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Anionic detergents and dyestuffs, their byproducts and intermediates of production are the main sources of sulfonated aromatic pollutants, rather recalcitrant to biodegradation. The steric and polar properties of sulfonate part are maily responsible for recalcitrance, but microbial degradation of different sulfonoaromatics is possible. The purpose of the present research was to investigate the fate of several sulfonated compounds by cells isolated from plants known to possess enzymes likely to cope with. Rhubarb cells were cultivated in shake flasks or in bioreactor in the presence of anthraquinones possessing one or two sulfonate groups in different positions. Cells were separated from growth medium and filtrate was analyzed by capillary electrophoresis, HPLC and spectrophotometry for its content in the parent compound and metabolites derived from it. Large differences observed between compounds of the same family were interesting from a biochemical point of view and could be of great significance for environmental applications. In the future, immobilized plant cells could be used for the treatment of industrial effluents, whereas whole plants grown in the field could help to decontaminate polluted soils or groundwaters.

S08-24

A PHENYLALANINE AMMONIA-LYASE FROM MAIZE HAS ALSO TYROSINE AMMONIA-LYASE ACTIVITY.

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A full-length cDNA encoding phenylalanine ammonia-lyase (PAL) from maize was isolated. A fusion protein with glutathion *S*-transferase (GST) was expressed in *E. coli*, purified, the GST moiety cleaved off and the resulting PAL enzyme analyzed. In contrast to PAL from dicot plants, the maize PAL catalyzed not only the deamination of phenylalanine to cinnamic acid but also converted tyrosine to *p*-coumaric acid (TAL activity). This is the unequivocal proof that PAL and TAL activities reside in the same polypeptide. The physiological implications of this additional activity will be discussed.

S08-25

INTERACTIONS OF SOME PLANT PROTEINS WITH PECTINS

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A small number of proteins from zucchini hypocotyls bind to polygalacturonic acid (PGA) or pectins, provided these latter are not highly esterified. The binding occurs only in the presence of calcium which induces the "egg-box" conformation of PGA or pectins. It has been demonstrated by co-sedimentation of proteins and calcium-pectate gel upon centrifugation, by gel filtration, by assays in wells of a micro-titration plate, or by affinity chromatography through a column filled with a PGA/polyacrylamide gel. In each case, proteins attached to the Ca^{2+}-pectate gel may be released by Ca^{2+} withdrawal with EGTA or by ionic strength increase with NaCl. The effect of NaCl indicates that the binding is based on ionic interactions, but as isolated highly anionic PGA chains do not retain proteins in the absence of Ca^{2+}, a recognition phenomenon between Ca^{2+}-pectate gel and the binding proteins is likely to exist. In addition, some of these proteins are anionic and should not be attracted by PGA. Chemical modifications of proteins have shown that lysine and/or arginine residues are involved in the binding mechanism of both anionic and cationic proteins.

S08-26

ANALYSIS OF THE ORGAN-SPECIFIC AND ELICITOR-INDUCED EXPRESSION OF A 3-DEHYDROQUINATE SYNTHASE GENE IN TOMATO.

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cDNA clones for all enzymes of the prechorismate pathway of higher plants have previously been cloned in our laboratory, with the exception of the second enzyme of the pathway, 3-dehydroquinate synthase (DHQS). Here we describe the isolation of a cDNA encoding a DHQS from tomato which was identified by complementing a DHQS-deficient *E. coli* strain with a tomato cDNA library. The deduced amino acid sequence contains a putative N-terminal plastid-specific transit peptide. The abundance of DHQS-specific transcripts differs in the organs of tomato plants analyzed, and in cultured tomato cells transcript levels increased 9-fold within 4 to 5 hrs of elicitor treatment.

S08-27

FLORAL STEM EXTENSION RATE MEASUREMENT IN *ARABIDOPSIS THALIANA*

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Floral stem extension rate (FSER) of *Arabidopsis thaliana* (Line Landsberg erecta) was measured with a very sensitive computer assisted auxanometer. The installation uses linear voltage differential transformers (LVDTs) as sensors. It monitors the growth of up to 12 plants together with light, temperature and relative humidity. During long-day light (16:8 L:D) FSER fluctuates considerably over the whole growth span. During light exposure SFER was higher than in dark periods. In continuous light exposure succesive periods of low and high SFER were also observed.

S08-28

PURIFICATION AND PARTIAL CHARACTERIZATION OF SOYBEAN GLYOXYSOMAL CITRATE SYNTHASE

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The conversion of fatty acids to carbohydrates in germinating oilseeds involves various cellular compartments: glyoxysomes (ß-oxydation and glyoxylate cycle), mitochondria (citric acid cycle) and cytosol (gluconeogenesis). The glyoxylate and the citric acid cycles share the citrate synthase, aconitase, and malate dehydrogenase activities. Citrate synthase (CS, EC 4.1.3.7) catalyzes the condensation of oxaloacetate and acetyl-CoA to form citrate and CoA. In soybean (*Glycine max*. L.), CS has glyoxysomal and mitochondrial isoforms (gCS and mCS) encoded by different genes.
gCS is induced in cotyledons at germination, disappears when glyoxysomes are converted into peroxisomes at the onset of photosynthesis, and is apparently reinduced at senescence. gCS and mCS can be differentiated by their optimum activities at different pHs, and by the aggregation/deaggregation behavior characterizing gCS (first purification step). Purification of gCS is then pursued using hydroxylapatite and hydrophobic interactions chromatographies. Kinetic studies demonstrated that gCS is significantly inhibited by DTNB (the disulfide compound used to monitor CoA evolution), and this inhibition is hysteretic (*i.e.* it develops gradually). This phenomenon may be indicative of a general regulatory property of the enzyme.

S08-29

RECONSIDERATION OF THE ROLE OF THE 20S PROTEASOME IN THE DEGRADATION OF MULTIUBIQUITIN-PROTEIN CONJUGATES

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The 26S proteasome is involved in the disassembly of proteins tagged with multiubiquitin chains. Many of the biochemical steps of this fundamental process are known, but less is known about how the ubiquitin-protein conjugates are recognized by the proteolytic complex and the fate of the multiubiquitin chain. Because attempts to purify the 26S proteasome of the lower plant *Physcomitrella patens* were unsuccessful, we carried out experiments to determine whether the moss 20S proteasome also exerts its proteolytic activity as an independent free proteolytic complex in the degradation of ubiquitin-protein conjugates. We find that the 20S proteasome of moss as well as human, unlike *T. acidophilum*, proteolyze the multiubiquitin.K48 chains and cleave the peptide bond at the ubiquitin-protein junction of UbCEP52, in a reaction that does not require ATP, releasing functional ubiquitin. Electron microscopy of ice-embedded proteasomes during the process of degradation revealed the central channel of many particles is filled suggesting that the multiubiquitin chain is positioned in the central channel of the proteasome. In addition, we show that subunit 1 of both moss and human 20S proteasome binds multiubiquitin chains. We conclude that the 20S proteasome plays a novel and essential role in the degradation of ubiquitinated proteins.

S08-30

INDUCTION OF MALATE SYNTHASE (EC 4.1.3.2) IN SENESCING NODULES OF SOYBEAN

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Senescence in higher plants may be defined as the series of events leading to the organized disassembly of biological functions at various levels, from individual cells or specific organs to the entire plant.
The glyoxylate cycle, which directs the carbon flow from β-oxidation to gluconeogenesis, is (re)activated in senescing organs such as cotyledons, leaves, roots and petals. Isocitrate lyase (ICL) and malate synthase (MS), two enzymes specific to this cycle, were therefore chosen in order to study signals controlling senescence in symbiotic nodules.
Soybean plants were infected with *Bradyrhizobium japonicum* 110 and used 20 days after inoculation. Senescence was induced in the nodules reversibly (*via* dark treatment of the whole plant) or transiently (by removal of 90% of leaf material). ICL and MS abundances and activities were detected using antibodies that recognize the plant ICL and MS (but not the bacterial enzymes), as well as by enzymatic assays.
MS activity shows dramatic increases 3 d after forage or 3-6 d after dark treatment, whereas ICL activity was not significantly affected. A novel approach is now to equate nodule senescence with a plant defense mechanism, using bacterial mutants.

S08-31

A CYTOSOLIC ACONITASE PARTICIPATES IN THE GLYOXYLATE CYCLE AT GERMINATION IN SOYBEAN COTYLEDONS.

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Aconitase (EC 4.2.1.3) is known as an enzyme of the Krebs and glyoxylate cycles (occurring in mitochondria and glyoxysomes, respectively). Localization and developmental changes of aconitase isoforms have now been analyzed throughout germination in soybean cotyledons (*Glycine max.* L.) using sucrose gradients (to purify organelles) and agarose zymograms (to differentiate the multiple isoforms).
No glyoxysomal aconitase activity is detected in cotyledons at germination even though all the other enzymes of the glyoxylate cycle are located in glyoxysomes.
Five isoforms of aconitase are identified on zymograms. Two of them are of mitochondrial origin, whereas the other three forms seem to be cytosolic. One of the cytosolic isoform appears during germination and gradually disappears in cotyledons on the onset of photosynthesis. These data therefore suggest that a cytosolic aconitase participates in the glyoxylate cycle during germination concurrently with four other enzymes strictly located in glyoxysomes.

S08-32

LOCALIZATION OF SULFATE ASSIMILATION IN MAIZE

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The intercellular distribution of assimilatory sulfate reduction and glutathione synthesis between mesophyll (MC) and bundle sheath cells (BSC) was analyzed in maize leaves. Cross-contamination of the cell preparations were determined by quantification of marker enzymes. The enzymes catalyzing the first steps of assimilatory sulfate reduction are exclusively present in the bundle sheath cells. Tracer experiments with ^{35}S indicate cysteine being the transport metabolite between BSC and MC.

S08-33

TISSUE-SPECIFIC ACTIVITY OF ADENYLATE KINASE IN TOBACCO

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Adenylate kinase (ATP:AMP phosphotransferase) is a key-enzyme of energy metabolism involved in the interconversion of adenylate nucleotides. In spite of this physiological importance, its localization in plant tissues is largely unknown. Tissue-distribution of adenylate kinase activity in rigid tobacco tissues was examined with the tissue blot technique and native activity staining. Adenylate kinase activity was found to be highly restricted to specific tissues. In callus cultures, high activity was correlated with green and growing tissues, situated in distinct cell aggregations or cell layers near the callus surface (*J. Plant Physiol.* 144: 400-409, 1994). In stems of tobacco plants, activity was restricted to the vascular bundle, especially cambium and phloem. These results suggest that high adenylate kinase activity in plants is, as in vertebrates, related to a high energy turnover. They also indicate that the AK reaction in plants could be involved in high energy phosphoryl transfer as recently proposed for AK in vertebrate tissues (*J. Biol. Chem.* 270: 7311-7319, 1995).

S08-34

AMINO ACID SEQUENCE OF THE VARIABLE SUBUNIT OF MAIZE FERREDOXIN:THIOREDOXIN REDUCTASE

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The complete amino acid sequence of the variable subunit of maize (*Zea mays*) ferredoxin:thioredoxin reductase (FTR) was established by protein sequencing. This subunit is a 97 residue peptide with a Mr of 10,939. A comparison with the sequences of the variable subunits from *Anacystis nidulans* and spinach reveals the presence of three homologous domains, one near the N-terminus, one in the middle of the subunit and one near the C-terminus. Two of these domains contain positive charges whereas the C-terminal domain is mainly negatively charged. Results suggesting the involvement of the N-terminal domain in the interaction with the catalytic subunit will be presented.
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S08-35

GENE TRANSFER TO RECALCITRANT WHEAT CULTIVARS

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By far most of the chemical fungicide spraying in Switzerland is used to control fungal pathogens in wheat. Therefore, improved fungal resistance in wheat contributes to sustainable agriculture in Switzerland. Genes which improved fungal resistance already in cereals are available, like chitinase. Therefore gene transfer to Swiss wheat cultivars would be the approach of choice. However, gene transfer to wheat, which was published in highly regenerable model varieties, is still far from routine. Currently no publication is available reporting transfer of an agronomical important gene into an agronomical important wheat cultivar. All the important Swiss cultivars which we tested proved to be recalcitrant to regeneration. We report gene transfer to a wheat variety, which has a comparably low regeneration potential than the Swiss cultivars. This paves the way to improve elite wheat in Switzerland and in collaboration with CIMMYT also for sustainable agriculture in developing countries.

S08-36

THE SYNTHESIS OF SALICYLIC ACID AND SYSTEMIC ACQUIRED RESISTANCE IN POTATO

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Spraying potato leaves with arachidonic acid (AA) at 1500 ppm gives rise to systemic acquired resistance to *Phytophthora infestans* and *Alternaria solani*. Rapid local synthesis of salicylic acid (SA) and accumulation of a SA conjugate, that was shown to be 2-*O*-β-glucopyranosylsalicylic, acid occurs. Radiolabelling studies with untreated leaves showed that SA was synthesized from Phe and that both cinnamic and benzoic acid were intermediates in the biosynthesis pathway. Using radiolabelled phenylalanine as precurseur, the specific activity of SA was found to be lower when leaves were treated with AA than in control leaves suggesting a second pathway which did not directly involve phenylalanine. Similar results were obtained when leaves were fed with the labelled putative intermediates, cinnamic acid and benzoic acid. Application of 2-aminoindan-2-phosphonic acid (AIP) at 40μM, an inhibitor of PAL, prior to treatment with AA reduced the local accumulation of salicylic acid and inhibited the incorporation of radioactivity from Phe into salicylic acid and its conjugate. When the putative intermediates were applied to leaves, instead of Phe, in the presence of AIP acid about 40% of the expected accumulation of free salicylic acid was recovered, but the amount of the conjugate remained constant.