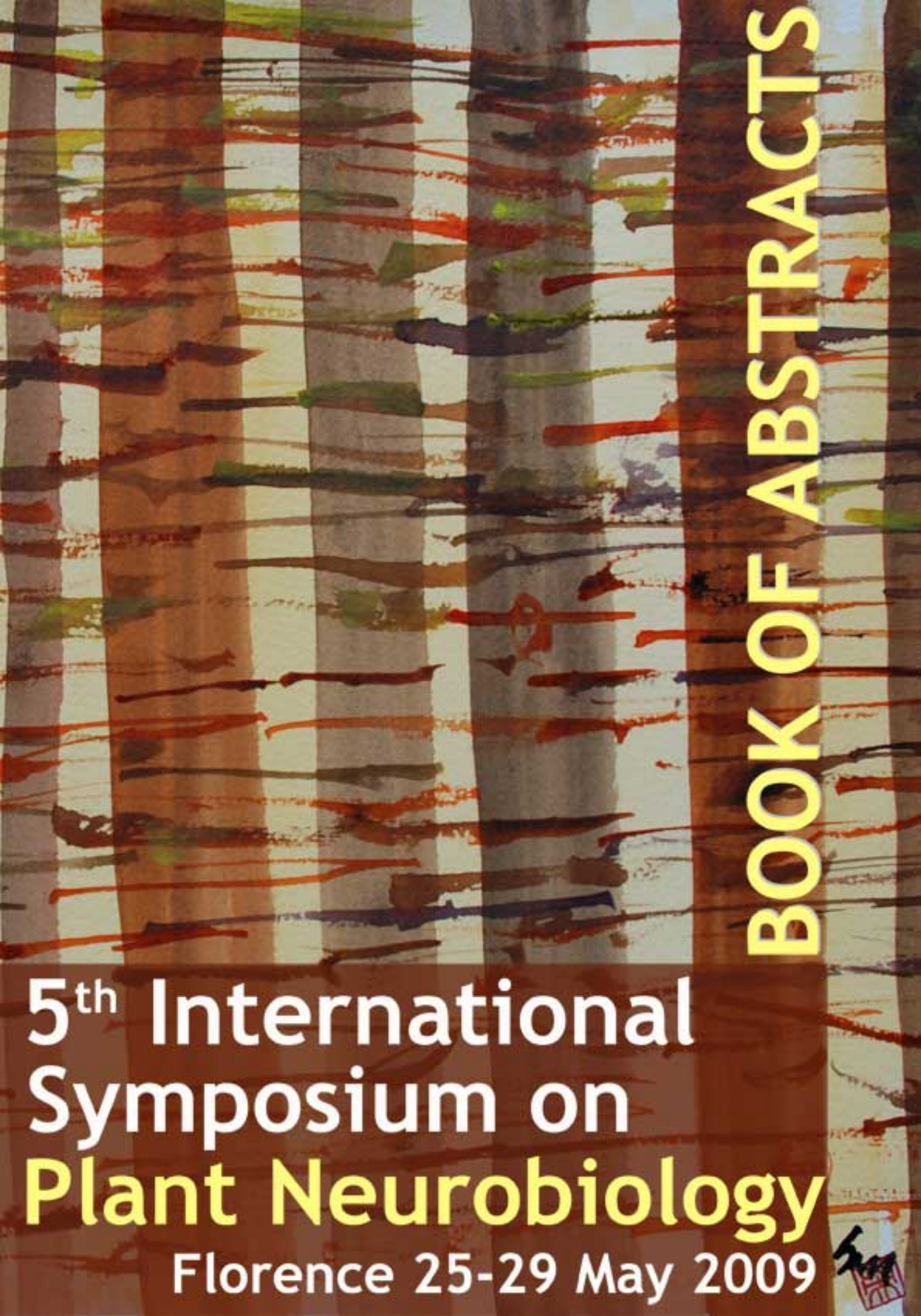
BOOK OF ABSTRACTS
5th International Symposium on
Plant Neurobiology
Florence 25-29 May 2009

Proceedings

5th Symposium on Plant Neurobiology

25 – 29 May 2009
Florence – Italy

Programme

Monday 25th May

11:30 – 14:00		**Registration**
14:30 – 15:00	**Stefano Mancuso, Liz Van Volkenburgh**	**Opening and welcome addresses**
15:00 - 15:40	**Frantisek Baluska**	Animal-like behavior in plants: avoidance and escape tropisms of roots
15:40 – 16:20	**Peter Barlow**	The influence of the lunar-solar tidal acceleration on trees gives a glimpse of how the plant-neurobiological system came into being
16:20 – 17:00	**Markus Geisler**	Regulation of the auxin export complex
18:00 - 20:00		**Welcome drink**

Tuesday 26th May

9:30 – 10:10	**Aart Van Bel**	Forisomes: molecular mammoths unveil calcium-associated sieve-element biology
10:10 - 10:40	**Clement Thomas**	Plant LIMs: simple proteins, intricate functions
10:40-11:10		**Coffee break**
11:10 - 11:50	**Miguel Botella**	The role of plant synaptotagmins in plasma membrane integrity and cell survival
11:50 - 12:20	**Narendra Tuteja**	Cloning and identification of a novel function of pea lectin receptor-like kinase in salinity stress tolerance
12:20 - 12:50	**Luciana Renna**	AGD5, an ARF-GAP, interacts with ARF1 GTPase at the Trans-Golgi Network
12:50 - 14:30		**Lunch**
14:30 - 15:10	**Tomonori Kawano**	Development of model peptides as the research tools for studying the dynamic signaling events in living plant cells
15:10 - 15:40	**Axel Mithofer**	Plant-insect interactions: General aspects and dissection of mechanical and chemical challenges
15:40 - 16:10	**Haiyun Ren**	AtFH8, an actin filament nucleator and bundler, has a relationship with root development in arabidopsis
16:10 - 16:40		**Coffee break**

16:40 - 17:20	**Manuela Giovannetti**	Flow of nutrients and information in mycorrhizal networks
17:20 - 17:50	**Ton Timmers**	Cellular mechanisms and signal transduction associated with endosymbiotic root infection

Wednesday 27th May

9:30 -10:10	**Sergey Shabala**	GORK and NSCC channels as components of salinity tolerance mechanism in plants
10:10 - 10:40	**In Sun Yoon**	Sucrose nonfermenting1 (SNF1)-related protein kinase 2 (SnRK2) family function in hyper-osmotic stress signaling of rice
10:40-11:10		**Coffee break**
11:10 - 11:30	**Beom-gi Kim**	Na^+ measurements using a fluorescent dye in plant
11:30 - 12:00	**Veronique Bergougnoux**	The 7B-1 mutation in tomato confers a blue light-specific lower sensitivity to coronatine
12:00 – 12:30	**Maria-Carmen Risueno**	Changes in cell wall polymers and pectin methyl esterase expression are developmentally regulated during pollen maturation and embryogenesis
12:30 – 12:50	**Jinxing Lin**	Actin turnover is required for myosin-dependent mitochondrial movements in Arabidopsis root hairs
12:50 - 14:30		**Lunch**
14:30 - 16:00		**Poster session**

Thursday 28th May

9:30 – 10:10	**Rainer Hedrich**	Sensory transduction in guard cell
10:10 - 10:40	**Mathias Rudi Zimmerman**	System potentials, a novel electrical long distance apoplastic signal in plants induced by wounding
10:40-11:10		**Coffee break**
11:10 - 11:50	**Patrick H. Masson**	WDL proteins control root growth behavior and anisotropic cell expansion in Arabidopsis
11:50 - 12:20	**Paul Jarvis**	Genetic analysis of the protein import machinery of Arabidopsis plastids
12:20 - 12:50	**Vladimira Hlavackova**	Both electrical and chemical signals may act in a triggering of rapid systemic responses of tobacco or tomato plants to local burning

12:50 - 14:30		**Lunch**
14:30 - 15:10	**Bettina Hause**	Jasmonates functions in symbiotic interactions and plant response to wounding
15:10 - 15:40	**Cristian Mazars**	Nuclear calcium signaling in plant cells
15:40 - 16:10	**Doreen Schachtschabel**	Apocarotenoids - signaling compounds of Zygomycetes and plants?
16:10-16:40		**Coffee break**
16:40 - 17:10	**Lorella Navazio**	Sensing plant symbiotic signals by nitrogen-fixing bacteria
17:10 - 17:40	**Yoko Nakamura**	Chemical Biology of Leaf-movement of *Albizzia saman*
20:00		**Gala dinner**

Friday 29th May

9:30 -10:10	**Mary Beilby**	Membrane potential fluctuations in *Chara australis*: a characteristic signature of high external sodium.
10:10 - 10:40	**Francois Bouteau**	Anion channel activation is an early event in ozone-induced cell death in Arabidopsis cell suspension
10:40-11:10		**Coffee break**
11:10 - 11:50	**Fernando Migliaccio**	Arabidopsis root movements and symmetry
11:50 - 12:20	**Mark Staves**	Increasing the density of the external medium inhibits and reverses root gravitropism
12:20 – 12:50	**Joseph Neumann**	Panpsychism - past and present
12:50 - 13:00		**Closing of the meeting**
13:00 – 14:30		**Lunch**

ABSTRACTS

Oral Presentation

Poster

Animal-Like Behavior in Plants: Avoidance and Escape Tropisms of Roots

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In the last two-three decades, plants have been demasked as very sensitive organisms, monitoring and integrating immense amount of abiotic and biotic parametres from their environment. Plants are unique as their development and morphogenesis are plastic throughout their life. Plants retrieve properties of their environment via sensory perceptions which are critical for their survival. Especially light and gravity are essential in this respect. Plants seem to actively experience environment and can both store and retrieve memories in order to drive active life-style evident especially in growing roots. There are several critical situations requiring 'centralized' decisions in growing roots, such as, for instance, search for water and avoidance of dangerous soil patches. Although these root decisions are based on information retrieved preferentially at the root cap, they imply some central 'processor' which would reliably control the root tropisms. Importantly, any wrong decision at root apices would have detrimental consequences for the whole plant.
Recently, dramatic salt-induced modification of root growth direction has been reported which represent new salt-avoidance tropism of root apices. This salt-avoidance behavior of growing root apices might represent an active adaptive mechanism for roots approaching saline areas. Similarly as in the root gravi- and photo-tropisms, polar auxin transport and PIN2 auxin exporter play the central role in this new type of root tropism. It was also shown that root apices recognize in advance dangerous substrate (soil) patches having high aluminium content and avoid them using similar active avoidance root tropism. Interestingly in this respect, root apices exposed to light have been reported to increase their growth rate in association with more active role of PIN2 in driving auxin transport. These responses are mediated by blue-light receptor PHOT1 which is localized to PIN2-enriched synaptic domains in root cortex cells. As roots are evolutionarilly optimised for exploitation of the dark environment in soil, this blue light induced root escape tropism emerge as serious factor affecting *in vivo* studies using Arabidopsis roots in confocal microscopy. All authors using living roots in confocal microscopy should be aware of this phenomenon and keep this in mind when intrerpreting their data obtained *in vivo*.
Our recent study revealed that neuronal molecules like synaptotagmins are relevant for coping of plant cells with salt stress and these proteins are relevant also for adaptation to cold stress. All these reports document that plant roots have a fine and sophisticated sensory and communicative systems that enable them to dynamically and efficiently cope with rapidly changing environment via animal-like active behavior.

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The influence of the lunar-solar tidal acceleration on trees gives a glimpse of how the plant-neurobiological system came into being

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"The Universe in which we find ourselves and from which we cannot be separated is a place of Law and Order. It is not an accident, nor chaos. It is organized and maintained by an Electro-dynamic Field capable of determining the position and movement of all charged particles." H. S. Burr

Two separate and independent systems in plants appear to be affected by the lunar-solar tidal acceleration, δg. The first is the bean leaf (of Phaseolus spp., Canavalia ensiformis) whose 'sleep', or nastic, movements inspired the idea of an endogenous physiological 'clock'. The dilatation cycles (diurnal variations of trunk diameter, δDi) of tree trunks and branches comprise the second system; these cycles have also provoked controversy as to whether or not they are governed by δg (Zürcher et al. 1998).

The time courses of the leaf movements of bean plants grown in darkness were recorded in the 1990s by the late Dr Gunter Klein. He proposed that the initiation of rapid upward or downward movements of leaves occurred at times when δg was changing from low to high, or vice versa (Klein 2007). In order to place Klein's proposal on a firmer footing, we re-examined his original and unpublished data and used the statistical methods of cosinor analysis and cross-'correlation' in conjunction with estimates of δg, derived from a geophysical-mathematical program, as they applied to the geographical location and times when leaf movements were recorded (Barlow et al., 2008). Other data were extracted from published literature (notably results obtained by Anthonia Kleinhoonte in the 1930s) in which individual leaf movements (i.e., not averaged results) were recorded at specified times, dates and location (Barlow 2007). In all these cases, temporal leaf turning points coincided with temporal turning points of δg.

Published time courses of δDi for trunks of Picea abies (Zürcher et al. 1998) were examined, also with the aid of statistical methodologies (Barlow et al. 2009a, b). The varying values of of δDi of trees placed in darkness were positively correlated with the pattern of δg (the paper of Zürcher et al. 1998 contains an erroneous conclusion in this respect). Analogous data for δDi from trunks of other species of trees showed similar correlations with δg. Illumination seemed to regulate the dilatation cycle, apparently over-riding the effect of lunar-solar gravity. Other physiological features that seem to be affected by lunar gravity are the onset and termination of sapflow, transpiration, and the related rapid rises and falls of electrical potential. All data sources are given in Barlow et al. (2009b).

The conclusion drawn from the observations on the two mentioned biological systems is that when plants are screened from 'strong', solar-driven environmental influences, 'weak', lunar gravitational forces can be perceived and responses initiated. In the particular cases discussed, the lunar tidal accelerations probably regulate hydrostatic pressures within the plant. As commented by Harold Saxton Burr (1945), Professor of Anatomy at Yale University and one of the pioneers of plant bioelectricity, "... since growth in trees is in part a matter of hydration, it may eventually turn out that the effect of the lunar cycle on the growth of the tree is as direct as on the tide level". How do these conclusions impinge upon 'Plant Neurobiology'? Burr's comments above may give us a clue. First, according to the data sets which we analysed, sapflow follows changes in the electrical potential of the young secondary xylem. Second, the stem dilatation cycle seems to be brought about by the rhythmic concertina-like changes to the radial structure of the secondary phloem (Zweifel et al. 2000). These changes could affect, also in a rhythmical manner, the internal pressures within the secondary phloem. As Fensom et al. (1994) have proposed, pressure waves in the phloem might regulate the movements of H+ and K+ in and out of the sieve elements and companion cells. Pressure waves initiated in the phloem may also be transmitted to the secondary xylem via the rays and thereby

initiate electrical signalling. And for certain simple algae, Bisson et al. (2006) have reviewed the variations in the electrical potentials of their protoplasts which can be induced by turgor changes (i.e. variations in pressure exerted upon the cytoplast membrane). The variation δDi in trees, driven by δg, may therefore provide a constant and ever-present internal physiological condition by means of which electrical potentials are supported and caused to exhibit rhythms. Cycles of δDi may also be linked with sapflow via internal water movements in the manner proposed by De Pauw et al. (2008) and, indeed, this process may even be re-inforced by the action of 'muscular' groups of cells in the bark (Holdheide 1951), such as the phloem fibre sclereids (in Populus, e.g.). Explorations have also begun of the correlations between certain geomagnetic indices (Thule index and Disturbance storm index) and δg, and their link with sapflow. It could be that the lunar tidal acceleration δg is important for 'focussing' geomagnetic fluxes upon the Earth's surface and these fluxes can be perceived by living systems. The effect of the aurora borealis on plant growth is suggestive in this respect [see Lodge (1908) reporting on the work of Karl Selim Lemström].

Perhaps it is not fortuitous that the relationships between sapflow, stem dilatation and electrical potential have been discovered in trees! And it may even be safe to speculate that an autonomic plant 'neural' system for the transmission of action potentials developed in the giant progymnosperms which appeared in the mid-Devonian era, 374 million years BP. Before this time, vascular cambium, if present at all, was unifacial and produced secondary xylem only; and this xylem may or may not have possessed electrical variation potentials. However, the progymnosperms (e.g., Triloboxylon hallii) developed a bifacial cambium and were therefore a new cell type – the secondary phloem – could be produced (Scheckler and Banks 1971). Besides enabling more sugars from photosynthesis to be transported between the extremities of the plant, we can speculate that secondary phloem became the prototypic neural channel of plants due to the electrical properties inherent to cells of this type. The development of a 'neural' channel might also have been an evolutionary adaptation to the increasing dryness of the primitive soil and provided the means of communicating stress signals which, in the form of action potentials passing from the roots to more remote regions of the plant, became more critical than would have been the case in earlier epochs when the equally large, tree-like Lycopsids flourished in wetter and more steamy environments.

As mentioned, the tidal force due to the Moon has been ever-present, even during those earlier epochs when sunlight to the planet was blotted out by clouds of volcanic ash, or when persistent clouds deposited the ages of ice and snow upon the Earth. Life on Earth – ranging from the most ancient archaic forms to the forms of today – evolved always and everywhere in the company of lunar-solar tidal acceleration, δg. Besides the 'gift' of a neural system from the Moon to plant life, another gift from the Moon is the ability of organisms to keep time – viz. the clock-like bean leaf movements – and maybe also the ability to anticipate events within the flow of time. Perception of the cosmic lunar δg rhythm has become a fundamental feature of living systems, and has perhaps also become internally assimilated into their physiology. We propose that to the five Aristotelian 'senses' should be added the 'sensing of the passage of time'.

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Regulation of the auxin export complex

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Cellular efflux provided by the independent and interactive action of ABCB/PGP- and PIN catalysts is the rate-limiting step of polar auxin transport.
Here, we summarize recent progress of ABCB interaction with immunophilin-like FKBP42, TWISTED DWARF1 (TWD1).
Using yeast and in planta BRET (bioluminescent resonance energy transfer) assays, we show that ABCB1-TWD1 interaction is disrupted by synthetic and native auxin transport inhibitors, like NPA and quercetin, leading to inactivation of ABCB1. NPA binds to ABCBs but surprisingly also to the N-terminus of TWD1. As a consequence, auxin fluxes and gravitropism of twd1 roots are NPA insensitive while gain-of-function alleles perform faster bending kinetics.
Our data demonstrate that the TWD1 and ABCB1 are key components of the NPA-binding protein complex. Moreover, we suggest a protein-protein interaction feedback loop building the basis for the establishment and control of plastic asymmetric auxin fluxes.

Forisomes: molecular mammoths unveil calcium-associated sieve-element biology

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Cutting paradermal windows in leaf veins of *Vicia faba* allowed in vivo observation of events in intact sieve elements (SEs). In SEs, we found contractile protein bodies (forisomes) of considerable size (10-100 microns in length), which disperse in response to Ca^{2+} supply and re-contract after Ca^{2+} withdrawal. Forisome genes are exclusively expressed in the SE/CC precursors of legumes. Forisomes may possess nanobiological potential as shown by their behaviour in artificial silicate-based sieve tubes. Forisomes have further been used as in vitro indicators for Ca^{2+}-binding capacity of aqueous saliva. Their calcium-binding proteins appear to prevent sieve-tube occlusion in response to wounding (inflicted by insertion of the stylet) and, hence, help to maintain food supply. Presence of forisomes enabled to identify and select SE protoplasts for physiological tests. In these protoplasts, touch and osmotic treatments induced forisome dispersion, most likely as result of changes in calcium concentration. Forisomes also demonstrated Ca^{2+}-induced sieve-tube occlusion in response to remote stimuli and the apparent involvement of Ca^{2+}-channels in long-distance signalling. Passage of an electric potential wave (EPW) often coincided with dispersion of forisomes. The sensitivity of forisome dispersion was dependent on its intracellular location which conforms to an uneven distribution of Ca^{2+}-channels over SEs. Ca^{2+} channels were found to be located both on the PM and EM cisternae. Both channels may cooperate in amplifying the amounts of calcium released into the SE mictoplasm. The Ca^{2+} threshold for forisome dispersion may only be reached in the ER interstices, where the forked forisome ends are inserted. That nature and strength of the stimuli were related to forisome behaviour led to a model in which the release of Ca^{2+}-ions during EPW propagation results in various local reactions in SEs and adjacent cells.

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Plant LIMs: simple proteins, intricate functions

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The LIM domain defines a tandem zinc finger motif that primarily functions as a protein-protein binding interface in eukaryotic cells. In animals, LIM domain-containing (LIM) proteins are abundant and display a wide range of cellular functions as regulators of gene expression, cytoarchitecture, cell adhesion, cell motility and signal transduction. Although LIM proteins are less numerous and diverse in plants, a comprehensive picture of their biological roles only starts to emerge. This presentation highlights central information regarding the biology of plant LIM proteins and discusses the most recent advances in the field.
Plant LIM proteins are relatively short (~200 AA) proteins consisting of two LIM domains, an inter-LIM spacer and a C-terminal domain. In the cytoplasm they bind to, stabilize and bundle actin filaments into thick actin cables. Based on its expression pattern and specific responsiveness to regulatory factors such as calcium and pH, one LIM protein subset is expected to play a central role in actin cytoskeleton organization of growing pollen tubes. Another subset of LIM proteins is assumed to respond to mechanical cues by massively accumulating along actin filaments thereby reinforcing their strength via bundle formation. In a transient protoplast system, one LIM protein was found to enhance the activation of a target promoter. A direct interaction between the LIM protein and cis-elements of this target promoter is supported by in vitro data. In conclusion, plant LIM proteins appear as multifunctional proteins that connect the cytoskeleton to the nucleus. We speculate that they serve as biosensors to modulate the actin cytoskeleton organization/dynamics and gene expression in response to abiotic signals.

The role of plant synaptotagmins in plasma membrane integrity and cell survival

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Calcium dependent vesicular trafficking (CDVT) is involved in many essential physiological processes in animals. Synaptotagmins, proteins containing a transmembrane domain and two C2 domains in tandem, have been identified as key players in CDVT. Plasma membrane of animal cells can rapidly reseal disrupted sites through a tightly regulated CDVT process that is dependent on Synaptotagmin VII. In fact, this process is essential for survival and defective plasma membrane repair produces muscular dystrophies, a diverse group of myogenic disorders characterized by progressive loss of muscle strength and integrity.

Despite the importance of plasma membrane repair in animals, this process has not been reported in plants. In our screening for salt hypersensitive Arabidopsis mutants we identified that mutations in the SYT1 gene, that shows homology and conserved domains similar to animal synaptotagmins, produce hypersensitivity to different abiotic stresses by decreasing the integrity of the plasma membrane. This result implicates CDVT as an essential uncharacterized process in plant abiotic stress tolerance. We made an exhaustive analysis of the mutant and a biochemical characterization of the SYT1 protein reporting its Ca2+ and phospholipid binding characteristics. The SYT1 protein is localized predominantly to the plasma membrane, an aspect that is likely to be critical for its function. We also found that the homologous SYT3 gene has partially redundant function with SYT1 as the doble mutant shows decreased plasma membrane integrity than single mutants. Our data indicate that Ca^{2+} dependent plasma membrane repair mediated by SYT1 and SYT3 is essential for plasma membrane integrity and cell survival.

Cloning and identification of a novel function of pea lectin receptor-like kinase in salinity stress tolerance

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The plant lectin receptor-like kinases are involved in various signaling pathways but their role in salinity stress tolerance has not heretofore been described. Salinity stress is one of the major factors which negatively affects plant growth/productivity and threatens food security worldwide. Based on functional gene-mining assay, we have isolated 34 salinity tolerant genes out of one million *Escherichia coli* (SOLR) transformants containing pea cDNAs grown in 0.8 M NaCl. Sequence analysis of one of these revealed homology to lectin receptor-like kinase (LecRLK), which contains N-myristilation site and N-glycosylation sites thus corroborating that the protein is a glycoconjugate. Structurally the plant RLKs are similar to animal receptor kinases because both consist of: 1) N-terminal, extracellular, ligand-binding domain, 2) a hydrophobic transmembrane domain and 3) a C-terminal intracellular, kinase catalytic domain (Ser/Thr). The homology based computational modeling of the kinase domain suggested the high degree of conservation with the protein already known to be stress responsive in plants. The NaCl tolerance of LecRLK in bacteria was further confirmed by using another strain of E. coli (DH5α) transformants. In planta, the expression of LecRLK cDNA was also upregulated in response to NaCl. However, there was no significant effect of K+ and Li+ ions on the expression level of the gene in planta as well in E coli, suggesting the Na+ ion specific response. Transcript of the PsLRK gene accumulate mainly in roots and shoots. The purified 47 kDa recombinant pea LecRLK protein has been and shown to contain autophosphorylation activity and also phosphorylate the MBP substrate. This suggests that the cellular response to high salinity stress is conserved across prokaryotes and plant kingdom. Overall, this study urges to develop novel concepts about the role of plant LecRLK in high salinity stress tolerance and this study shall provide a highly significant new contribution for our better understanding of stress tolerance in plants. It remains to be tested whether the expression of these genes will confer durable resistance to high salinity tolerance in crops, but the successful identification of the salinity stress induced gene reveals a clear new pathway for the direction for further experimentation.

AGD5, an ARF-GAP, interacts with ARF1GTPase at the trans Golgi network

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Eukaryotic cells are characterized by a complex of endomembranes. The endomembranes system identity and interconnection is preserved by an active intracellular trafficking mediated by vesicles, which shuttle cargo molecules such as proteins, polysaccharides, and lipids between these organelles. During the intracellular trafficking, vesicle fusion and budding is driven by the assembly and disassembly of the coat proteins from the membrane. In turn, assembly and disassembly of coat proteins are regulated by other proteins called ADP-Ribosylation Factors (ARFs) [1]. As in animal and yeast cells, ARFs have different effectors and regulator proteins that can control the trafficking pathway, like the ARF-GAP proteins. In general, GAP proteins play a crucial role in regulating the disassembly and dissociation of vesicle coats. In the *Arabidopsis thaliana* genome there are 15 proteins with an ARF-GAP domain, which are classified as ARF-GAP Domain proteins (named AGD1-15) [2]. Here we characterized an Arabidopsis ARF-GAP (AGD5) that contains the AGD domain at the amino terminus. This protein is structurally related to the yeast ARF-GAPs (Age2p, Gcs1p and Glo3p) who perform their function at the trans-Golgi network (TGN) [3,4].This study provide evidence that AGD5 represent a new interactor for the small GTPase ARF1 protein at the TGN organelle suggesting a role in vesicle transport along the endocytic pathway. Furthermore, analysis of transgenic Arabidopsis plants for this protein, showed various defects in root growth, as well as root hairs and pollen tubes development.

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Development of model peptides as the research tools for studying the dynamic signaling events in living plant cells

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Recently, we have been engaged to the development of oligopeptide based artificial model signaling peptides behaving as artificial enzymes and/or ion channels. This presentation focuses on the development of artificial redox-active enzyme mimics capable of catalyzing the redox reactions such as decomposition of hydrogen peroxide, oxidation of amines and phenolics and generation of superoxide anion, by analogy to the redox nature of human prion protein and plant peroxidases. By designing artificial peptides encompassing the prion-like metal-binding domain, a series of peroxidase mimics with thermo-stable (heat-tolerant and freezing/thawing-tolerant) nature was materialized. Among such metal-binding and redox active peptides synthesized and tested, some acted as pro-oxidants and some behaved as anti-oxidants. Pro-oxidative peroxidase mimics were capable of catalyzing the phenol-dependent robust superoxide generation (Kawano et al., 2007; Yokawa et al 2009a, b) and the aniti-oxidative peptides were capable of plant cell protection from the metal-induced cell death possibly by removal of both the toxic metals and resultant reactive oxygen species (Kagenishi et al., 2009). Furthermore, by inserting certain motifs into the artificial peroxidase sequences, the mode of reaction could be converted from the phenol-requiring peroxidative mode to the catalase-like phenol-independent mode by decomposing hydrogen peroxidase and evolving molecular oxygen.In addition to the redox-active nature of above artificial enzyme mimics, we found that these peptides can actively interact with other proteins or peptides. Such intermolecular interactions include oxidation of amino acid residues (enzyme mimics as catalysts) and modification by phosphorylation (enzyme mimics as substrates), suggesting that controls of signal transduction pathway by properly designing the peptide sequences. For an instance, by designing the oligo-peptides composed of artificial peroxidase moiety and MAP kinase phosphorylation domain, we could develop a novel articial enzyme capable of altering the catalytic activity by phosphorylation of tyrosine and threonine. Lastly, application of these artificial peptides for the studies of plant signaling mechanism will be discussed.

Plant-insect interactions: General aspects and dissection of mechanical and chemical challenges

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Interactions between plants and insects are central to almost all ecosystems. Although numerous types of interaction have evolved, there is considerable overlap in recognition, signal transduction and gene expression events that orchestrate the plants' reactions. The activation of any specific responses requires efficient recognition of the interacting organism, conversion of the perceived signal into downstream signalling cascades, and eventually, the onset of appropriate reactions. Responses to insect herbivore damage can be triggered by simple wounding or insect-derived elicitors such as certain enzymes, fatty acid-derived conjugates, other low molecular weight aliphatic compounds, and peptides generated from degradation of ingested plant material. Early induced signalling processes in host plants are characterised by membrane depolarization, intracellular [Ca2+] transients and ROS production, followed by the activation of protein kinases and downstream phytohormone networks that coordinate particular pathways leading to defences. These defences include physical factors (barriers) as well as the deployment of toxic or harmful phytochemicals that are constitutive or induced. Such defensive phytochemicals belong to different classes of secondary metabolites, such as phenylpropanoids, alkaloids, terpenoids, or are fatty acid derivatives. In addition, diverse indirect and direct strategies that plants have evolved to defend themselves against phytophagous insects will be addressed. For example, emissions of induced volatile organic compounds influence local and long range interactions by repelling herbivores and attracting parasites and parasitoids from a distance, thus employing a third trophic level.
I will present data on the early phase of plant-insect interactions: from recognition and subsequent signalling processes to the activation of defence related genes and appropriate defence responses towards an attacking enemy. In detail, we studied the individual contributions of wounding and chemistry, which is introduced by any feeding insect. Therefore, we tried to dissect mechanical and chemical treatments during herbivory in order to understand their particular impact on the induction of the whole set of plant responses.

AtFH8, an Actin Filament Nucleator and Bundler, Has a Relationship with Root Development in *Arabidopsis*

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Formins have been paid much attention for their potent nucleating activity. In addition to nucleate actin filament assembly, some formins can bundle actin filaments. In this study, we characterized the bundling activity of AtFH8 (*Arabidopsis thaliana* Formin Homologue 8) *in vitro*. Biochemical analysis showed that AtFH8(FH1FH2) could form dimers and bundle preformed actin filaments. However, during the polymerization processes, it not only bundled actin filaments but also induced stellar structures consisting lots of actin bundles. To investigate the localization and function of AtFH8 *in vivo*, full-length cDNA and truncated forms of AtFH8 were expressed as GFP fusion protein in Arabidopsis. It was found that AtFH8 localized to nuclear envelope in interphase and to the forming cell plate during cytokinesis, which was significantly dependent on its N-terminal transmembrane domain. The immunolocalization of AtFH8 confirmed the nuclear envelope and cell plate localization. Overexpression of AtFH8 promoted mitosis of root tip cells and increased the primary root growth rate in the young transgenic seedlings and vise verse in its N-terminal transgenic lines. The lateral root initation of T-DNA insertion mutant seedlings was partially inhibited by a F-actin-depolymerizing drug, latrunculin B, treatments for 8 days, and the wild-type AtFH8 transgene complements the lateral root phenotype, indicating that AtFH8 linked stabling of actin filament structures may associate with the initiation of lateral roots. Our results suggest that AtFH8 is a potent actin bundle organizer that contributes to primary root growth and lateral root initiation in Arabidopsis young seedlings.

Flow of nutrients and information in mycorrhizal networks

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Arbuscular mycorrhizal fungi (AMF) are soil microorganisms which live in symbiosis with the roots of most land plants and are fundamental for plant nutrition and ecosystem biodiversity and productivity. Their extraradical mycelium is the key structure for soil nutrient uptake and transfer to the host plants: it can spread from mycorrhizal roots into the soil and simultaneously colonise many different plants, representing a means for nutrient exchange and resource allocation in plant communities. Recent studies revealed that hyphal fusions (anastomoses) between compatible hyphae represent the main mechanism allowing the formation of large interconnected mycorrhizal networks, whose structure directly influence the nutrition and growth of host plants (1, 2). Anastomoses allow also mycorrhizal networks originating from plants belonging to different species, genera and families to become interconnected, thus giving raise to indefinitely large mycelial webs (3, 4). The continuous bidirectional protoplasmic flow established between self-compatible hyphae, easily detected by time-lapse video microscopy and vital staining, shows that cytoplasm, cellular organelles and nuclei can migrate in AMF mycelium by means of anastomosis. Since genetic exchange has been recently shown between genetically different individuals, and specific genetic markers were transmitted to the progeny (5), a considerable promiscuity can be assumed to occur in the mycorrhizal network, where important nutritional, genetic and information flows are active.

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Cellular mechanisms and signal transduction associated with endosymbiotic root infection

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The mutual beneficial relationship between plants and nitrogen-fixing bacteria known as rhizobia is very important both from the agricultural and ecological viewpoint. Rhizobia possess the enzyme complex nitrogenase which enables them to convert atmospheric nitrogen into ammonia, which can then be utilised by the plant host. In exchange, the plant provides the bacteria with a carbon source and a privileged ecological niche in a newly formed plant organ, the root nodule. The rhizobial symbiosis is limited to leguminous plants for which the interaction between Medicago truncatula and Sinorhizobium meliloti is a model system for nodulation. Rhizobia enter the M. truncatula root hair following entrapment within the curled tip and gain access into the root internal tissues via a specialised intracellular plant-derived apoplastic compartment called the infection thread (IT). The path of the IT is determined by the transient formation of a nuclear-driven cytoplasmic assembly which precedes the construction of the IT. The formation of this assembly requires a major intracellular reorganisation involving the plant cytoskeleton and endomembrane system. By using an in vivo confocal microscopy approach we have recently discovered a number of novel and important characteristics of the rhizobial infection process (Fournier et al. 2008, Plant Physiol. 148: 1985-95). These include the relationship between the position of the migrating plant cell nucleus and the progression of the IT, as well as the mechanisms of rhizobial colonization of the thread.
One important observation is the frequent presence of a bacterial-free space behind the tip of the extending IT, which shows that direct physical contact between the two organisms is not required for IT growth. Time-lapse studies indicate that a precise coordination exists between IT extension and progressive bacterial invasion. Together with genetic and molecular evidence, this strongly suggests that IT initiation and progression requires continued signalling between the two symbionts. A likely candidate for this signalling is a rhizobial secreted molecule, the Nod factor, a lipochitooligosaccharide, which has been shown to play a prominent role in the host-bacteria recognition and early events of nodulation. When applied externally to plant roots, purified Nod factors elicit a number of plant responses including changes in the cytoskeleton and cytoplasmic Ca^{2+} levels. By using live-cell imaging and fluorescent microtubule markers we have shown that Nod factors elicit a reorganisation of the microtubular array and modifies microtubule dynamics in root hairs (Sieberer et al. 2005, MPMI 18: 1195-1204; Timmers et al. 2007, Eur J Cell Biol. 86: 69-83). NF-induced cytoplasmic Ca^{2+} spiking has been reported to arise in the perinuclear region of root hairs (Oldroyd and Downie 2006, Curr. Opin. Plant Biol. 9: 351-357). In order to evaluate NF-elicited Ca^{2+} responses in the nuclear compartment we have developed a nuclear-targeted cameleon sensor. Our experiments reveal that Ca^{2+} spiking also occurs within the root hair nucleus and that this response is cell-autonomous. Nuclear Ca^{2+} spiking is highly variable in terms of frequency and spike duration. Future work will focus on the role of, and the spatio-temporal relationship between the plant cytoskeleton and Ca^{2+} spiking during rhizobial infection in different root tissues and the potential role of NFs within this process.

GORK and NSCC channels as components of salinity tolerance mechanism in plants

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Salinity stress tolerance is mediated by multiple physiological and biochemical mechanisms, implying the orchestrated regulation of numerous membrane transporters. In this talk, I summarise our recent findings to show that both outward-rectifying depolarisation-activated K^+ (GORK) and non-selective cation (NSCC) channels play a key role in plant salinity responses. We show that salt tolerance correlates with K^+ retention, and GORK channels are primarily responsible for mediating salt-induced K^+ loss [1]. Interestingly, GORK channels density was similar in barley cultivars contrasting in their salt tolerance, highlighting the importance of channel regulation, not their physical number [2]. Voltage gating was one of the key features of this regulation. We show that tolerant plants had superior control of membrane potential by intrinsically higher H+-pump activity, thus reducing K^+ loss through GORK, as well as improving the thermodynamics for K^+ uptake. We also show that GORK channels are the downstream target for all substances known to ameliorate detrimental effects of salinity on plants (e.g. exogenous application of calcium or compatible solutes). Furthermore, a link between intracellular potassium homeostasis and Reactive Oxygen Species (ROS) signalling (a well known component of salinity stress) is established and discussed. We show that application of ROS (either H_2O_2 or $OH^{\bullet}$-generating Cu^{2+}/ascorbate mixture) to plant tissues results in a massive, dose-dependent efflux of K^+. Pharmacological experiments [2, 5] and patch-clamp data [3] indicate outward-rectifying K^+ (KOR) and NSCC as downstream targets of such signalling. We further show that expression of animal CED-9 anti-apoptotic gene significantly increases plant oxidative stress tolerance by regulating KOR and NSCC activity [4], thus providing the first link between 'ion flux signatures' and mechanisms involved in regulation of PCD in plants. Finally, we show that physiologically relevant concentrations of various compatible solutes significantly reduce ROS impact on major ion transporters [5]. Importantly, we demonstrate a significant reduction in K^+ efflux (associated with increased stress tolerance) using osmolytes with no reported free radical scavenging activity. This indicates that compatible solutes must play other (regulatory) roles in addition to free radical scavenging in mitigating the damaging effects of oxidative stress.

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Sucrose nonfermenting1 (SNF1)-related protein kinase 2 (SnRK2) family function in hyper-osmotic stress signaling of rice

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Plant SnRK2 family has been implicated as important regulators of abiotic stress and ABA signaling. Although rice genome contains 10 SnRK2 members, their biological function is largely unknown yet. In the present study, we have characterized biochemical properties and physiological functions of rice SnRK2 subclass 1a proteins. GST-fused recombinant proteins showed autokinase activity, strong substrate preference for rice bZIP transcription factors and cofactor requirement for Mn2+ over Mg2+. Autophosphorylation of catalytic domain was important for enzyme activation and C terminus domain was involved in activity regulation. Noticeable differences in optimum pH, autokinase activity, substrate specificity and inhibitor sensitivity were found among different SnRK2 proteins. We made transgenic rice over-expressing each SnRK2 proteins under the control of 35S promoter and hyper-osmotic stress responses were analyzed. Two SnRK2 transgenic rice showed similar phenotype, i.e. salt-sensitive, but drought-tolerant phenotype. Salt-responses of rice roots were further analyzed. In gel kinase assay revealed that salt and ABA responsive protein kinase are highly activated in the roots of 35S-OSRK1 transgenic rice. Comparison of proteome changes during early salt response of roots showed that enzymes associated with glycolysis, pentose phosphate pathway, nitrogen assimilation and branched amino acid catabolism were rapidly induced by salt treatment. Furthermore, it was noted that those salt-induced enzymes were constitutively up-regulated in roots of 35S-OSRK1 transgenic rice under non-stressed condition. Our data suggests that OSRK1 is upstream regulator of salt stress signaling and possibly be involved in stress-induced carbon and nitrogen metabolic changes. This work was supported by the grants from National Academy of Agricultural Sciences, RDA.

Na^+ measurements using a fluorescent dye in plant

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Fluorescent indicators for Na+ are valuable for nondestructive monitoring of spatial and temporal distribution of Na+ in plants. We tested whether CoroNa Green fluorescent dye, a newly developed sodium indicator, is suitable for measuring the relative concentration of Na^+ in planta. To determine the ideal conditions for measuring Na^+ content in planta using CoroNa Green dye, NaCl pre-treated Arabidopsis thaliana seedlings were incubated with different concentrations of CoroNa Green, and fluorescence in each organ was visualized using a fluorescein isothiocyanate (FITC) filter. When 50 μM of dye was used, we found that fluorescence was distributed more uniformly and intensely in root tip than in other organs. Under these conditions, we showed that fluorescence gradually increased in root tip upon binding of Na^+ to CoroNa Green for concentrations up to 100 mM NaCl. Consequently, confocal fluorescence microscopy revealed that when Arabidopsis seedlings were incubated with the same concentration of NaCl, the sos1 mutant exhibited much stronger fluorescence than that of wild type. This study marks the first report of the properties of CoroNa Green used to measure Na^+ in intact plants, and demonstrates the usefulness of this technique for investigating the mechanism of Na^+ homeostasis in plants.

The 7B-1 mutation in tomato confers a blue light-specific lower sensitivity to coronatine

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Male sterility of crop species, spontaneous or induced, is a criterion of importance for breeders. In almost all crop species male-sterile mutants have been reported but their use in breeding programs has been limited as some of them were sensitive to abiotic stresses, such as drought or cold temperature. In tomato (*Solanum lycopersicum L.*), one of the most important crops worldwide, the spontaneous mutant 7B-1, isolated for its photoperiod-dependent male-sterility, has been described as resistant to various abiotic stresses specifically under blue light. Since this finding improved potential of 7B-1's use in breeding programs, its susceptibility to coronatine (COR)-induced stress, the phytotoxine produced by several Pseudomonas syringae strains, was assessed in this study. The 7B-1 mutant was found to be less sensitive than the corresponding wild-type (WT) to COR treatment in a blue light dependent manner. Treatment of WT and 7B-1 plants with COR induced a strong accumulation of salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA) in hypocotyls. Interestingly, accumulation of ABA and SA in the 7B-1 mutant was distinctly greater than in WT, especially in blue light. Based on the cross-talk between SA- and JA-signaling pathways, expression analysis of NPR1 and COI1 genes, respectively involved in these pathways, was investigated in COR-stressed plants. The blue light-specific lower sensitivity of 7B-1 plants to COR was found to be associated with blue light-specific over-expression of the NPR1 gene. This data suggests that the SA-dependent NPR1-dependent pathway could be involved in the lower sensitivity of the 7B-1 mutant to COR. The role of anthocyanins and ABA accumulation during the response to COR is also discussed.

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Key-words: blue light-specific response, COI1, coronatine, growth, NPR1, SA-signaling pathway, 7B-1 mutant, tomato (*Solanum lycopersicum L.*).

Changes in cell wall polymers and pectin methyl esterase expression are developmentally regulated during pollen maturation and embryogenesis

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Plant cell walls are involved in many mechanisms of growth and development but the specific roles of the different plant cell wall polymers are still unclear. They are the repository of chemical signals and also contain information for the direction of cell fate. Changes in the composition and mechanical properties of the cell wall seem to be crucial for coordinated growth and development during morphogenesis. Pectins are complex polysaccharides of the cell walls and are formed by different structural domains (HG, RGI and RGII) which can be modified. The methyl-esterification degree of pectins has a crucial role in various growth and development processes in plants. Pectin methylesterases (PME, EC. 3.1.1.11) are the enzymes involved in the de-methyl-esterification of plant cell wall pectins in muro. Modifications in pectin residues, oligosaccharides and other wall components have been reported as potential signals regulating growth and development.
In the present work in situ analysis of the presence and distribution of various cell wall components has been performed to monitor cell wall changes during two different developmental programmes followed by the pollen: the gametophytic development, a differentiation process, and stress-induced reprogramming to embryogenesis, in *Capsicum annuum L* and *Brassica napus L.* Specific antibodies recognizing the major hemicellulose, xyloglucan (XG), the rhamnogalacturonan II (RGII) domain of pectins, and high and low-methyl-esterified pectins were used for dot-blot and immunolocalization assays at light and electron microscopy levels at defined developmental stages. Also, the expression profile of the PME was analysed during the pollen developmental processes of *B. napus* by semiquantitative RT-PCR.
Results showed differences in the presence and abundance of these molecular complexes, as well as changes in the esterification level of pectins at specific developmental stages of gametophytic pollen differentiation and pollen embryogenesis. An increase in the expression of PME was found accompanying embryogenesis progression and differentiation events. These changes were related to cell wall growth and maturation during proliferation and differentiation processes followed by both pollen developmental programmes, suggesting that cell wall complexes could contain information on the cell fate and the direction of the cell development.

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Actin turnover is required for myosin-dependent mitochondrial movements in *Arabidopsis* root hairs

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Background: Previous studies have shown that plant mitochondrial movements are myosin-based along actin filaments, which undergo continuous turnover by the exchange of actin subunits from existing filaments. Although earlier studies revealed that actin filament dynamics are essential for many functions of the actin cytoskeleton, there are little data connecting actin dynamics and mitochondrial movements.

Methodology/Principal Findings: We addressed the role of actin filament dynamics in the control of mitochondrial movements by treating cells with various pharmaceuticals that affect actin filament assembly and disassembly. Confocal microscopy of *Arabidopsis thaliana* root hairs expressing GFP-FABD2 as an actin filament reporter showed that mitochondrial distribution was in agreement with the arrangement of actin filaments in root hairs at different developmental stages. Analyses of mitochondrial trajectories and instantaneous velocities immediately following pharmacological perturbation of the cytoskeleton using variable-angle evanescent wave microscopy and/or spinning disk confocal microscopy revealed that mitochondrial velocities were regulated by myosin activity and actin filament dynamics. Furthermore, simultaneous visualization of mitochondria and actin filaments suggested that mitochondrial positioning might involve depolymerization of actin filaments on the surface of mitochondria.

Conclusions/Significance: Base on these results we propose a mechanism for the regulation of mitochondrial speed of movements, positioning, and direction of movements that combines the coordinated activity of myosin and the rate of actin turnover, together with microtubule dynamics, which directs the positioning of actin polymerization events.

Key words: mitochondrial movements, actin turnover, FABD2-GFP, evanescent wave microscopy, velocity, myosin

Sensory transduction in guard cell

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Stomata of higher plants close in response to darkness, drought and CO2. The plant hormone abscisic acid (ABA) is involved in the transmission of environmental changes. This process is induced by the activation of guard cell anion channels. To online record changes in ion fluxes across the plasma membrane of guard cells in intact plants, we have developed a method, based on multi-barrelled microelectrodes introduced into the cytoplasm of guard cells in combination with calcium imaging. Using this online, in planta approach, we have been able to identify signalling elements required for fast ABAinduced stomatal closure. Recently mutants were shown to lack a gene encoding a putative guard cell anion transporter named SLAC1. SLAC1 function and stomatal closure-related signaling components leading to anion channel activation, however, remained still unknown. Using protein-protein interaction assays we identified a protein kinase and -phosphatase within the ABA transduction pathway as regulators of SLAC1. Our studies demonstrate that SLAC1 represents the slow inactivating, weak voltage-dependent anion channel of guard cells controlled by phosphorylation- dephosphorylation. A model on the ABA-based regulation of guard cell ion transport will be presented at the meeting.

System potentials, a novel electrical long distance apoplastic signal in plants induced by wounding

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We present a recently discovered electrical long-distance signal which, unlike action potentials or variation potentials, is transmitted systemically through hyperpolarization. These signals, called 'system potentials' (SPs), are induced by cut-wounding and simultaneously added agents like inorganic salts or glutamic acid. Although these agents produce a depolarization at the stimulation site, the plants respond with a transient hyperpolarization which is transmitted at 5 to 10 cm min-1 to the target (leaf). It is suggested that this hyperpolarization is due to a stimulation of the plasma membrane H+ATPase (H^+-pump), a notion which is supported by the actions of fusicoccin and vanadate. Ion movements, measured within the leaf apoplast of the target leaf using ion-selective ion electrodes (K^+, Ca^{2+}, Cl^-), follow the SP-voltage changes.
So far, SPs have been demonstrated in all thereupon tested plants : *Hordeum vulgare, Vicia faba, Nicotiana tabacum, Phaseolus vulgaris, Zea mays*. This indicates that SPs may be a universal long-distance signal which is used by plants in general to respond to injuries.

WDL proteins control root growth behavior and anisotropic cell expansion in Arabidopsis

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In plants, anisotropic cell expansion is a tightly regulated process that contributes to morphogenesis. In addition to modulating overall growth rates, this process orchestrates directional growth responses to both endogenous and external cues, allowing plant organs to grow toward environments that are better suited for their primary functions. To better understand how mechanical information and other surface-derived stimuli modulate root growth behavior and anisotropic cell expansion, we screened a population of Arabidopsis thaliana activation-tagging mutants for root behavioral defects on tilted hard-agar surfaces. Under such conditions, wild-type roots are exposed to a combination of gravity, touch and other surface-derived stimuli. Consequently, they tend to skew their growth toward the right of the gravity vector (when viewed through the medium), and they wave on the surface, a phenomenon that is accompanied by reversible helical growth. This complex growth behavior is believed to facilitate obstacle avoidance by growing roots. wvd2-1 was identified in this screen, showing opposite root skewing and dampened or no waves on tilted hard-agar surfaces. This mutant also displayed altered anisotropic cell expansion in all organs of the plant, and enhanced thigmomorphogenesis suggested a role in the control of mechano-transduction or response. WVD2 encodes a microtubule (MT)-associated protein that promotes the bundling of MT in vitro, and affects the organization and dynamic instability of cortical MTs in expanding cells of the root. It shares a 95 amino-acid motif with 7 other Arabidopsis proteins, called WDL1-7, and initial phenotypic analyses of over-expression and knockout mutants suggest distinct, though overlapping roles for the WDL proteins in the regulation of MT-dependent morphological processes. Interestingly, brassinosteroids, which appear to modulate root waving on hard-agar surfaces and anisotropic cell expansion, may regulate WVD2 and WDL proteins activity, as suggested by in vitro studies of WVD2/WDL – BRI1 kinase-domain (KD) interactions and of BRI1-KD-mediated phosphorylation of WVD2 and at least some of the WDL proteins. We hypothesize that WVD2 and related WDL proteins contribute to the control of organs growth behavior by regulating the organization and/or dynamic properties of cortical MTs in expanding cells, thereby modulating the patterns of anisotropic cell expansion and complex growth responses to the environment.

Genetic analysis of the protein import machinery of Arabidopsis plastids

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While plastids retain a fully-functional genetic system, the plastid genome encodes less than 10% of the proteins required to build a fully-functional organelle. The majority of plastidic proteins are encoded in the nucleus and translated on free cytosolic ribosomes. They are synthesized in precursor form, each one bearing an amino-terminal targeting signal, or transit peptide. The transit peptide directs the protein through a post-translational targeting pathway, and is cleaved upon arrival inside the plastid. This targeting or import process is mediated by the coordinate action of two proteinaceous import machines, one in each of the envelope membranes. The import machinery of the outer envelope membrane is called the TOC complex, and that in the inner membrane is called the TIC complex. Over the last decade, several components of the TOC and TIC complexes have been identified using biochemical approaches and isolated pea chloroplasts. Interestingly, many of these components (particularly receptor components of the TOC complex) have been found to have multiple homologues in Arabidopsis. We have used genetic approaches to dissect the functional significance of these different TOC protein isoforms. Our results suggest that the different isoforms operate in different import pathways with distinct precursor recognition specificities; i.e., different import pathways exist for different precursor protein classes. The existence of such substrate-specific import pathways might play a role in the differentiation of different plastid types, and act to prevent deleterious competition effects between abundant and non-abundant precursors.

Both electrical and chemical signals may act in a triggering of rapid systemic responses of tobacco or tomato plants to local burning

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Higher plants respond to adverse environmental factors by various defence processes, e.g. stress protein accumulation, changes in stomata aperture, changes in rates of respiration or photosynthesis. An important characteristic of the defence processes is their occurrence also in undamaged plant tissues that are distant from the damaged ones. This feature suggests an existence of a mobile signal that is transmitted from the damaged part of the plant to the distant undamaged tissues where it evokes systemic stress responses.
We studied mainly short-term (up to one hour) systemic reactions of tobacco (*Nicotiana tabacum* cv. Samsun) or tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) to local wounding (burning) of an upper leaf. In both plant species, significant changes were detected in gas exchange parameters of undamaged leaf growing under the burned one. In tobacco, a decrease of the stomatal conductance and the rates of transpiration and CO_2 assimilation has already been observed 5-7 minutes after local stimulation. In tomato, an initial (within 5 min) increase of the stomatal conductance and the rate of transpiration was reversed by their marked decrease up to 25 min after local burning when steady-state values were reached. Values of the rate of CO_2 assimilation only slowly decreased to the steady-state levels. We detected no considerably rapid systemic changes in chlorophyll a fluorescence parameters in tobacco. Similarly, no significant changes were detected in systemic chloroplast movement in the non-treated tobacco leaf after local burning of the distant leaf.
Electrical and chemical signals that could be involved in a triggering of the above mentioned systemic responses were investigated. A sharp decrease followed by an increase (seconds to minutes) of surface electrical potential (SEP) was detected in several leaves growing below the burned one in tobacco plants. In tomato, an increase of SEP was detected in distant untreated leaves within seconds after burning of the upper leaf. Our low-noise multi-channel device for the monitoring of SEP propagation throughout plants will be presented.
SEP changes in tobacco were followed by rapid systemic increases in endogenous concentration of abscisic acid (ABA, 8-15 min) and jasmonic acid (JA, 15-60 min) after local burning. In tomato mutants - sitiens (ABA-deficient plants), SEP changes propagated more rapidly down the leaf trace of the wounded leaf and SEP amplitude was dependent on the position of the measured leaf with respect to the leaf trace of the wounded leaf. An amplitude of the SEP wave in sitiens mutants was approximately twice lower (30 mV) than that in wild-type (60 mV).
Our results indicate a possible participation of both, electrical (SEP) and chemical (ABA, JA) signals in rapid long-distance (systemic) responses of plants to local stress. ABA seems to be involved in the electrical signal generation and its long-distance propagation after local wounding of tomato. A scheme of possible signaling pathways including generation of the chemical and electrical signals, their interactions and participation in triggering of the responses will be discussed.

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Jasmonates functions in symbiotic interactions and plant response to wounding

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Jasmonic acid (JA) and its derivatives, commonly termed jasmonates, are hormonal regulators involved in plant responses to abiotic and biotic stresses as well as in plant development. Jasmonates are lipid-derived signals, they are synthesized by the octadecanoid pathway via the allene oxide synthase branch of the so-called lipoxygenase pathway. The role of jasmonates is well established as part of a complex signal transduction pathway activated upon wounding of leaves by insects and interaction of plants with microorganisms. Among these interactions, also the mutualistic interactions between plants and arbuscular mycorrhizal (AM) fungi or nitrogen-fixing rhizobacteria are believed to be regulated from the plant side among other signals by action of JA. To get deeper insights, functional analyses by transgenic approaches were performed to investigate the role of JA during the interaction between *Medicago truncatula* and *Glomus intraradices* or *Shinorhizobium meliloti*.
The capacity of *M. truncatula* roots to synthesize JA was changed by transformation with *Agrobacterium rhizogenes* leading to chimeric plants. This was achieved by modulation of the transcript level of the *MtAOC1* gene encoding the allene oxide cyclase (AOC), a crucial enzyme involved in JA biosynthesis. Transgenic roots exhibiting partial suppression of *MtAOC1* and lower JA levels showed a significant delay in the process of colonization with *G. intraradices*. Analyses of global transcript profiles by microarrays in non-mycorrhizal and mycorrhizal roots with modulated *MtAOC1* expression, revealed a large number of regulated genes, among them are those encoding enzymes involved in phenylpropanoid metabolism. Analyses of isoflavonoids showed that mycorrhizal roots with enhanced and decreased *MtAOC1* expression exhibited decreased and increased levels, respectively. In contrast to mycorrhization, a role of JA in the interaction of *M. truncatula* with *S. meliloti* leading to the formation of nodules could not be demonstrated. Here, overexpression and partial suppression of *MtAOC1* did not lead to an altered nodule phenotype: Neither the morphology of nodules nor the number of nodules were different in these plants in comparison to the empty vector control.
As wounding of plants leads to endogenous rise of JA accompanied by the expression of a distinct set of genes, we addressed the question, how roots and shoots of *M. truncatula* respond to wounding. Surprisingly, *M. truncatula* plants responded very sensitively to mechanostimulation caused by harvest. Mechanostimulation led to a rapid increase in JA levels followed by increased transcript accumulation of JA-responsive genes. Even repeated mechanostimulation performed by touching obviously changed the phenotype of plants. Moreover, repeated wounding of leaves affects mycorrhization of *M. truncatula* with *G. intraradices* pointing to a systemic effect of JA raised in shoots. In conclusion, our results indicate that jasmonates act as positive regulator of mycorrhization, but do not have a role in nodule formation of *M. truncatula*.

Nuclear calcium signalling in plant cells

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In plants, Ca^{2+} is a ubiquitous intracellular second messenger, known to couple a diverse array of signals and responses such as the plant responses to biotic and abiotic stress [1,2]. Using tobacco BY-2 cell suspension cultures harbouring the bioluminescent Ca^{2+}-responsive protein aequorin in the nucleoplasm, we show here that isolated plant nuclei generate Ca^{2+} signals on their own in response to mechanical and thermal stimuli, independently of a cytosolic environment (3).By combining mathematical modeling and pharmacological approaches, we predict that the buffering capacity of the nucleoplasm and the activities of putative calcium transporters/exchangers located on the inner nuclear membrane might be highly coordinated (4).
We have also addressed the possible connections between sphingolipid metabolites and the modulation of $[Ca^{2+}]$nuc in tobacco BY-2 cells and in isolated nuclei. Experimental evidences suggest that an active metabolism of sphingolipid takes place in the nucleus. Thus, exogenously supplied LCBs, elicited increases in $[Ca^{2+}]$nuc in intact cells and isolated nuclei in a dose-dependent and structure-related manner. In contrast, sphingosine-1-phosphate and C2-ceramide were completely inactive. Importantly, we found that pre-treatment of isolated nuclei with transient receptor potential (TRP) channel inhibitors significantly inhibited the effect of DMS on $\Delta[C^{a2}+]$nuc (5). We also show that jasmonate derivatives or their synthetic mimics differentially induced calcium transients in the cytosol and the nucleus. We show further that some of them were active on $[C^{a2}+]$nuc only giving a physiological relevance to the calcium signalling autonomy of the nucleus(6). Collectively, our results reinforce the theory suggesting that the nucleus is autonomous in terms of calcium signaling and may impact on response specificity by controlling downstream nuclear Ca^{2+}-dependent events. Current work investigates the role of calcium compartmentation in sphingolipid-induced cell death-processes.

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Apocarotenoids - signaling compounds of Zygomycetes and plants?

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Zygomycetes are common heterotrophic microorganisms which naturally occur on terrestrial habitats. For sexual reproduction and parasitic interaction, the zygomycete fungi interact via an elaborate series of carotene derived compounds, namely trisporic acids and their biosynthetic precursors. However, details of their metabolism and the biological significance of the various intermediates remained unclear.[1] Therefore we generated a trisporoid library including deuterium labeled intermediates by a combination of synthesis and biotransformation using cultures of *Blakeslea trispora*.[2] These references enabled us to study the biosynthesis and the biological function of individual trisporoids in more detail.[2-4] The results prompted us to postulate a new sequence of molecular interaction between both mating partners, which includes two different metabolic pathways.[5] Moreover, apocarotenoids are strongly discussed as new branching hormones in plants and fungi.[6] Therefore we decided to determine an influence on plant cells and mycorrhizal interaction. Amazingly the β-C_{18}-ketone (D'orenon), an early trisporoid precursor, strongly inhibited root hair development of *A. thaliana* as results of disruption of the auxin signaling network.[7]

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Sensing plant symbiotic signals by nitrogen-fixing bacteria

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The rhizosphere represents a highly dynamic forefront for interactions between plant roots and soil microbes. In this space, encompassing few millimeters, complex biological and ecological processes occur, such as the exchange of chemical signals among organisms. Under nitrogen-limited conditions, the Gram-negative soil bacteria rhizobia are able to establish a symbiotic interaction with leguminous plants. This host plant-specific association results in the development of root nodules in which rhizobia differentiate into nitrogen-fixing bacteroids. A successful symbiosis is the result of an elaborate developmental program, regulated by the exchange of molecular signals between the two partners. During growth in the rhizosphere of the host plant, rhizobia sense compounds such as flavonoids secreted by the host root and respond upon induction of the nodulation genes by synthesizing the Nod factors. These diffusible rhizobial signal molecules of lipochito-oligosaccharide nature are perceived by plant roots and trigger a Ca^{2+}-dependent signaling pathway leading to specific physiological responses, which culminate in the nodule organogenesis. In comparison with the large knowledge of the signalling pathway active in plants, limited information is available on the rhizobial perception and transduction of symbiosis-inherent signals generated by the plant host.
By using aequorin-expressing rhizobia (Mesorhizobium loti and Rhizobium leguminosarum bv. viciae) we demonstrated that host plant root exudates, and signal molecules therein contained, such as flavonoids (nod gene inducers), are sensed by nitrogen-fixing bacteria through transient intracellular Ca^{2+} elevations. The significant inhibition of nod gene expression obtained when the Ca^{2+} response is blocked indicates that an upstream Ca^{2+} signal is required for nod gene activation. Ca^{2+} changes were not triggered by flavonoids unable to induce nod gene expression (anti-inducers). A rhizobium strain cured of the symbiotic plasmid, impaired in flavonoid-induced nod gene expression, retained its ability to respond to flavonoids with an unchanged Ca^{2+} response. The possible placement of the Ca^{2+} signal within the NodD-flavonoid gene expression paradigm will be discussed. These data indicate a newly described early event in the molecular dialogue between plants and rhizobia and are consistent with a crucial role played by Ca^{2+} in the symbiotic signalling.

Chemical biology of leaf-movement of *Albizzia saman*

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Albizzia plants close their leaves in the evening, as if to sleep, and open them in the morning according to the circadian rhythm. Potassium β-D-glucopyranosyl-12-hydroxyjasmonate (1) was isolated as leaf-closing factor (LCF) of *Albizzia saman*. We developed molecular probes consisting of modified LCF 1 in order to identify its mode of action. We have already demonstrated that a specific binding protein is involved in the motor cell of *A. saman*[1)]. We synthesized natural-type photoaffinity probe and biologically inactive enantiomer-type probe. We utilized them for photoaffinity labeling of the receptor for LCF 1. By using protoplasts of motor cell, we found membrane protein of 38 kDa which strictly recognizes the stereochemistry of 1, and it is highly likely that the protein is the specific receptor for LCF[2)].
Recently, we observed that LCF shrank motor cell protoplasts prepared from A. saman. And comparing the results of several bioassay using glucosyl jasmonate-type LCF and jasmonic acid, it is also interesting that the mode of action of LCF is completely different with that of jasmonate[3)].

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Membrane potential fluctuations in *Chara australis*: a characteristic signature of high external sodium

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We have studied fluctuations in membrane potential difference (PD) in *Chara australis* at frequencies between 1 and 500 mHz, by classical noise analysis and by inspection of the PD time-course. The former shows (1) a quasi-Lorentzian ($1/f^2$) rise of noise power as frequency falls, and (2) a marked increase in noise power when the cell is exposed to high salinity (*Chara australis* is a salt-sensitive species). Inspection shows that as well as initiating depolarization, exposure to high Na^+ usually initiates a continuous but random series of small depolarizations with median amplitude of 2 mV, frequency of 0.2 Hz, depolarization rate of 2 mV/s and repolarization of 0.9 mV/s (7 cells). The noise is abolished by substituting Mg^{2+} for Na^+, but remains unchanged by exchanging Cl^- for SO_4^{2-}. We hypothesize that the noise is caused by transient openings of H^+ or OH^- (H^+/OH^-) channels in the early exposure to sodium. With time in saline medium the membrane PD depolarizes due to inactivation of the proton pump. The noise diminishes, as progressively greater numbers of the H^+/OH^- channels remain open, modifying the current-voltage characteristics of the membrane and shifting the membrane PD towards zero.
The involvement of H^+/OH^- channels in salt sensitivity has not been considered before. Similarities between charophyte cells and roots of land plants are discussed.

Anion channel activation is an early event in ozone-induced cell death in *Arabidopsis* cell suspension

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Ozone (O_3) produced by a complex series of photochemical reactions from primary precursor emissions of nitrogen oxides and volatile organic compounds, is a major secondary air pollutant often reaching high concentrations in urban areas under strong daylight. By exposing cells to the pulse of ozonized air, O_3 induced acute cell death was observed in suspension cells of Arabidopsis thaliana depending on the exposure time. In this study we demonstrated that the activation of plasma membrane anion channel is an early key component of the O_3-induced cell death in A. thaliana. Previous data obtained on tobacco cells suggested the action of Ca^{2+} as a secondary messenger initiating the oxidative cell death. As in tobacco cells, impairment of Ca^{2+} influx with BAPTA or La^{3+} allowed to decrease the extent of O_3-induced cell death. The increase in anion channel activity also seemed to be dependent on an increase in cytosolic Ca^{2+} since BAPTA or La^{3+} allowed to decrease the O_3-induced depolarization and anion channel activity increase. Reactive oxygen species scavengers also allowed to decrease O_3-induced cell death, but failed to inhibit anion channel increase suggesting that the involvement of ROS in O_3-induced cell death doesn't concern the early events.

Arabidopsis root movements and symmetry

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When Arabidopsis plants are grown on an agar dish inclined at an angle, the primary roots do not grow straight down but by making a characteristic wavy pattern, which direction, in the wild-type, slants to a side that was defined as the right-hand. This because the half-waves made to the right-hand are deeper than those made to the left-hand. In addition, the waves normally show a twsting that is alternatively left-handed when the wave is made to the right-hand, and right-handed when the wave is made to the left hand. Furthermore, when the roots are grown on a plate set horizontally they make large or strict clockwise coils, that show also a strong torsion to the left-hand on themselves. The reason of all these movements are still not totally clear, and there is more than a single hypothesis about, but generally they are supposed to be the consequence of three forces acting together on the root, i.e. positive gravitropism, circumnutation and a form of thigmotropism. Gravity in fact controls the direction down the plate, the waving seems to be the consequence of typical circumnutation, with the complication of an alternative switching from the right-handed to the left-handed symmetry, that seems to be induced by negative thigmotropism. As concerns the coils they seems to be circumnutation circles flattened on an agar dish, that possibly show a strong torsion due to the necessity of discharging the tension produced by the flattening of the space right-handed helix. Following a different hypothesis the coils, however, could be also a consequence of thigmotropism. These are the results of several researches made both on the wild-type and on mutants, in 1g , in simulated microgravity, and in one case also in space. The various possibilities are discussed and confronted with data coming from different plants.

Increasing the density of the external medium inhibits and reverses root gravitropism

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As an alternative to the statolith model, we have presented a model for plant gravity sensing in which the entire protoplast functions as the gravity sensor. This gravitational pressure model was developed as a result of experiments with the large, statolith-free, intermodal cells of Chara. These cells exhibit a polarity of cytoplasmic streaming in response to gravity such that, under normal conditions when the density of the external medium is less than that of the protoplast, streaming takes place ca. 10% faster in the downward direction than in the upward direction. However, when the density of the external medium is equal to that of the protoplast the polarity of cytoplasmic streaming is abolished. When the density of the external medium is greater than that of the protoplast (and the protoplast becomes buoyant within the extracellular matrix) the gravity response is reversed.
The question remains whether the gravitational pressure model can explain the gravity responses of higher plants containing statocytes. We tested the gravitational pressure model by monitoring gravitropic curvature of statolith-containing roots in media of differing densities. Changing the density of the external medium will affect the static buoyancy of the protoplast but not the sedimentation of intracellular particles. We find that increasing the density of the external medium inhibits, and in some cases reverses the direction of gravitropic curvature of these roots. These data are consistent with the gravitational pressure model for plant gravity sensing and inconsistent with the statolith model.

Panpsychism - Past and Present

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Panpsychism is the doctrine that all matter has a psychical aspect. Not only human beings and animals, but also plants and even inanimate objects. It was a favored doctrine in the past, echoed in many prescientific cultures.
In the 18-th and 19-th century panpsychism was related to the idealistic philosophy. Among the supporters of panpsychism should be mentioned - Fechner who is known for establishing the science of psychophysics, and who wrote a book about the soul of plants, and Wilhelm Wundt . Other supporters include the important psychologist and philosopher William James, and the philosopher Alfred Whitehead.
Most people would say that plants are not conscious, yet a case could be made that the first stirrings of consciousness came with having senses and interacting with the world, and plants can sense the world; they respond to gravity, light temperature and moisture.
An argument in favour of panpsychism is related to the theory of evolution. How so enormous a jump from one creature to another should have occurred, as the introduction of a fact entirely different from the physical fact. Panpsychism became much less attractive with the ongoing powerful scientific picture of the physical world and the assumption (based on a very large amount of evidence) about the closure of the physical world. Maintaining otherwise would amount to believe in miracles .As a result of our immense scientific knowledge of the physical world, panpsychism has become an implausible view. However, recently the possibility of panpsychism surfaced in relation to the problem of consciousness. The philosophy of mind in the modern era begins with Rene Descartes in the 17-th century. His famous doctrine was dualism, the idea that the world divides into two different substances : the mental (consciousness) and the physical (being extended in space). Humans are composite beings, consisting both of a mind and a body; animals and plants are mere machines with no mental life. The problem of dualism is how can there be an interaction between two different substances, despite the undeniable fact that that such an interaction does take place. Because of the failure of dualism (in its different forms) there is in contemporary philosophy a turn to physicalism – according to which, the only reality that exists is physical. Mental states are either eliminated, reduces to physical states or identified with them. An attractive theory is Searle\'s biological naturalism, which avoids both dualism and physicalism; consciousness is caused by the behavior of neurons in the brain and at the same time, it is realized in the brain system, which is composed itself of neurons, but there still remains the question how exactly to integrate consciousness with the brain. There are two possibilities: panpsychism and emergentism. It seems imperative to decide whether and how mind emerges; whether it exists only under some specifiable conditions in specific places (the brain) or whether it is a part of the fundamental structure of the world. The advances in science: the theoretical success of physical science which explained how chemical complexity arises from physical principles point to the latter. Thus, all modern physicalistic theories of mind implicitly rest upon a theory of emergence (either epistemological or ontological), but none is satisfactory. Until such an account will be achieved, panpsychism remains an open possibility.

Epigenetic memory in plant responses to environmental stimuli

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Epigenetics refer to heritable changes (during mitosis and meiosis) of genome function that occur without a change in DNA sequence. The advantage of epigenetic changes is that they are stable and also reversible. DNA methylation and histone modifications are thought to have crucial roles in organism adaptive response to environmental stimuli and in inheritance of stress memories. Our group is interested in characterization of regulatory factors involved in the deposition of histone modifications, such as methylation and ubiquitylation on histone lysine residues. We will show and discuss our recent data to highlight roles of histone modifications in plant growth and development and in plant responses to environmental stimuli, including abiotic and biotic stresses.

Selected recent publications from our group:

XU, L., MENARD, R., BERR, A., FUCHS, J., COGNAT, V., MEYER, D., and SHEN, W.-H. (2009) The E2 ubiquitin-conjugating enzymes, AtUBC1 and AtUBC2, play redundant roles and are involved in activation of FLC expression and repression of flowering in Arabidopsis thaliana. Plant J. 57, 279-288. (Epub online 14 october 2008)
XU, L. and SHEN, W.-H. (2008) Polycomb silencing of KNOX genes confines shoot stem cell niches in Arabidopsis. Curr. Biol.,18, 1966-1971.
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Sieve-element Ca^{2+} channels link remote stimuli and sieve-tube occlusion in Vicia faba

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This study addresses the role of Ca^{2+} as a link between propagation of electropotential waves (EPWs) and forisome dispersion in intact legume plants. Here, Ca^{2+}-induced dispersion of forisomes induced by a remote stimulus was used as an optical marker for Ca^{2+} influx during EPW passage as result of Ca^{2+}-channel activity. In response to a number of remote stimuli of different strength and nature, EPWs, activation of Ca^{2+} channels, Ca^{2+} influxes into sieve elements (SEs), and forisome reactions could be correlated semi-quantitatively. The magnitude of initial EPW phase was reduced by La^{3+} and Gd^{3+}, impermeant Ca^{2+}-channel antagonists, comparable with Ca^{2+} influx from the cell wall during EPW propagation. The permeant Ca^{2+}-channel blockers, nifedipine and verapamil had a more marked effect on the prolonged plateau phase which rather suggests interference with intracellular Ca^{2+} channels. All Ca^{2+}-channel blockers inhibited forisome dispersion. Resting levels of cytosolic Ca^{2+} around ~50 nM were measured using both Ca^{2+}-selective electrodes and Oregon Green BAPTA-1 (OGB-1). Transient increases in cytosolic Ca^{2+} were observed in intact phloem tissue in response to remote stimuli, but were below the threshold of forisome dispersion (~30 µM Ca^{2+}). The discrepancy between observed Ca^{2+} changes and Ca^{2+} threshold necessary for forisome dispersion implies that forisome ends must be located close to Ca^{2+} channels. We showed a tight association between forisomes and ER stacks at the EM level. We used high-affinity binding of fluorescent nifedipine (Kd 600 nM) to map the density of Ca^{2+} channels over the SEs. Ca^{2+} channel density was higher in the vicinity of sieve plates and pore plasmodesma units with the lowest densities in central parts of SEs. Fluorochrome studies revealed that Ca^{2+} accumulation and Ca^{2+}-channel patches overlayed the unevenly distributed ER stacks. Ca^{2+} channels that co-localize with the plasma membrane were deployed mainly on the plasma membrane at the companion cells. To reach the functional threshold, Ca^{2+} ions must remain limited to the SE margins, most likely in the unstirred interstices between ER stacks. Insertion of forked forisome ends between the ER stacks and a higher dispersion rate of ER-attached forisomes upon distant burning seem to demonstrate an optimal localization of Ca^{2+} stores and channels for occlusion reactions.

Short-time effects of coumarin along the maize primary root axes

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Coumarin, the simplest allelopathic compound, induces a broad range of effects on plant root morpho-physiology. Recent studies indicate that coumarin selectively inhibites or stimulates growth of individual roots of Zea mays [1] and Arabidopsis thaliana [2]. Nevertheless, coumarin-effects on different zones along the root axes are still missing. Thus, the main objective of the present study was to analyze the responses of three development regions (tip, middle and basal zone) of the primary maize root exposed to localized coumarin concentrations. For this purpose, tip (3 mm), middle (20 mm) and basal regions (50 mm) of primary maize root were exposed to 0, 25, 50 or 100 µM coumarin and the root elongation rate (RER), proton efflux (PE) and plasma-membrane potential response of each root region were evaluated.
The root tip treatment (3mm) with 25 and 50 µM coumarin did not modify the primary root RER, which, instead, was significantly increased by 100 µM coumarin (0.0274 mm/min) respect to the control (0.0146 mm/min). Conversely, the middle and basal zone treatment, at all coumarin concentrations, did not influence the primary root RER. The proton efflux was also enhanced when the root tip was exposed to the coumarin, but this effect was not observed on the other root zones.
An unchanged depolarization phase of plasma-membrane potential was observed in root tip zones, followed by an increased, coumarin concentration-dependent, hyperpolarization phase. In a different way, only an increased depolarization phase was induced by coumarin treatment in the middle root zones, which did not modify plasma-membrane potential of basal root zones.
In conclusion, the results show that tip is the root zone much more sensitive to coumarin. Further, coumarin-induced effect on proton efflux and plasma-membrane potential (hyperpolarization) on root tip could consider, as reported by Zimmermann et al (2009), a third systemic electrical signal in higher plants [3].

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Monoterpene-mediated modulations of *Arabidopsis thaliana* phenotype: effects on stomata, actin-cytoskeleton and on the expression of selected genes

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Monoterpenes are reported to have beneficial effects on plants as they protect them from oxidative damage, increase high temperature tolerance and are essential for plant defence strategies against herbivors. On the other hand, monoterpenes can act as allelochemicals by suppressing the growth of neighbouring plants. Those negative effects are developed during long term exposure and fumigation with higher concentrations of monoterpenes but also by prolonged exposure to volatiles of aromatic plants. Thus, the manifestation of positive or negative responses of a target plant exhibits a dose and time dependency.

In the presented study negative effects of camphor and menthol on 3 three weeks old *Arabidopsis thaliana* used as a target plant were investigated. Two fumigation periods of 96 h with 10 mg camphor / L resulted in phenotypic modifications such as reduced growth, alterations in plant body shape and rosette structure, as well as decrease of the quantum yield indicating a lowered photosynthetic performance.

Camphor and menthol, as well as volatile bouquet of aromatic plants, induced permanently opened stomata which were unable to close. By studying the actin-cytoskeleton of cotyledones of a stably transformed line of *Arabidopsis thaliana* transformed with the actin reporter, the *35S::GFP:FABD* construct, after fumigation with 10 mg / L camphor and 5 mg / L menthol; a reorganisation and partial disruption of F-actin was observed. The aberrant organization of the actin cytoskeleton is supposed to be the major reason for the unability to accomplish closure of stomata after monoterpene fumigation, resulting finally in desiccation and plant death.

The response of *Arabidopsis* plants on fumigation with camphor (10 mg/L) and menthol (5 mg/L) is also characterized by time dependent alterations in the expression of several genes involved in stomatal movement and stress response, such as *RD29B* (At5g52300); *AREB1, AREB2* (accession No. AB017160; AB017161), *CER5* (*At1g51500*); *CER6* (*At1g68530*); *LOX2* (*At3g45140*; *MPK3* (*At3g45640*); *PEPCase* (*At2g42600*).

These results demonstrate the deleterious effects of monoterpenes, when the target plants are permanently exposed to higher concentrations as they appear naturally; for instance in the *Citrus* orchards or in the High Chaparral vegetation of South California

Effects of acetylcholine on blue-light response of dark-grown Arabidopsis seedlings: nutrition, light quality, and the effect of mutations in the pigment cryptochrome

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Dark-grown (etiolated) Arabidopsis plants grow rapidly in the dark, and this high growth rate is rapidly inhibited in response to blue light. The pigment cryptochrome has been implicated in this pathway. The earliest response to light is a transient depolarization, which has been proposed to be due to activation of a chloride channel in a ligand-gated channel family (Lewis & Spalding, 1998). They report that blocking this channel function prevents the light-dependent growth inhibition. At a previous meeting (Slovakia, 2007) we reported that the neurotransmitter acetylcholine (ACh) prevented the light-dependent depolarization and increased decreased the inhibitory effect of light. We suggested that ACh (or a related endogenous molecule) could act as a ligand that modulates the effect of light on the channel in question. We report here further experiments that eliminate some confounding possibilities. We employ blue light instead of white, to narrow range of pigments that might be responsible. We also minimize the use of green as a safe light, since it has been suggested to be antagonist to blue light (Folta 2004). We investigated the possibility that the nitrogen in choline might be acting as a nutrient in increasing plant growth. Choline alone had less effect than ACh, which suggests that this is not true. We also utilized more nutrient-rich media, to relieve any nitrogen limitation on growth. In all cases, we show that ACh inhibits the growth response to light. We also utilize double mutants in the two cryptochrome genes implicated in the pathway (cry1cry2) to determine whether the effect is due to inhibition of the cryptochrome-initiated pathway or a competing pathway that masks the cryptochrome response. We explore models of the mechanism of ACh action, taking into account new models of broader activities of ACh in animals.

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Lectins as determinants of cell recognition in cyanolichens through *Peltigera canina*

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Lichen are symbiotic between a fungus and a cyanobacterium (cyanolichens) or green alga (chlorolichens), joined to form a new biological entity different from its individual components. Both bionts appear in nature among a mixture of millions of non-symbiotix microorganisms, and mechanisms of compatible combination are required. Thus, specificity is required for the lichen association. Specificity can be defined in this context as the preferential, but not exclusive, association of a biont with another. Recognition of compatible algal cell is carried out by specific lectins produced and secreted by the potential mycobiont. Some lectins from phycolichens have been characterized as glycosylated arginases which bind to an algal cell wall receptor indentified as an α-1.4-polygalactosylated urease. The binding is improved by Ca^{+2} and Mn^{+2} in a similar way to that described for legume lectins. Two forms of glycosylted arginases seem to be involved in cell contact between phyco- and mycobiont, whereas a second, secreted arginase produces recruitment of compatible algal cells near the surface of fungal arginase is internalised, increasing the levels of algal putrescine, which promotes chloroplast disorganization, activation of glucanases and breakdown of the cell wall with loss of the prototoplast. Evolution of symbiotic relationships implies then the synchronization of cell division and lectin receptor production, probably as a consequence of the perception of environmental factors. The structural and functional similarities between lectins from cyano- and phycolichens, it could be expected that the recognition mechanism in phycolichens could be expanded to cyanolichens.

We worked with the cyanolichen *Peltigera canina* which contain a blue-green alga (a Nostoc sp.). This lichen secreted arginase ti the incubation media, that was labelled with FITC showing lectin activity, since it bind to the cell wall cyanobionts, apparently using a polygalactosylated urease as specific cell wall ligand. Desorption of arginase from the algal cell wall is achieved by 50 mM α- D-galactose, indicating that lectin use the same polygalactosilated ligand that foun in chloroplichens. In addition, other receptors can be developed by both photobionts since concanavalin A, a lectin from *Canavalia ensiformis* specific from α- D-glucose and α- D-mannose also binds to several cyanobionts and chlorobionts. The nature of the ligand, definided as glycosylated urease, has been confirmed by affinity chromatography using a stationary phase constituted by arginase immobilized on agarose, resulting in the elution of only one fraction enriched in urease activity. Urease was cytochemically located by using recently isolated cyanobionts from *P. canina* thalli incubated with 40 mM urea and 10mM cobalt choride. Images obtained show that black deposits of cobalt carbonate, revealing urease activity, are mainly located at the cell wall of cyanobionts, although the occurrence of some polydisperse, small granules inside the cells indicates intracellular urease activity.

The binding of fungal arginase to glicosylated urease in the algal cell wall implies an affinity mechanism between some specific amino acid residues of the lectin and specific sugar residues of the glycoside moiety of the ligand. In any way, the ability of fungal lectins developing arginase activity seems to be functional not only for chlorobints but also for cyanobiont containing thalli

Scald-susceptible cultivars of sugarcane promote signalling to induce the synthesis of a virulence factor in *Xanthomonas albilineans*

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Leaf scald is a vascular disease of sugarcane plants caused by *Xanthomonas albilineans*. Scalded leaves show white-yellowish streaks alternating with green zones in parallel to the main veins. The white-yellowish streaks show both phloem and xylem completely occluded by the gum and the overall mesophyll appears to be full of this bacterial secretion, as revealed by scanning electron microscopy. The gum in conducting tissues has been purified from juices obtained from scalded stalks and it was identified as a xanthan-like polysaccharide composed by repeated tetrameric units containing two rests of fructose, one of mannose and one of glucuronic acid. Hydrolysis of xanthan with selective mannosidases and β-1,4-glucanases reveals that the macromolecule consists of a linear, β-1,4-backbone of β-glucose units to which mannose in β-1,3 bonds is linked.
Since xanthans contain glucuronic acid, the ability of *Xanthomonas* to produce an active UDP glucose dehydrogenase is often seen as a virulence factor. X. albilineans produces a UDP-glucose dehydrogenase growing on sucrose. The enzyme oxidizes UDP-glucose to UDP-glucuronic acid by using molecular oxygen and NADPH. Kinetics of enzymatic oxidation of NADPH is linearly dependent on the amount of oxygen supplied. N-Terminal sequence has been determined as IQPYNH.
X. albilineans axenically cultured does not secrete xanthans to liquid media but they are produced in inoculated sugarcane tissues. This host-dependence can be explained on the basis of the action of bacterial proteases upon the dehydrogenase. In vitro enzymatic assay of UDP-glucose dehydrogenase from X. albilineans requires the addition of a protease-inhibitors cocktail to cell-free extracts, since bacterial proteases rapidly hydrolyses the enzyme in solution. The addition of low amounts of 8-azaguanine and chloramphenicol to the culture medium do not impede the production of the dehydrogenase that requires concentrations higher than 0.3 mM of both antimetabolites to inhibit its synthesis, concentration that is sufficient to inhibit the production of proteases. Glycoproteins from sugarcane, the natural host of the bacterium, that are produced as a response to the secretion of bacterial elicitors, also assure the production of the active enzyme by inhibiting bacterial proteases.

Cytology of compatible and incompatible hyphal interactions in arbuscular mycorrhizal fungi

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Arbuscular mycorrhizal (AM) fungi are 400 million years old obligate biotrophs, which live symbiotically in the roots of most land plants. Fossil records and DNA sequence data confirmed that these ancestral organisms have coevolved with their host plants since the early colonization of land. Efficient survival strategies are active in AM fungi, i.e. a wide host range, the ability of multiple germination, chemotropic guidance to host roots, hyphal morphogenesis elicited only by host-derived signals. Further mechanisms evolved by AM fungi to increase their fitness involve the phenomenon of hyphal fusion (anastomosis), which occurs widely between hyphal tips belonging to the same individual and to different individuals of the same isolates (Giovannetti et al., 1999; 2003; 2004). Recently, anastomoses were also detected in interactions between hyphae belonging to genetically different isolates of Glomus intraradices (Croll et al., 2009). Living-cell microscopy revealed protoplasmic streaming in anastomosing hyphae, while viability staining showed protoplasmic continuity and nuclear migration in perfect fusions.

Hyphal tips of individuals belonging to different species and to geographically different isolates of G. mosseae never underwent anastomoses: most hyphal contacts led to intermingling without interference and a few interactions showed pre-fusion incompatible responses. The main feature of pre-fusion incompatibility was represented by growth arrest followed by protoplasm withdrawal and septa formation in contacting hyphae, prior to or after interactions. Pre-contact tropism and growth reorientation suggested the involvement of specific signals guiding early hyphal recognition events, occurring before incompatible interactions.

Analyses of interactions among genetically different isolates of G. intraradices showed some perfect fusions and pre- and post-fusion incompatible responses, characterised by hyphal protoplasm withdrawal following anastomosis, with septa formation between involved hyphae.

The experimental system used allowed the detection of cytological changes during compatible or incompatible interactions among AM fungal populations and of their ability to undergo genetic recombination, fundamental for the maintenance of AM fungal diversity.

Lectins as determinants of cells recognition in cyanolichens through peltigera canina

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Lichen are symbiotic between a fungus and a cyanobacterium (cyanolichens) or green alga (chlorolichens), joined to form a new biological entity different from its individual components. Both bionts appear in nature among a mixture of millions of non-symbiotix microorganisms, and mechanisms of compatible combination are required. Thus, specificity is required for the lichen association. Specificity can be defined in this context as the preferential, but not exclusive, association of a biont with another. Recognition of compatible algal cell is carried out by specific lectins produced and secreted by the potential mycobiont. Some lectins from phycolichens have been characterized as glycosylated arginases which bind to an algal cell wall receptor indentified as an alfa-1.4-polygalactosylated urease. The binding is improved by Ca^{+2} and Mn^{+2} in a similar way to that described for legume lectins. Two forms of glycosylted arginases seem to be involved in cell contact between phyco- and mycobiont, whereas a second, secreted arginase produces recruitment of compatible algal cells near the surface of fungal arginase is internalised, increasing the levels of algal putrescine, which promotes chloroplast disorganization, activation of glucanases and breakdown of the cell wall with loss of the prototoplast. Evolution of symbiotic relationships implies then the synchronization of cell division and lectin receptor production, probably as a consequence of the perception of environmental factors. The structural and functional similarities between lectins from cyano- and phycolichens, it could be expected that the recognition mechanism in phycolichens could be expanded to cyanolichens.

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Towards functional characterization of plant class II formins: first lessons from outliers.

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Plant cells are able to diversify their surface by re-structuring their cytoskeleton and directing exocytosis to distinct portions of the cell cortex, which we have recently termed \"activated cortical domains \" (ACDs; Zárský et al., New Phytologist, in press). ACD diversification is a central mechanism of plant cell morphogenesis, contributing also to cell and tissue polarity, which is, in turn, crucial for developmental processes, including but not limited to exploratory growth both on the level of single cells (e.g. root hairs or pollen tubes) and whole organs. A number of proteins that may participate in ACD diversification have been identified, including members of several large protein families such as e.g. Rop GTPases and their interactors, subunits of the exocyst complex, receptor-like kinases, enzymes locally modifying membrane composition, or actin-organizing FH2 proteins. Combinatorial use of paralogs could thus contribute to generation of ACD diversity.
We are focusing on members of one such a large protein family – the formins (FH2 proteins). Formins of higher plants can be divided into two clades; according to publicly available data (www.arexdb.org), some representatives of both clades exhibit differential gene expression between arabidiopsis trichoblasts and atrichoblasts, i.e. between cell types that differ by the presence of a single specific ACD. For one of the plant formin clades, Class I, several experimental reports have confirmed a role in actin nucleation and other aspects of cytoskeletal organisation, previously documented for yeast and metazoan formins. However, plants possess also a second, considerably divergent and hitherto uncharacterized, FH2 protein group, the Class II formins, whose members exhibit complex gene structure and usually low expression levels. Since experimental study of formin function is hampered by genetic redundancy within the large gene family, we have focused on two arabidopsis Class II formins that represent outliers of this gene family in terms of either domain structure (AtFH16) or overall sequence divergence (AtFH12). We have cloned a cDNA encoding AtFH16, and verified that AtFH12 is expressed under conditions predicted from publicly available microarray data. Heterologous expression of AtFH16 in budding yeast leads to a phenotype suggesting interference with endogenous formin function. Disruption of the AtFH16 gene does not produce an obvious phenotype, indicating functional redundancy within the Class II formin family. However, T-DNA insertion within AtFH12 inhibits root growth and aggravates the toxic effects of GFP-tagged mouse talin (GFP-mTalin) expression, known to cause extensive actin bundling. Taken together, these observations suggest that, despite substantial sequence divergence, plant Class II formins retained the conserved function in cytoskeletal organisation, shared with other FH2 protein clades.

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Involvement of auxin-binding proteins and auxin in response of maize seedling to blue light

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Auxin-Binding Protein 1 (ABP1), a putative auxin receptor has been extensively studied. It was found that in Arabidopsis, ABP1 is essential for embryo development, and it participates in auxin-mediated cell elongation in different species. In maize, several ABPs have been identified but their roles are still not understood. The aim of this study is to contribute to the understanding of the role of maize ABP1 and ABP4 during growth and development, with special reference to seedlings developed in blue light (BL). We have observed, that BL decreases the level of free IAA in maize aerial organs. Using maize abp1 and abp4 single mutants, and the abp1abp4 double mutant we have found that ABP1 and/or ABP4 regulate this BL-induced response. However, extent of the elongation of coleoptile and mesocotyl in BL does not correlate with the levels of free IAA. Interestingly, we observed that BL inhibits root elongation in WT plants, but not in abp1, abp4 single and double mutants. Our results indicate that in maize, ABPs positively influence elongation growth of etiolated seedlings, and that ABP1 and ABP4 are involved in BL-signaling pathway that regulates auxin accumulation. Additionally, the data suggest that ABP1 and ABP4 are engaged in BL-induced inhibition of root elongation.

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Light alters plant elongation responses to auxin

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Despite the recent advances, many fundamental issues of interaction between light and hormone signaling pathways involved in plant growth remain to be uncovered. In model plants Arabidopsis, tomato and maize we investigated effects of light on plant growth responses to exogenous auxins. In conditions in vitro and in the dark, blue light (BL) and red light (RL), NAA and 2,4-D in concentration-dependent manner reduced elongation of hypocotyl in intact Arabidopsis and tomato plants, and reduced coleoptile growth in intact maize seedlings. When Arabidopsis WT plants developed in BL, hypocotyl responsiveness to auxin strongly diminished, whereas in RL, the hypocotyls responded to auxin similarly like in the dark. Weak responses to exogenous auxin under BL were also observed in Arabidopsis mutants, cry2 and hy2. Unlikely, mutant cry1 grown in BL responded to auxin in the same extent like in the dark. These data indicate that functional photoreceptor CRY1 is involved in BL-induced reduction of hypocotyl responsiveness to exogenous auxin. In tomato, NAA inhibited hypocotyl elongation significantly more in etiolated plants than in BL- and RL-grown seedlings. Low seedling responsiveness to NAA in BL and RL was associated with dramatically higher levels of free IAA in light-grown seedlings in comparison with etiolated plants. Compared to corresponding WT, etiolated seedlings of 7B-1 mutant affected in BL responses exhibited resistance to NAA-induced inhibition of hypocotyl elongation. The response was not affected by BL or RL, and it was associated with weak BL- or RL-induced augment in the level of endogenous IAA. Other analyses suggest that BL- and RL-induced reduction of sensitivity to the inhibitory effect of NAA is mediated by photoreceptor CRY1. Surprisingly, BL amplified hypocotyl sensitivity to the inhibitory effect of 2,4-D, and the response was not affected by cry1 mutation. These data suggest that the responsiveness of tomato hypocotyls to the inhibitory effects of NAA and 2,4-D is likely regulated by different mechanisms. In older dense-sensitive maize hybrids, relative inhibition of coleoptile elongation by NAA in intact seedlings was essentially smaller in BL or RL than in the dark. Interestingly, the lower responsiveness of coleoptiles to NAA in BL correlated with distinct decline in the level of free IAA in BL-grown seedlings. In comparison with old hybrids, coleoptile in modern dense-resistant hybrid was less sensitive to NAA in the dark, which corresponded with low level of IAA found in etiolated coleoptiles. In opposite, coleoptile in the modern hybrid grown in BL was more sensitive to NAA than that in the older maize lines. It was associated with higher amount of free IAA in BL-grown coleoptiles in modern than in older hybrids. The data support our hypothesis that in maize, the selection of modern hybrids led to the alteration in interaction between light and auxin signaling involved in elongation growth. Finally, analysis of elm1, a phytochrome-deficient mutant in maize indicated that phytochromes are involved in BL- and RL-induced reduction of coleoptile growth responses to exogenous auxin. Our results confirmed the existence of interaction between light and auxin signaling in plant growth. Analyses also suggest the existence of diverse mechanisms of the cross-talk between light and auxin in different plant species.

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Shotgun proteomics of protein complexes using mass spectrometry.

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Interactions between proteins are crucial for most of the molecular processes in cells.
Therefore, identification of protein-protein interaction will yield a better understanding of the cellular machinery on the molecular level. However, the development of such networks for plant proteins has been slowed, because there was no useful high-throughput technique.
To address this challenge, we have developed a powerful method to identify the intracellular protein network using LTQ-Orbitrap XL.
In this study, complex proteins were purified using affinity column coupled with antibody. The protein samples were directly digested in solution, and then performed shotgun analysis.
We would like to discuss about usefulness of this method.

Reaction of a light-induced chloroplast movement to local and systemic stimuli and relation to photoinhibition.

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Light-induced chloroplast movement in leaves is a very conservative and wide spread phenomenon in plant kingdom. Chloroplast movement is assumed to protect chloroplasts against photoinhibition. We have studied this movement in leaves of tobacco (Nicotiana tabacum cv. Samsun) in relation to local and systemic stimuli and to photoinhibition processes.
The local stimulus was realized by strong light, the systemic one by strong light or burning. Two methods of chloroplast movement detection have been developed. The first method is based on the application of the SPAD chlorophyll-meter. The other method combines cold white light, optical filters and light guides with spectral radiometer LI-1800. The method is called transmittance in partially collimated light Tc, because only apart of the transmitted light is detected. A stronger light (above 400 µmol photons m-2 s-1) caused the movement of chloroplasts to the anticlinal cell walls and the light of lower intensity (50 µmol photons m-2 s-1) causes reverse changes. Successive application of the two light intensities enabled to study the chloroplast movement in an oscillating regime. The state of photosynthetic apparatus at the thylakoid membranes in the chloroplasts was detected by the fluorescence induction method (O-J-I-P curve)
As far as systemic stimuli are concerned, it has been shown that systemic stimuli do not influence the light induced chloroplast periodic movement in a detectable range. The light was an essential signal governing the movement with no detectable influence of long-distance signaling pathways. This indicates a control of this motion at the intracellular level.
On the other hand, the local irradiance by light of two intensities (400 and 800 µmol photons m-2 s-1 of PAR) and three spectral regions (blue, white, red) has indicated an effect of light intensity and spectrum on both chloroplast movement and photosynthetic performance. The red light did not induce the chloroplast movement but caused changes in the maximal photochemical efficiency of photosystem II (Fv/Fp) similar to those caused by white and blue light.
Concerning the relation of chloroplast movement and photoinhibition processes, we have found no component of changes in Fv/Fp correlating with the chloroplast movement. Two fast phases of Fv/Fp changes, a decrease in strong light and an increase in lower light, were the same for blue (chloroplast movement) and red (no chloroplast movement) lights. Thus, the detected changes of Fv/Fp are of photochemical origin. A part of the Fv/Fp decrease could be ascribed to a photoinhibitory effect. The weak blue light was more effective in a long lasting regeneration phase (100 to 300 minutes) in which no detectable chloroplast movement was registered. Chlorophyll fluorescence gives information only about the state of chloroplasts located close to the illuminated surface. These chloroplasts were exposed to the same photoinhibitory damage independently on their movement.
Our data indicate a pivotal role of the intensity and spectral composition of the incident light on chloroplast movement of tobacco instead of an effect of a systemic signal. Furthermore, it seems that chloroplast movement regulates the light distribution in the leaf but does not protect the exposed chloroplasts from photoinhibition.

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Blebbistatin inhibits chemotactic response of smut teliospores towards high and middle molecular mass glycoproteins present in sugarcane juices

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Sporisorium scitamineum (Syd.). Piepenbr. & Oberw. (= Ustilago scitaminea Sydow & P. Sydow), wich causes sugarcane smut, is one of the most important pathogens of this crop. It produces important looses in yields. This phytopathogen is a basiodiomycete belonging to the order Ustilaginales. Sugarcane smut was one of the first diseases of sugarcane to be recognized because of the conspicuous whip-like sorus produced by the fungus in infected plants. Sori arise either from the terminal meristem or from side shoots of infected stalks and contain a central core of parenchyma and vascular host tissue around which a thin cylinder of teliospores is produced. The dycariotic mycelium is the pathogenic cell type of the life cycle. It exhibits mycelial growth and colonizes meristematic tissues. Thus, colonization of the stalks takes place and diploid teliospores are formed in the host tissues. The ability of sugarcane glycoproteins to induce teliospore cytoagglutination has been reported yet. This ability is related to de capacity of high and middle molecular mass glycoproteins (HMMG and MMMG) obtained from sugarcane juices to bind selectively to smut teliospore wall receptors. After binding, cell aggregation occurs in parallel to a loss of the germination ability of recruited teliospores. However, the interaction between glycoproteins and receptors present in the smut teliospore wall is different depending on the resistance of the cultivar to smut. HMMG and MMMG from Mayarí, resistant cultivar, show high affinity to bind N- acetyl-D-glucosamine residues in glycoproteins of the spore wall whereas HMMG and MMMG from Barbados, sensitive to infection, interact less efficiently. Therefore, production of HMMG and MMMG and interaction with receptors present in teliospore wall is a plant response to smut disease that proves how specific and complex is the relation between host and pathogen.

Chemotaxis is involved in a wide variety of biological processes including host-pathogen recognition. Smut fungus teliospores are chemotactically sensitive to HMMG and MMMG. Spores suspended in distilled water are able to move towards sugarcane juice fractions containing HMMG or MMMG. This phenomenon has been reported in two cultivars, Mayarí, resistant, and Barbados, sensitive to smut. In both cases movement of teliospores occurs and is in Barbados where a higher chemotactic index has been obtained. On the other hand, teliospores are more sensitive to HMMG than MMMG. The aim of this study is to verify a direct role of cytoskeleton in this response of teliospores to chemoattractants such as HMMG and MMMG. To achieve this objective, teliospores were treated with blebbistatin, a specific inhibitor of myosin II-dependent cell processes. A suspension of teliospores on blebbistatin was added to a plate where a sealed capillary containing glycoproteins at different concentrations had been inserted. After 15 hours, capillary content was observed under light microscopy. Photographs were obtained in order to quantify the amount of teliospores that had moved toward HMMG and MMMG fractions. In addition, we have assessed effect of blebbistatin upon cell polarization by fluorescence microscopy to verify a direct role of blebbistatin in the reorganization of cytoskeleton that take place previous chemotactic displacement.

Regulation of aquaporin by protein phosphorylation in fruit and flower

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Aquaporins are responsible for water transport across biomembranes and play a crucial role in the regulation of water status in plants. Recent reports showed that aquaporins transport substances other than water, such as glycerol, urea, ammonium, boron, silicon, arsenate, carbon dioxide, hydrogen peroxide and nitrogen monoxide. Therefore aquaporin function not only for water transport but also for many solute transports. More than 30 aquaporin genes were identified in Arabidopsis and rice. Plant aquaporins are classified into 4 groups: plasma-membrane intrinsic protein (PIP), tonoplast intrinsic protein (TIP), Nodulin 26-like intrinsic protein (NIP) and small basic intrinsic protein (SIP). PIPs and TIPs are responsible for water transport across plasma membrane and vacuolar membrane, respectively. PIPs are further classified into two subgroups, i.e. PIP1 and PIP2. PIP2 has higher water transport activity than PIP1.
Our interests are regulation and function of aquaporins in horticultural crops, such as fruits and flowers. Gene expression and protein level of PIPs and TIPs were detected in pear fruit development. Gene expression and protein level of TIPs were especially high in young fruit and dramatically decreased in the middle of fruit development. On the other hand, gene expression and protein level of PIPs were almost constant in fruit development. Therefore we considered that water transport PIPs is regulated by post-translational regulation.
Post-translational modification of aquaporins has been suggested, such as phosphorylation, methylation, glycosylation, oligomerisation, vesicle trafficking and non-covalent protein modification by pH and calcium ion. It has been suggested that water transport activity of PIP2 is increased by phosphorylation and decreased by dephosphorylation. So we focused to protein phosphorylation of PIP2 in this study. We tried to prepare phospho-specific antibodies against putative phosphorylation sites, i.e. Ser115 and Ser280. Although the phospho-specific antibody against Ser115 did not react to microsomes from pear fruit, that against Ser280 recognizes phosphorylated Ser280 specifically. We determined phosphorylation state in fruit, suspension cells and flower using this antibody. Phosphorylation state of PIP2 in pear fruit changed dramatically with fruit diurnal growth. Phosphorylation state of PIP2 decreased in pear suspension cells one hour after salt and osmotic stress treatments. Phosphorylated PIP2 increased with flower opening of Japanese morning glory. These results show the presence of phosphorylational regulation of aquaporins in many events of plants, including fruit growth and flower opening.

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Ozone-inducible glycine-rich peptides as plant prions?

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Ozone-inducible (OI) genes have been isolated from *Atriplex canescens* (saltbush) and shown to be responsive to exposure to ozone, SO_2 and water deficit. OI peptides contain repeat sequence (YGHGGG) tandemly repeated for 8-10 times, which tightly binds copper. The putative role for OI has been proposed to be active anti-oxidants by chelating the redox-active metals.
Our previous study concerning the action of prion protein has revealed that the copper-binding regions in prion protein (such as VNITKQHTVTTTT) interacts with free tyrosine or tyrosine-containing peptides (including tyrosine-rich sequence within prion protein) in the presence of H_2O_2, finally leading to robust generation of superoxide. We found that tyrosine-residues on peptide sequences contribute as a substrate for this peroxidative ROS generating reaction. The key common structure among the redox-active peptide sequences found in mammals and chicken is the presence of Cu-anchoring His residue(s) placed at the vicinity of glycine-rich sequences.
In the present study, we examined the peroxidative catalytic activity of the OI peptides leading to generation of superoxide and break-down of H_2O_2. Here the generation of superoxide was assessed with the superoxide a specific chemiluminescence of *Cypridina* Luciferin Analog (CLA). This result suggests that the OI peptides in plants may play role as both pro-oxidants and anti-oxidants thus the fate of plant cells under oxidative stress.

Role of auxin, auxin-binding proteins and light in the development of maize seedlings

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Modern maize (*Zea mays L*) hybrids developing erect leaves show less sensitivity to dense planting and reduced responsiveness to auxin and light in comparison to older, density sensitive varieties. In addition it has been found that in modern maize hybrid the expression of Auxin-Binding Protein 4 (ABP4), a hypothetical auxin receptor, is impaired. In this study we investigated the role of auxin, ABPs and light in maize growth and development. In vitro, etiolated maize mutants affected in ABP1 and/or ABP4 genes showed less sensitivity to auxin in comparison to wild type (WT). Analysis of endogenous auxin in etiolated coleoptiles revealed that abp1/abp4 double mutant contained the highest concentration of free IAA, indicating that ABP1 and ABP4 cooperate in decreasing the content of free IAA in this maize organ. Interestingly, we found that in abp4 and abp1/abp4 double mutant red light (RL) strongly promoted elongation of coleoptile whereas in WT and abp1 RL did not have any effect on coleoptile elongation. In the greenhouse (in vivo), abp1 and abp4 single mutants developed more and less erect leaves, respectively, while abp1/abp4 double mutant had leaves less vertical than WT. abp4 plants developed the tallest stature with longer leaves than WT, although abp1 mutant developed the longest and the widest leaves. Our data suggest that auxin regulates development of maize seedlings through ABPs and that ABP4 mediates RL-induced inhibition of coleoptile elongation. The data support the existence of interaction between auxin and light in maize growth and development. In addition, these findings suggest the involvement of ABPs in leaf angle development, as well as an important role of ABP1 in maize leaf morphology.

Key words: maize (Zea mays L), plant development, light, auxin, Auxin-Binding Proteins (ABPs).

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Role of acetylcholine in plant cell elongation

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We have investigated the role of acetylcholine (ACh) in cell elongation, and the possible influence of ACh on the action of auxin in its plant cell growth-regulating activity.
Hypocotyl segments represent an ideal experimental system to look for the contribute of different factors influencing some plant cell behaviour. Tomato (*Solanum lycopersicon*) hypocotyls elongate when treated with auxin (acid growth theory), and this auxin induced elongation correlates with the expression of an expansin gene (*LeEXPA2*) (Catalá et al. 1997, Caderas et al. 2000), member of a multigene family of extracellular proteins that mediate cell wall extension and relaxation during growth (McQueen-Mason et al. 1992; McQueen-Mason and Cosgrove 1994, Cosgrove 2000). We explored the possible signaling role of ACh in mediating the auxin-induced expansin gene expression. We cut 1 cm long hypocotyl segments just below the apical hook of 5 days old etiolated tomatoes, and treated them with different combinations of ACh 50 uM, sucrose, and auxin 5 uM.
After 2h of treatment, ACh seems to barely induce expansin mRNA expression (about 1,5 fold change with respect to control), although when ACh is supplied with 2% sucrose, LeEXPA2 transcript expression is clearly induced (about 4 fold). But the most interesting result cames out when ACh and auxin are supplied together. Their action is more than additive: where auxin alone determines a 16-fold expansin transcription, the transcript abundance grows up to 39 times when auxin is supplied with ACh. More than this, when sucrose is present in the medium, there is the major induction registered: about 50-fold change with respect to control. These results deserve to be more carefully investigated because of the potential of their implications, and at the same time they open new perspectives and possibilities in unravelling the role of sugars as signalling molecules.

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The model of cell recognition in phycolichens through a fungal lectin that binds to an algal ligand could be expanded to cyanolichens.

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Lichens are intimate and long-term symbioses of photosynthetic, unicellular algae or cyanobacteria and heterotrophic fungi joined to form a new biological entity different from its individual components. Specificity required for the lichen association can be defined in this context as the preferential, but not exclusive, association of a biont with another. Recognition of compatible algal cells is carried out by specific lectins produced and secreted by the potential mycobiont. However, lichen phenolics are not involved in the recognition process, in contrast to that found for other plant symbioses, such as mycorrhizal or Rhizobium legume associations.
Some lectins from phycolichens have been characterized as glycosylated arginases which bind to an algal cell wall receptor identified as an α-1,4-polygalactosylated urease. The binding is improved by Ca^{2+} and Mn^{2+}, in a similar way to that described for legume lectins. Two forms of glycosylated arginases seem to be involved in the recognition process, one of them, particulated in the cell wall of fungal hyphae and involved in cell contact between phyco- and mycobiont, whereas a second, secreted arginase produces recruitment of compatible algal cells near the surface of fungal hyphae. When glycosylated urease is lacking from the algal cell wall, fungal arginase is internalized, increasing the levels of algal putrescine, which promotes chloroplast disorganization, activation of glucanases and breakdown of the cell wall with loss of the protoplast. Evolution of symbiotic relationships implies then the synchronization of cell division and lectin receptor production, probably as a consequence of the perception of environmental factors (light and temperature).
In this work we attempt to validate this arginase-urease interaction as the universal base not only for phycobionts but also for cyanobionts recognition by using a cyanolichen from the Collemataceae family: Leptogium corniculatum (Hoffm.) Minks, containing Nostoc as cyanobiont. It has been found that recently collected thalli of L. corniculatum secrete arginase after 2h of incubation on 40 mM arginine. The enzyme is efficiently retained by activated agarose beads on which galactosylated urease from the phycolichen Evernia prunastri has previously been attached. Leptogium arginase is completely eluted from the bead using 50 mM D-galactose. This implies that an interaction between the cyanolichen lectin and the phycolichen ligand would justify the recognition process when the cyanobiont was able to synthesize urease and to retain a part of this galactosylated enzyme attached to its cell wall.
In addition, Nostoc cells showed intense emission of green fluorescence when they were incubated with secreted arginase from Leptogium labelled with FITC (Fluorescein Isothiocyanate). Similar results were obtained when phycobionts recently isolated from E. prunastri were incubated with FITC-arginase from Leptogium, although fluorescence emission was strongly lower than that observed from the homologous cyanobacteria. Desorption of the lectin from the corresponding cell wall was achieved by incubation of FITC-lectin-labelled cells with 100 mM D-galactose for 1 h, resulting in almost total recovery of fluorescence in the supernatant.

Scald-susceptible cultivars of sugarcane promote signalling to induce the synthesis of a virulence factor in *Xanthomonas albilineans*.

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Leaf scald is a vascular disease of sugarcane plants caused by *Xanthomonas albilineans*. Scalded leaves show white-yellowish streaks alternating with green zones in parallel to the main veins. The white-yellowish streaks show both phloem and xylem completely occluded by the gum and the overall mesophyll appears to be full of this bacterial secretion, as revealed by scanning electron microscopy. The gum in conducting tissues has been purified from juices obtained from scalded stalks and it was identified as a xanthan-like polysaccharide composed by repeated tetrameric units containing two rests of fructose, one of mannose and one of glucuronic acid. Hydrolysis of xanthan with selective mannosidases and β-1,4-glucanases reveals that the macromolecule consists of a linear, β-1,4-backbone of β-glucose units to which mannose in β-1,3 bonds is linked.
Since xanthans contain glucuronic acid, the ability of *Xanthomonas* to produce an active UDP glucose dehydrogenase is often seen as a virulence factor. X. albilineans produces a UDP-glucose dehydrogenase growing on sucrose. The enzyme oxidizes UDP-glucose to UDP-glucuronic acid by using molecular oxygen and NADPH. Kinetics of enzymatic oxidation of NADPH is linearly dependent on the amount of oxygen supplied. N-Terminal sequence has been determined as IQPYNH.
X. albilineans axenically cultured does not secrete xanthans to liquid media but they are produced in inoculated sugarcane tissues. This host-dependence can be explained on the basis of the action of bacterial proteases upon the dehydrogenase. In vitro enzymatic assay of UDP-glucose dehydrogenase from X. albilineans requires the addition of a protease-inhibitors cocktail to cell-free extracts, since bacterial proteases rapidly hydrolyses the enzyme in solution. The addition of low amounts of 8-azaguanine and chloramphenicol to the culture medium do not impede the production of the dehydrogenase that requires concentrations higher than 0.3 mM of both antimetabolites to inhibit its synthesis, concentration that is sufficient to inhibit the production of proteases. Glycoproteins from sugarcane, the natural host of the bacterium, that are produced as a response to the secretion of bacterial elicitors, also assure the production of the active enzyme by inhibiting bacterial proteases.

System potentials, a novel electrical long distance apoplastic signal in plants induced by wounding

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Systemic signalling has been investigated on both a dicot (*Vicia faba L.)* and a monocot (*Hordeum vulgare L.*) plant. Stimuli were applied to one leaf (S-leaf) and apoplastic responses were monitored on a distant leaf (target; T-leaf) with micro-electrodes positioned in sub-stomatal cavities of open stomata. Cut-injury of leaves and subsequent addition of a variety of different cations caused voltage transients at the T-leaf, which are neither action potentials nor variation potentials: with respect to the cell interior the initial polarity of these voltage transients is hyperpolarizing; they do not obey the all-or-none rule, but depend on both concentration and type of the added substance, and propagate at 5 to 10 cm/min. It is argued that this response is due to stimulation of the plasma membrane H^+-ATPase, a notion supported by the action of fusicoccin which also causes such voltage transients on the T-leaf, whereas ortho-vanadate prevents its propagation. Moreover, apoplastic ion flux analysis reveals that, in contrast to action- or variation potentials, all of the investigated ion movements (Ca^{2+}, K^+, H^+, Cl^-) occur after the voltage change has started. We suggest that these wound-induced 'system potentials' represent a new type of electrical long distance signaling in higher plants.

Microgravity alters the expression of actin and tubulin genes, of some ROS scavenging factors, and of the auxin transport genes *aux1* and *eir1* in *arabidopsis*

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Microtubules are common to all eukaryotic organisms, are higly conserved, and display specialized biological functions. Microtubules are organized in different patterns, relying on a variety of mechanisms for assembly, orientation and function. Recent studies suggest that cells may sense mechanical stimuli, including those due to gravity, through changes in the balance of forces in the cytoskeleton. A study of gene expression in simulated microgravity conditions (Random Positioning Machine, is a laboratory facility equipped to randomly change the gravity vector of an accommodated experiment in a 3-dimensional space), made through qPCR, was performed on seedlings of *Arabidopsis thaliana*, CV *Landsberg*, and two auxinic mutants (*aux1, eir1*) of the same plant. The plants were kept in microgravity for different periods of time: 3, 6 and 24 hours, then stored in RNAlater, and subsequently subjected to mRNA extraction. With the mRNA a qPCR analysis was performed, revealing the expression of the chosen genes involved in actin and tubulin synthesis, in senescence processes and auxin transport. The studied genes were actin (*ACT8*), tubulin α (*TUA4, TUA6*), tubulin β (*TUB2*), *FeSOD, CAT1, CAT2, AUX1, EIR1*). The results indicated that *ACT8, TUA4 TUA6* and *TUB2* are notably upregulated by microgravity, even though the effect appeared only after a 3 hour run of the seedlings with fluctuation of their expressions, while *AUX1* and *EIR1* showed a strong upregulation in the transcript levels after 6 hours. The effect on cell perturbation was seen also on genes notoriously activated by oxidative stress, such as *FeSOD*, *CAT1*, and *CAT3*, which are responsible for ROS scavenging at the chloroplastic and peroxisomal level. These genes were up regulated as the previous ones, but only after 24 hours. This results suggest that when the ultrastructure of the cells starts to be compromised, as it is indicated by the effect on the actin and tubulin genes, also the plant cell metabolism is compromised, and ROS-scavenging proteins are activated.

Serotonin and melatonin influence somatic embryogenesis in *Coffea canephora* P.ex.Fr.

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Melatonin(MEL)and Serotonin (SER) are prominent indoleamines which participate in neural transmission in animals. They are also found to occur in various genera of plants. The precise function of these compounds in plant system at physiological level has not been worked out so far. In the present study, the effect of SER, MEL and calcium channel activator calcium ionophore (A23187) on somatic embryogenesis was investigated in *in vitro* cultures of *Coffea canephora*. Embryogenic callus cultured on EG (embryogenic) medium comprising MS basal salts (Murashige & Skooge 1962) along with IAA+BA, only 5% explants responded for embryogenesis. SER/ MEL at 100 µM (without IAA) showed 60% and 65% explants response for somatic embryogenesis, wherein 62±0.1 and 84±2.9 embryos produced from each callus mass respectively. Addition of indoleamine inhibitors viz. p-chlorophenylalanine (p-CPA) at 40µM, and Prozac (fluoexitine hydrochloride) at 20 µM reduced induction of somatic embryogenesis and also endogenous pools of SER, MEL and IAA levels by 40-70%. EG medium with MEL / SER at 100 µM + 50 µM CaCl2/ 0.1mM calcium ionophore A23187 induced 75% and 85% explants responded for somatic embryogenesis. EG medium with calcium channel blocker verapamil hydrochloride at 1mM and a calcium chelator EGTA at 1mM reduced somatic embryogenesis. The results clearly demonstrated that the endogenous profiles of indoleamines and IAA levels positively influenced somatic embryogenesis response. This response was further substantiated by calcium imaging studies. SER was found to be localized in vascular tissues of the coffee stem, root and somatic embryos, also in endocarp region (husk) of the coffee immature fruits. The outcome of this study will certainly help to further realize the multiple roles of the indoleamines in plant morphogenesis and elucidating SER and MEL dependent cellular signaling mechanisms in plants.

Network connectance and autonomy analyses of the photosynthetic apparatus in tropical tree species from different successional groups under contrasting irradiance conditions.

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Biological systems are complex dynamical systems whose relationships with environment have strong implications on their regulation and survival. From the interactions between plant and environment can emerge a quite complex network of plant responses rarely observed through classical analytical approaches. The objective of this current study was to test the hypothesis that photosynthetic responses of different tree species to increasing irradiance are related to changes in network connectances of gas exchange and photochemical apparatus, and alterations in plant autonomy in relation to the environment. The heat dissipative capacity through daily changes in leaf temperature was also evaluated. It indicated that the early successional species (Citharexylum myrianthum Cham. and Rhamnidium elaeocarpum Reiss.) were more efficient as dissipative structures than the late successional one (Cariniana legalis (Mart.) Kuntze), suggesting that the parameter DT (T ºCair – T ºCleaf) could be a simple tool in order to help the classification of successional classes of tropical trees. Our results indicated a pattern of network responses and autonomy changes under high irradiance. Considering the maintenance of daily CO_2 assimilation, the tolerant species (C. myrianthum and R. elaeocarpum) to high irradiance trended to maintain stable the level of gas exchange network connectance and to increase the autonomy in relation to the environment. On the other hand, the late successional species (C. legalis) trended to lose autonomy, decreasing the network connectance of gas exchange. All species showed lower autonomy and higher network connectance of the photochemical apparatus under high irradiance.

β-1,3 glucanase activity as a response to xanthomonas signalling in permeabilized leaf discs from healthy and scald-diseased sugarcane plants

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Sugarcane plants develop different defence mechanisms to combat the invasion by pathogenic organisms. Resistance of plants to disease seems to be a multifactorial process and implies constitutive (structural) and active (biochemical) processes according to their function. Several proteins are involved in these mechanisms by playing a predominant role in the process of resistance against pathogens. Enzymes degrading microorganism walls (chitinases, glucanases), and the production of elicitation compounds (e.g. salicylate, jasmonates), are some of the active plant biochemical defence mechanisms. Production of systemic plant β-1,3-glucanase, effected by bacterial signals, and the action of probable elicitors of these defensive proteins are studied as a process of sugarcane plant defence.

Leaf discs from five cultivars of sugarcane exhibiting different degree of susceptibility to leaf scald were used to measure β-1,3-glucanase activity before and after experimental infection with Xanthomonas albilineans. Leaf discs were permeabilized with iso-propanol to facilitate the uptake of the enzyme substrate by intact tissues and to improve the enzyme assay. Sugarcane leaves usually produced the enzyme β-1,3-glucanase and its activity can be in vivo measured by using permeabilized leaf cells.

Bacterial infection significantly enhanced β-1,3 glucanase activity of susceptible cultivars whereas a significant decrease was observed for the resistant one. Nevertheless, the degree of increased activity was different according to the cultivar.

The major increase of activity after the infection were obtained for cv. Louissiana 55-5 (25 %) and Barbados 42-231 (20 %) by comparing it to the activity measured in the controls obtained of healthy plants. Lower increases could be observed in the cv. Cuba 236-51 (7 %) and Jaronú 60-5 (11 %). Nevertheless, the experimental infection of leaves from the resistant cv. Mayarí 55-14 diminished a 16% glucanase activity with regard to the non-infected controls.

Low concentrations of exogenous salicylate increased hydrolase activity whereas exogenous jasmonic acid did not act as an elicitor of the enzyme.

Action of neurotoxic peptide in plant cells

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Prion protein (PrP) is the causative agent of the transmissible spongirform encephalopathies. In general, PrPs from mammals have six to seven putative Cu-binding sites consisted of 4 distinct sequences. Among the copper binding regions, the neurotoxic region in the PrP (106-126) is reportedly binds copper to form the toxic complex & damage the cells. However, Among four distinct Cu-binding peptides derived from human PrP (KTNMKHMA) corresponding to a partial sequence of the neurotoxic region was shown to lack the pro-oxidant activity while other three peptides catalyze the robust production of ROS in the presence of some biological components. Therefore, in the present study, we tried to use this sequence for positive purpose in plant biotechnology, or plant environmental studies.
In plant cells, induction of cell death by oxidative stresses accompanying the generation of reactive oxygen species (ROS) is often mediated by early signaling events such as calcium influx via ROS-mediated activation of calcium channels on the plasma membrane. Copper is known to be one of such oxidative stress inducer. This phyto-toxic metal actually induces an increase in cytosolic fee calcium concentration ($[Ca^{2+}]_c$) followed by cell death in tobacco cells.
In this study, impact of copper on the oxidative and calcium signal transductions leading to cell death in suspension culture tobacco cells (*Nicotiana tabacum* L., cell line BY-2, expressing the aequorin gene) and the effects of the copper-binding peptide derived from the human PrP as a novel plant-protecting agent were assessed. The role of early oxidative events induced by copper was proven by the action of specific ROS scavengers blocking the calcium responses and the calcium signature was monitored by the aequorin luminescence and the calcium events was blocked in the presence of specific channel blockers. Following these early events completed within 10 min, the development of copper-induced cell death was observed during additional 1 hour in a dose-dependent manner. Addition of synthetic peptide (KTNMKHMA) corresponding to the neurotoxic sequence in human PrP, prior to the addition of copper effectively blocked both the calcium influx and cell death induced by copper. Since the agent tested here is a peptide, genetic modification of plants for overproduction and excretion of this or related peptidic agents is one of the possible choices in order to minimize the phytotoxicities of various metals in the future environments.

The 7B-1 mutation in tomato confers a blue light-specific lower sensitivity to coronatine

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Male sterility of crop species, spontaneous or induced, is a criterion of importance for breeders. In almost all crop species male-sterile mutants have been reported but their use in breeding programs has been limited as some of them were sensitive to abiotic stresses, such as drought or cold temperature. In tomato (*Solanum lycopersicum L.*), one of the most important crops worldwide, the spontaneous mutant 7B-1, isolated for its photoperiod-dependent male-sterility, has been described as resistant to various abiotic stresses specifically under blue light. Since this finding improved potential of 7B-1's use in breeding programs, its susceptibility to coronatine (COR)-induced stress, the phytotoxine produced by several *Pseudomonas syringae* strains, was assessed in this study. The 7B-1 mutant was found to be less sensitive than the corresponding wild-type (WT) to COR treatment in a blue light dependent manner. Treatment of WT and 7B-1 plants with COR induced a strong accumulation of salicylic acid (SA), jasmonic acid (JA) and abscisic acid (ABA) in hypocotyls. Interestingly, accumulation of ABA and SA in the 7B-1 mutant was distinctly greater than in WT, especially in blue light. Based on the cross-talk between SA- and JA-signaling pathways, expression analysis of NPR1 and COI1 genes, respectively involved in these pathways, was investigated in COR-stressed plants. The blue light-specific lower sensitivity of 7B-1 plants to COR was found to be associated with blue light-specific over-expression of the NPR1 gene. This data suggests that the SA-dependent NPR1-dependent pathway could be involved in the lower sensitivity of the 7B-1 mutant to COR. The role of anthocyanins and ABA accumulation during the response to COR is also discussed.

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Key-words: blue light-specific response, COI1, coronatine, growth, NPR1, SA-signaling pathway, 7B-1 mutant, tomato (*Solanum lycopersicum L.*).

Investigation of BL-mediated de-etiolation in the spontaneous 7B-1 mutant in tomato

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In dark condition, plants grow rapidly in order to place meristem in favorable conditions to ensure autotrophic growth. As soon as plants perceive light, growth rate decreases and cotyledons open and accumulate active chloroplasts. This process, known as de-etiolation, is mostly regulated by blue light (BL), through the photoreceptors cryptochromes (CRY) and phototropins (PHOT). Studies of BL signaling pathway are mainly conducted on the plant model Arabidopsis, whereas less researches are developed on important crop species, like tomato (*Solanum lycopersicum L.*), one of the most important crop species worldwide.

In tomato, the spontaneous 7B-1 mutant has been selected originally for its photoperiod-dependent male sterility. This mutant presents essentially taller phenotype than the corresponding wild-type (WT) and previous results suggest that it is defective either in BL perception or signaling pathway. We investigated the BL-mediated de-etiolation in a comparative study involving the 7B-1 mutant and the cry1-1 mutant, defective in CRY1 photoreceptor.

When plants were grown in greenhouse, the taller size of the two mutants compared to the WT was correlated with longer internodes, suggesting a difference in cell expansion or cell number. An analysis of leaf surface demonstrated that 7B-1 developed larger leaves than the WT in opposite to cry1-1, suggesting that the 7B-1 mutation, even affecting BL signaling pathway, is different from the cry1-1 mutation. When grown in vitro, 7B-1 and cry1-1 were less sensitive to BL-induced de-etiolation compared to the corresponding WT. Once again, the higher size of plants was correlated with longer cells. Moreover, cell expansion under BL was driven by a higher osmotic pressure in the mutants compared to the WTs. Expression of LeEXT gene, encoding a xyloglucan endotransglycosylase and involved in cell wall loosening, was investigated by semi-quantitative RT-PCR. We found that in 7B-1, LeEXT expression was less inhibited by BL than in WT; in cry1-1 LeEXT expression was stimulated by BL. Finally, expression of PHOT1 and CRY1 genes was investigated by qRT-PCR. Whereas no significant difference was observed mutants and WT for CRY1 expression, expression of PHOT1 was less reduced by BL in the mutants compared to WT, especially in the 7B-1 mutant.

Our results show that the mutation in 7B-1 affects BL-mediated cell expansion but in a different manner compared to the cry1-1 mutation. Results obtained from expression analysis led us to the hypothesis that 7B-1 is affected in PHOT1 signaling pathway, or in the photoreceptor itself. PHOT1-signaling pathway during de-etiolation process in tomato is under further investigations.

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Key-words: tomato, *Solanum lycopersicum L.*, 7B-1 mutant, blue light (BL), phototropin (PHOT), cryptochrome (CRY) de-etiolation, cell expansion

Changes in abscisic acid distribution in heat-stressed pepper seedlings

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The heat stress-responsive changes in both abscisic acid (ABA) subcellular distribution and soluble content in pepper mesophyll and root cap cells were investigated by colloidal gold labeling technique and HPLC technique. The results showed that the ABA was localized in the nucleus and the cytoplasm of two cell types in the seedlings under normal temperature, with a higher accumulation in the root cap cells, relatively. As the seedlings were transferred to 40°C for heat stress, the ABA levels in both mesophyll and root cap cells increased markedly, especially in the later. With a sustained heat stress, the ultrastructure of mesophyll cell was damaged severely, and more ABA accumulated in the nucleus of mesophyll cells; comparably, the root cap cell maintained intact ultrastructurally, and a concomitant drastic increase in ABA in the nucleus of root cap cells was also observed. The HPLC quantization demonstrated that the soluble content of ABA in the tissue of root tip was higher than that of leaves under normal temperature, and with the heating time,the content of ABA in leaves increased, but decreased in the root tissue. The above results imply that ABA might be one of the heat stress signaling members in plant cells, whereas the mechanism by which ABA functions during this process remains poorly understood.

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